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SHORT COMMUNICATION

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Fever in honeybee colonies

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Abstract Honeybees, *Apis* spp., maintain elevated temperatures inside their nests to accelerate brood development and to facilitate defense against predators. We present an additional defensive function of elevating nest temperature: honeybees generate a brood-comb fever in response to colonial infection by the heat-sensitive pathogen *Ascosphaera apis*. This response occurs before larvae are killed, suggesting that either honeybee workers detect the infection before symptoms are visible, or that larvae communicate the ingestion of the pathogen. This response is a striking example of convergent evolution between this "superorganism" and other fever-producing animals.

Introduction

Colonies of honeybees, *Apis* spp., maintain elevated temperatures inside their nests (Seeley 1985). Among other potential benefits, high temperature accelerates brood development (Milum 1930) and facilitates defense against predators: honeybees engulf invaders in defensive balls, which they heat to lethal temperatures (Ono et al. 1995). Since many animals produce fever when ill (Kluger 1979), we examined the temperature response of *Apis mellifera* to colony infestation of the heat-sensitive pathogen *Ascosphaera apis*.

A. apis causes a fungal disease known as "chalk brood" (Maassen 1913; Deans 1940). Chalk brood, transmitted through ingestion of food containing *A. apis* spores, affects larvae (Maassen 1913; Deans 1940; De Jong and Morse 1976; De Jong 1977; Bailey 1981). Once infected, larvae die and dry into white lumps resembling chalk (commonly called "mummies"; Maassen 1913). However, the larvae must be chilled to about 30 °C (thus just a few degrees below the normal 33–36 °C brood-comb temperature; Seeley 1985) for the disease to develop (Bailey 1981). The slightly lower temperature enables oxygen to penetrate the larval gut, which activates mycelium growth (Bailey 1981). Accordingly, chalk brood is most common in the spring (Bailey 1981) and in small colonies which are unable to maintain high temperature (Deans 1940). It would be beneficial for honeybees to recognize colonial infestation by the pathogen and increase brood-comb temperature to limit the pathogen's effect.

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Methods

To determine whether honeybees (*A. mellifera*) produce fever in response to *A. apis* infestation we collected the queen, approx. 3750 bees, and one frame of brood-comb from each of three colonies. The bees and brood-comb (21 × 42 cm) were placed into two-frame observation hives (outer dimensions: 47 × 44 × 9 cm) containing one frame of empty honeycomb (14 × 42 cm). The brood and honey were kept separate by inserting a queen excluder between the upper and lower combs (Seeley 1995). Observation hives were insulated with 2.54-cm-thick Styrofoam sections. Using thermistor probes (sensitive to 0.25 °C) positioned inside (along the brood- and honeycomb) and immediately outside the hive, temperatures were measured with an IT 660 electrotherm digital thermometer (Electromedics). Temperatures were recorded from each colony at 00:00, 08:00, and 16:00 hours daily from 25 May 1998 through 23 June 1998.

The experiment contained four distinct periods: (a) *prefeed*, colonies were unfed from 00:00 on day 1 until 00:00 on day 10; (b) *feed*, colonies were fed 50% solution of sugar-water from a container on the observation hive from 00:00 on day 10 until on 00:00 day 15; (c) *treatment*, colonies were fed four 100-ml vials of 50% sugar-water solution containing 1% ground sporulating "mummies," which is sufficient to cause the disease (De Jong 1977; De Jong and Morse 1976), from 00:00 on day 15 through 00:00 on day 17 and from 00:00 on day 18 through 00:00 on day 22 colonies were fed a 50% solution of sugar-water, and; (d) *post-treatment*, colonies were unfed through day 30. To control for extraneous variables, temperatures were collected during the feed

and treatment periods from an additional colony, which was prepared identically to the treatment colonies, but not inoculated.

Regression analysis was performed to determine the relationship between ambient and brood-comb temperatures. The resulting regression equations were used to determine the expected brood-comb temperature for any given ambient temperature. Two-sample *t* tests were used to compare the brood-comb temperatures from different experimental periods within experimental colonies. Paired *t* tests were used to compare different experimental periods across experimental colonies. All data were analyzed using Excel 98 for Macintosh. All descriptive statistics are presented as means ± standard errors.

Results

Brood-comb temperature varied only slightly during the observation period (colony 1, 35.04 ± 0.04; colony 2, 34.31 ± 0.10; colony 3, 34.81 ± 0.10; control colony, 34.41 ± 0.05). However, the brood-comb temperature in all colonies was positively correlated with the respective ambient temperature (Fig. 1). Regression equations were used to determine the colony-specific expected brood-comb temperature for the observed ambient temperature. There was no relationship between ambient temperature and the observed minus expected brood-comb temperatures in any colony (linear regression: colony 1, df = 88, $r^2 = 0.00$, $P = 0.99$; colony 2, df = 88, $r^2 = 0.00$, $P = 0.99$; colony 3, df = 88, $r^2 = 0.00$, $P = 0.99$; control colony, df = 34, $r^2 = 0.00$, $P = 0.99$). This result indicates that our design effectively controlled for ambient temperature fluctuations.

Temperatures differed slightly between the *prefeed* and *posttreatment* periods (Figs. 2, 3), but no significant trend was detected across colonies (paired *t* test: $t_2 = 1.96$, $P = 0.19$; Fig. 3). Temperatures in the *feed* and *treatment* periods were significantly different within each (colony 1, *t* test: $t_{34} = 5.19$, $P < 0.0001$; colony 2, $t_{34} = 2.46$, $P < 0.02$; colony 3, $t_{34} = 5.53$, $P < 0.0001$; Fig. 2) and across all experimental colonies (paired *t* test: $t_2 = 9.94$, $P < 0.01$; Fig. 3). These results indicate an increase in brood-comb temperature after inoculation.

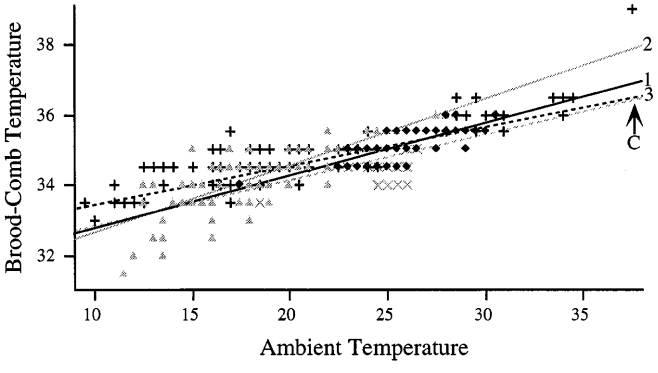

Fig. 1 Relationship between brood-comb and ambient temperatures for all colonies. The regression equations are as follows: colony 1 (◆), $y = 0.15x + 31.26$, df = 88, $r^2 = 0.46$, $P < 0.0001$; colony 2 (▲), $y = 0.19x + 30.72$, df = 88, $r^2 = 0.67$, $P < 0.0001$; colony 3 (+), $y = 0.12x + 32.34$, df = 88, $r^2 = 0.77$, $P < 0.0001$; control colony (×), $y = 0.13x + 31.55$, df = 34, $r^2 = 0.46$, $P < 0.001$

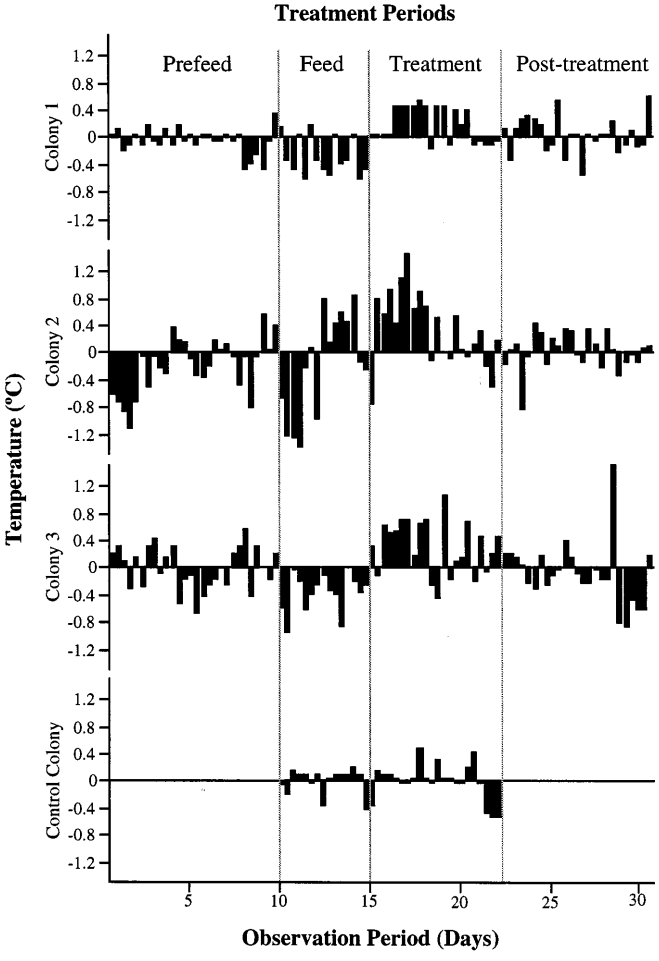

Fig. 2 Observed minus expected temperatures for each colony at each temperature collection period. Expected temperatures were calculated using regression equations (see "Methods"). Negative and positive values indicate lower and higher than expected temperatures, respectively

No such difference was observed in the colony controlling for extraneous variables ($t_{34} = 0.39$, $P = 0.70$; see Fig. 2); thus our data indicate a fever in response to infestation by *A. apis.*

Discussion

Although brood-comb temperatures were maintained within narrow temperature ranges, brood-comb temperature was positively correlated with ambient temperature for each colony (Fig. 1). This relationship, and the respective regression equations, enabled a colony-specific calculation of expected brood-comb temperature for any given ambient temperature. The expected brood-comb temperature was then compared to the observed temperature across the different treatment periods (Fig. 3).

Since each experimental colony effectively has control (*prefeed* and *posttreatment*) and experimental (*feed*

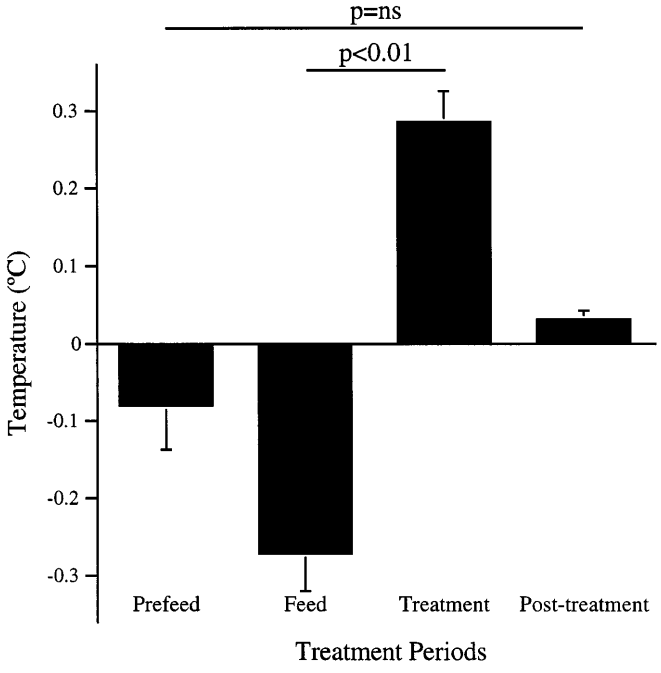

Fig. 3 Mean observed minus expected temperatures for the experimental colonies. Expected temperatures were calculated using regression equations. Negative and positive values indicate lower and higher than expected temperatures, respectively; columns means; error bars are standard errors. Both tests are paired *t* tests with two degrees of freedom

and *treatment*) periods, we report on three replicates of the experiment. The additional, control colony allowed for detection of any unknown extraneous variable influencing brood-comb temperature during the experimental period; no unexplained temperature fluctuations were observed (see Fig. 2).

Brood-comb temperature decreased in experimental colonies during the *feed* period (Figs. 2, 3). This result may have been due to the feeding protocol: a small opening at the top of the observation hive provided access to the sugar-water, thereby influencing some honeybees to exit the brood-comb region. This relationship was not observed in the control colony since the brood-comb and ambient temperature relationship was determined using only *feed* and *treatment* periods, i.e., those periods in which sugar-water was consistently available.

For all experimental colonies, brood-comb temperature was significantly greater after inoculation with the pathogen (see Figs. 2, 3). This result cannot be explained by the feeding protocol: brood-comb temperature was higher than all other treatment periods (Fig. 3). The average difference between observed and expected temperatures in the *feed* and *treatment* periods was 0.56 °C. Although at first glance this increase may appear small and ineffective, it should be noted that 0.56 °C is nearly 20% of the standard brood-comb temperature range (33–36 °C; Seeley 1985). Hence, the small temperature boost that we observed is likely to confer increased resistance to *A. apis*, which needs only a slight chilling of the larvae to cause disease (Bailey 1981). This up-regulation of brood-comb temperature occurred before larvae were killed, suggesting that the fever response was preventative.

Other than an adaptive, behavioral fever generated by the honeybees, we are aware of no other viable hypothesis to explain the postinoculation increase in brood-comb temperature. Since *A. apis* is a heat-sensitive pathogen, it is highly unlikely that *A. apis* benefits from higher brood-comb temperature. Indeed, only colony 1 produced "mummies," and this infection was minor. It is also unlikely that the elevated brood-comb temperature is optimal for uninfected brood: brood-comb temperature returned to normal by the end of the observation period (Fig. 3). Since brood-comb temperature returned to normal, it is also unlikely that the fever resulted from colony growth, i.e., an increase in the number of workers available for temperature maintenance.

In conclusion, our results indicate that (a) brood-comb temperature in honeybee colonies is positively correlated with ambient temperature, (b) honeybee colonies generate a brood-comb fever in response to inoculation with *A. apis*, and (c) this fever response is apparently preventative. These results suggest that either honeybee workers detect the infection before symptoms are visible, or that larvae communicate the ingestion of the pathogen.

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