Influence of Brood Temperature and Hygrometry Variations on the Development of the Honey Bee Ectoparasite *Varroa jacobsoni* (Mesostigmata: Varroidae)

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ABSTRACT The influence of different temperatures (from 26 to 39.5°C) and relative humidities (40 and 70%) on the development of *Varroa jacobsoni* (Oudemans) was studied by placing newly capped and parasitized worker bee broods into thermostatically controlled chambers. In one set of experiments the temperature was kept constant, and in the second set, the parasitized worker broods were placed at a temperature of 40, 41, or 42°C for a time varying from 0 to 24 h and then returned to 32.5°C. The optimal temperature for development of the mites was between 32.5 and 33.4°C, which corresponds to the brood temperature of *Apis mellifera* L. Above 36.5°C, reproduction of varroa females was significantly reduced, and above 38°C, mites began to die without reproduction. Jumps of temperature were unfavorable to the development of the mites. The regulation of brood temperature by bees and occasional temperature peaks may be key factors in resistance of honey bees to varroa mites.

KEY WORDS Insecta, *Varroa jacobsoni*, *Apis mellifera*, temperature

Varroa jacobsoni (Oudemans), an ectoparasite of the honey bees *Apis mellifera* L. and *A. cerana* F., is the main cause of significant bee (*A. mellifera*) losses, especially in temperate, Mediterranean, and subtropical climates. Honey bee colonies infected with varroa mites die within a few years if not treated with acaricides or some other means of mite control (Ritter 1981, De Jong et al. 1982).

Varroa mites were first identified on *A. cerana*, an Asian honey bee (Oudemans 1904, Delfinado 1963). In this particular honey bee species, mite populations remain at low levels and do not destroy the colonies. There are two explanations for this: first, Asian worker bees perform a series of cleaning behaviors that remove the mites (Peng et al. 1987), and second, fecund mites infest drone brood more frequently than the worker brood of this species (Koeniger et al. 1981, De Jong 1988).

In South America's tropical climates, especially in the warmest areas, which are populated to a large extent by Africanized bees, *A. mellifera* appears to be less vulnerable to the mite than in temperate areas (De Jong et al. 1984). As with *A. cerana*, the rate of the varroa mites reproduction on the Africanized worker bee brood *A. mellifera scutellata* is not as high as on European honey bees *A. mellifera mellifera* and *carnica* (Engels et al. 1986). Nevertheless, based on the rate of infertile varroa females in the worker brood cells, European honey bees in Uruguay, which has a subtropical climate, seem to be varroa resistant (Ruttner & Marx 1984).

Worker honey bees thermoregulate the brood nest maintaining a temperature between 33 and 36°C. The exact temperature depends on external conditions and on the species and races of bees (Ribbands 1953, Darchen 1973, Kronenberg 1979, Kronenberg & Heller 1982, Villa et al. 1987, Rosenkranz 1988). If the ambient temperature falls below that suitable for development of the brood, worker bees raise the temperature by producing metabolic heat (Esch 1960; Roth 1965; Simpson 1968, 225–234). If the temperature within the nest is too high, the workers begin fanning and evaporating collected water inside the hive to reduce the temperature (Lindauer 1954, Simpson 1968).

In an apiary located in India, *A. cerana* maintained a brood nest temperature of between 37.5 and 38.5°C during the warm season, and between 21 and 27.5°C in the cold season, whereas in the same apiary, the brood nest temperature of *A. mellifera* was maintained between 34 and 36°C (Verma 1970, Bisht et al. 1979). *A. cerana* does not display the same fanning behavior as *A. mellifera*, and comparative studies have shown differences in the thermoregulation of European and Africanized honey bees (Nunez 1979, Villa et al. 1987). Villa et al. (1987) have also reported peaks in temperature that may last 1–2 h in some parts of the brood nest. Temperature peaks occur more frequently in the nest of Africanized bees (20 peaks of 42°C over a 5-d period) than the nest of European honey bees (5 peaks of 41°C during the same period).

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The question is, could differences in the way the bees regulate the brood temperature affect the phenomenon of varroa resistance apparent in some honey bee populations?

To examine the relationship between varroa resistance and thermoregulation of the brood nest in which varroa breeds, the effect of temperature and relative humidity on reproduction and development of mites in worker brood cells was examined in this study.

Materials and Methods

Two strong colonies of *A. mellifera mellifera*, each with ≈10% of adult workers parasitized by varroa mites, were used in this study between May and July 1988.

Larvae of the same age were obtained by confining the queen on an empty comb for 24 h and then in a queen cage for 4 d. About 6–24 h after the capping of the cells, samples of brood containing 200–600 worker larvae were put into thermostatically controlled chambers until the worker nymphs reached the blue eyed-thorax pigmentation stage. At this stage, it is possible to distinguish between the different developmental stages of the mites. Each worker bee cell was opened and the number of mites, dead or alive, was counted and their stages of development (eggs, protonymphs, deutonymphs, adult males and females) were recorded. The worker bee pupae, dead or alive, were also counted. Control worker bee pupae were maintained in the two colonies for comparison.

Two sets of experiments were conducted to test the effect of temperature on the mites.

Constant Temperature. In the first set of experiments, the temperature of the thermostatically controlled chambers was held constant throughout the experiment. A range of temperatures between 26 and 39°C (±0.5°C) was tested at both 40 and 70% (±10%) RH.

Temperature Jumps. In the second set of experiments, samples of capped brood were first placed at 40, 41, or 42°C and 70% RH for times varying between 0 and 24 h and then returned to a chamber maintained at 32.5°C and 70% RH.

Test Parameters. The rate of mortality of the original mother mites, the fertility (percentage of females having at least one oviposition), the fecundity (number of eggs, nymphs and adults produced per fertile original mother), and the prolificity (number of alive protonymphs, deutonymphs, and adults produced per fertile original mother) were calculated for each brood sample used in the two sets of experiments. To measure the reproductive efficiency of the varroa females, the prolificity was calculated in relation to the total number of mother varroa females entering the cells. The rate of mortality for the worker bee pupae was also calculated.

Varroa Distribution. The number of mother varroas per cell from one brood frame was noted and analyzed using a Poisson distribution with a χ^2 test to examine the possibility of mite aggregation.

Statistical Analysis. The relationship between mortality and temperature, which was represented by sygmoid, was estimated by using the logit transformation: $P = 1/(1 + e^{-(\alpha+\beta x)})$ with $\alpha + \beta x = \log(P/Q)$, where P is the probability that a mite will die at a given temperature. Linear regression analysis was used to estimate the difference between observed and calculated values.

The equations of fertility, fecundity, and prolificity were established by the Gaussian formula: $y = (1/\sigma\sqrt{2\pi}) \cdot e^{-(x-\mu)^2/2\sigma^2}$ where μ and σ refer to means and standard deviations, respectively. A correlation coefficient was calculated between the observed and calculated cumulative data.

Comparison of effectives was determined using the χ^2 test. Differences of means and variances of each characteristic were compared using the t and F tests.

Results

Mortality. At 70% RH, varroa females were not affected by temperatures <28°C, even though losses of honey bee nymphs did occur ($\chi^2 = 46.15$, df = 1, $P < 0.001$) (Table 1). However, varroa females were severely affected by high temperatures. At 38.5°C, 100% mortality of the female mites was recorded at 40 and 70% RH. No concomitant bee losses were observed at this temperature. The normality of varroa female mortality was estimated by the logit transformation with $\alpha = -54.52$ and $\beta = 1.42$ ($r = 0.82$) for RH = 70% and with $\alpha = -93.78$ and $\beta = 2.53$ ($r = 0.81$) for RH = 40% (Fig. 1).

Fertility. The number of mites achieving at least one oviposition was strongly influenced by both temperature and hygrometry. Observed data are presented in Table 1. The Gaussian parameters $\mu = 33.22$, $\sigma = 2.42$ ($r = 0.99$) were calculated for 70% RH, and $\mu = 33.43$, $\sigma = 1.98$ ($r = 0.97$) for 40% RH. At 40% RH, the fertility levels were highest (68.3–66.7%) at temperatures between 33 and 35°C, and no oviposition was observed >37.5°C. At 70% RH, higher fertility levels were recorded than at 40% RH (93.8–97.4%) and peak values were observed between 32 and 34°C. The maximum fertility level at 70% RH was significantly higher than at 40% RH ($\chi^2 = 22.1$, df = 1, $P < 0.0001$). No oviposition at all was observed beyond the limits of 26–38°C.

Fecundity. The fecundity of fertile varroa females varied with temperature and hygrometry (Table 1). At 70% RH, no egg, nymph, or adult derived from original mother was observed at temperatures <26°C or >38°C. This was also the case at 40% RH at temperatures >37°C. Maximum fecundity was between 31 and 34°C at 70% RH and around 33°C at 40% RH, and was more significantly higher at 70% RH (2.75) than at 40% RH (2.27) ($t = 2.46$, $P < 0.02$). The equation of fecundity in

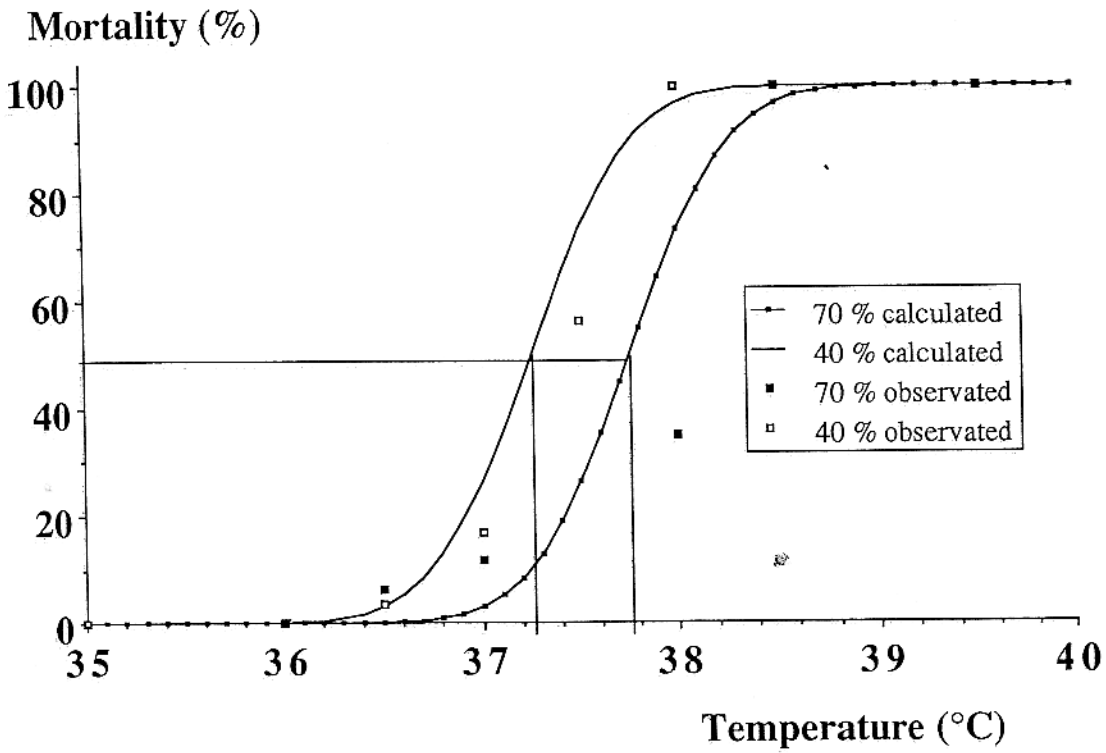

Fig. 1. Varroa mite mortality in the worker bee capped brood cells as a function of the ambient temperature.

relation to temperature was estimated with Gaussian parameters $\mu = 32.57$ and $\sigma = 2.73$ ($r = 0.94$) for 70% RH and $\mu = 33.27$ and $\sigma = 1.51$ ($r = 0.99$) for 40% RH.

Prolificity. At the tested temperatures, the rate of living protonymphs and deutonymphs per fertile mother varroa was higher at 70% RH than at 40% RH (Table 1). This higher prolificity was more

Table 1. Reproduction and development of *Varroa jacobsoni* in worker bee capped brood cells as a function of temperature and hygrometry (fecundity and prolificity: rate ± SD)

Temperature, °C	No. mother mites	% Varroa mortality	% Bee nymphs mortality	% Varroa fertility	Varroa fecundity	Mites prolificity/ fertile female
			40% RH			
30	97	0.0	14.2	38.1	0.27 ± 0.37	0
32	139	0.0	16.9	41.7	1.59 ± 0.82	0.64 ± 2.1
33	82	1.2	18.1	68.3	2.27 ± 1.27	1.00 ± 2.31
35	57	0.0	8.3	66.7	0.74 ± 0.55	0.32 ± 1.20
36.5	60	3.3	5.9	31.7	0.68 ± 0.51	0
37	133	17.0	13.9	5.2	—	—
37.5	71	56.3	2.9	0.0	—	—
38	38	100.0	18.7	0.0	—	—
			70% RH			
26	68	3.1	89.0	0.0	—	—
28	89	7.9	12.3	28.1	1.92 ± 0.61	0.12 ± 0.49
30.5	85	0.0	2.6	60.0	1.10 ± 0.48	0.08 ± 0.06
31	82	1.2	3.0	56.1	1.40 ± 0.80	0.13 ± 0.63
32	48	4.2	1.8	93.8	2.47 ± 1.24	1.16 ± 2.19
32.5	99	2.0	6.8	88.0	2.75 ± 1.35	1.79 ± 1.67
34	76	2.6	14.9	97.4	1.61 ± 1.12	1.04 ± 1.08
36	99	3.0	0.0	85.9	1.38 ± 1.06	0.68 ± 1.33
36.5	258	6.2	12.5	43.4	0.96 ± 0.79	0.46 ± 0.88
37	169	11.8	11.5	40.8	0.91 ± 0.42	0.28 ± 0.51
38	40	35.0	5.2	0.0	—	—
38.5	57	100.0	15.0	0.0	—	—
39.5	64	100.0	7.2	0.0	—	—
			Control			
	54	0.0	2.0	83.0	3.11 ± 1.28	1.86 ± 1.52

significant at 70% RH (1.79) than at 40% RH (1.00) ($t = 2.58$, $P < 0.01$). The optimum rate of prolificity was between 32 and 34°C at 70% RH and around 33°C at 40% RH. Fig. 2 shows the prolificity observed and calculated per original mother mite in relation to temperature.

Prolificity per fertile mother mite is represented by observed (Table 1) and calculated data with the Gaussian parameters $\mu = 33.35$ and $\sigma = 1.43$ for 70% RH ($r = 0.92$), and $\mu = 33.13$ and $\sigma = 1.12$ for 40% RH ($r = 0.99$). Maximum reproduction was observed at 33°C at 40% RH, and 32.5°C at 70% RH. Reproduction was significantly higher at 70% RH (1.58) than at 40% RH (0.68) ($t = 2.53$, $P < 0.01$). Beyond these temperatures, prolificity fell dramatically (Fig. 2). Satisfactory reproduction was limited to a temperature range at 30–36.5°C at 40% RH, and to temperatures between 31 and 37°C at 70% RH.

The mean optimal temperatures calculated for all of these characters lay between 32.6 and 33.5°C at 70% RH and between 33 and 33.43°C at 40% RH.

Control. Mortality and fertility levels in the controls did not differ significantly from the maximum levels observed in the experiments described above (Table 1). The prolificity per mother varroa in the controls did not differ from the maximum levels observed with a 70% RH but did with a 40% RH ($t = 2.25$, $P < 0.05$).

Temperature Jumps. Mite mortality reached significant levels after 24 h at 40°C, after 12 h at 41°C, and after 6 h at 42°C (Table 2). In all cases, mites were more susceptible to large increases in temperature than the bee nymphs.

Fertility and fecundity began to decrease significantly after 1 h at all three temperatures. Oviposition did not occur after 24 h at either 40 or 41°C, or after 6 h at 42°C.

Mite prolificity reduced to zero after 6 h at 41°C, and after only 1 h at 42°C, demonstrating that the immature varroa mites are very temperature sensitive. After 2 h of this treatment, the prolificity of the mites was decreased by 50% at 40°C, by 25% at 41°C and was reduced to 0 at 42°C.

Varroa Distribution. The observed number of mother varroa mites differed significantly from the number calculated with a Poisson's distribution in each category of cells (cells containing 0, 1, 2, 3, 4, 5 mites) ($\chi^2 = 152$, df = 5, $P < 0.0001$) (Table 3). Cells containing 3, 4, or 5 mites per cells were found more frequently than predicted.

Discussion

The dynamics of varroa populations are dependent on the mortality, fertility, fecundity, and prolificity of varroa females. We have shown that these factors are influenced by the temperature and hygrometry of the environment in which the host (bee brood) lives.

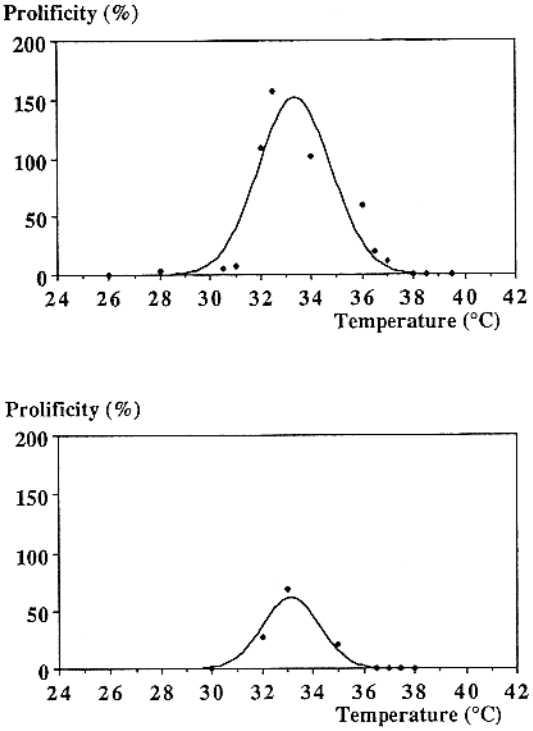

Fig. 2. Prolificity per original mother mite in the worker bee capped brood cells as a function of the ambient temperature at 70% RH (top) and 40% RH (bottom).

Relative humidity of 40% is less favorable for development than 70% RH. Normally relative humidity varies between 40 and 80% in the brood area of a honey bee colony (Wohlgemuth 1957). When the ambient temperature is high, worker bees bring water into the hive to help reduce the temperature by evaporative cooling, and when the ambient temperature is low, workers produce metabolic heat to raise the temperature levels. Both of these extremes in temperature lead to an increase in relative humidity (50–70%). When ambient temperature is close to brood temperature, the relative humidity decreases (40%) (Wohlgemuth 1957).

The temperatures at which varroa development and reproduction are best vary between 32.5 and 33.4°C, which correspond to bee brood temperatures (32–36°C) and more exactly, to the temperature of drone brood, which is located at the periphery of the brood area (Ribbands 1953, Darchen 1973, Kronenberg 1979, Kronenberg & Heller 1982, Villa et al. 1987). There is no reproduction above 37°C or below 28°C.

The brood temperature of *A. cerana* varies between 37.5 and 38.5°C in the warm season and 21 and 27.5°C in the cold season (Bisht et al. 1979). Under the same conditions, the brood temperature of *A. mellifera* remains between 32 and 36°C (Verma 1970). If we assume that the varroa mites, living on *A. cerana*, have the same genetic resistance to

Table 2. The effects of jumps in temperature on the reproduction and development of *Varroa jacobsoni* in worker bee capped brood cells (fecundity and prolificity: rate ± SD)

Time, hours	No. mother mites	% Varroa mortality	% Bee nymphs mortality	% Varroa fertility	Varroa fecundity	Mites prolificity per	
						Fertile female	Mother varroa
				40°C			
0	99	2.0	7.0	88.0	2.75 ± 1.35	1.79 ± 1.67	1.58 ± 2.66
1	46	2.0	4.6	73.9	2.24 ± 1.02	1.18 ± 1.8	0.87 ± 2.3
2	128	4.7	9.6	68.0	1.98 ± 1.19	1.09 ± 2.25	0.74 ± 1.81
3	29	3.5	6.6	79.3	1.91 ± 0.98	0.61 ± 0.96	0.48 ± 1.08
6	96	2.1	11.6	40.6	1.41 ± 0.65	0.64 ± 1.79	0.26 ± 1.6
24	82	59.8	14.4	0	—	—	—
				41°C			
0	99	2.0	7.0	88.0	2.75 ± 1.35	1.79 ± 1.67	1.58 ± 2.66
2	43	0.0	4.0	46.5	1.50 ± 0.94	0.80 ± 1.03	0.37 ± 1.06
6	52	1.9	2.1	19.2	1.10 ± 0.43	0	—
12	64	43.8	11.1	10.9	1.00 ± 0.31	0	—
24	60	100.0	15.3	0	—	—	—
				42°C			
0	99	2.0	7.0	88.0	2.75 ± 1.35	1.79 ± 1.67	1.58 ± 2.66
1	74	2.0	7.8	37.8	1.25 ± 0.65	0	—
2	69	1.5	1.3	30.4	1.00 ± 0.50	0	—
3	70	1.4	2.7	17.1	1.00 ± 0.33	0	—
6	29	96.6	1.6	0	—	—	—
12	66	90.9	4.8	0	—	—	—
24	72	100.0	17.0	0	—	—	—

high and low temperatures as the varroa mites examined in this study, thermal regulation of brood in *A. cerana* would seem to be responsible, in part at least, for varroa resistance in these bees. These results confirm those of Akimov & Piletskaya (1985) who found that in vitro there is no development of varroa eggs outside the limits of 31–37°C. Jumps of temperature to 41 and 42°C in our study reduced the prolificity of the varroa females to 75 and 100%, respectively.

There are differences in the thermal regulation of the brood between Africanized and European honey bees (Nunez 1979, Villa et al. 1987). The temperature peaks observed by Villa et al. (1987) in the brood of European honey bees (5 peaks of 41°C in 5 d) and of Africanized honey bees (20 peaks of 42°C in 5 d) moderate the development of the mite and could explain, at least partially, the phenomenon of varroa resistance in Africanized honey bees, and of varroa-resistant honey bees in tropical climates.

Varroa females are able to detect a 1.2°C change in temperature (Le Conte & Arnold 1987) and their preferred temperature (32.7 ± 2°C) corresponds to the optimal temperature for the development of the mites (Le Conte & Arnold 1988). Temperature, and especially the limits of 32.5–33.5°C, which correspond to the drone brood temperature, seems to be very important in the varroa life cycle. The mite, which is attracted to the drone brood of *A. mellifera* by kairomones and by ambient temperature cues, finds in these cells conditions that are the most suitable for development and reproduction.

Genetic variability influences thermoregulation in honey bees (Brückner 1975). The temperature limits for reproduction and development of varroa mites and temperature peaks in the bee brood lead us to conclude that thermoregulation by honey bee colonies could be a useful criterion in the selection of varroa-resistant honey bee strains.

The varroa mites are not evenly distributed in the brood comb, but tend to aggregate in some cells. There are kairomones produced by bee larvae, which are attractive to varroa mites at brood temperature (Le Conte et al. 1989). This suggests three possibilities: first, that the larvae in the cells in which the varroa females are concentrated may produce more kairomones than the other larvae, second, that a greater number of worker bees caring for selected larvae raises the probability of bringing varroa to those cells, and third, that the varroa females may produce a signal (pheromone) that attracts other varroa females.

Table 3. Distribution of the varroa mother mites in the brood capped cells

No. cells	No. varroa/cells					
	0	1	2	3	4	5
Calculated	700.7	377.5	203.4	54.9	10	1.5
Observed	735	338	180	78	36	15

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