2.0 Metabolic Physiology and the Energy and Nutritional Demands of the Parasitic Life of the Mite *Varroa destructor*

2.1 Abstract

The energy and nutritional demand of the ectoparasitic mite *Varroa destructor* (Anderson and Trueman) was investigated by calorimetry, respirometry and their resource utilization rate. Mites from worker brood, drone brood, and adult workers of the western honeybee *Apis mellifera carnica* were monitored in the absence of the host. Energy metabolism of the mites, elucidated by the heat production rate, was insignificant as a factor to cause honeybee colony death. The metabolic rates of mites ranged from 1.1% to 2.4% of that of the bee pupae, depending on the infestation level. The nutritional demand of the mites was, however, very high, owing to their inefficient metabolic machinery, utilizing up to 28% of reserve food of the pupa during the capped brood developmental stage. This contributes to the malformation and weakening of the bees and eventually death of the colony.

2.2 Introduction

The formidable ectoparasitic mite *Varroa destructor* (Anderson and Trueman) feeds on the hemolymph of the brood and adults of the western honeybee *Apis mellifera* L. and causes damage to the latter. The extent of harm is proportional to the degree of infestation. Varroa mites may involve different mechanisms in the weakening and obliteration of the honeybee colony. The role of *Varroa destructor* as a vector of bacterial and fungal disease as well as an inducer of latent viral infection in varroosis of the western honeybee *A. mellifera* L. has been demonstrated (Hargasm 1973, Ball 1983, Ball and Allen 1986, Wiegers 1986, Trubin et al. 1987, Gliński and Jarosz 1988, 1990a, 1990b, 1992, Ball 1994 and 1996, Liu 1996). Ball (1983 and 1996) mentioned that Varroa mites cause open wounds on the surface of the bees or brood, through which viral invaders get access to the hemolymph. These viral infections are commonly suspected to be the primary cause of bee mortality in colonies of *A. mellifera* severely infested with *V. destructor*. Destruction of tissue and impairment of the immune system of the host by the parasite can also induce the development of latent viral infections by releasing infectious agents from damaged tissues and stimulating replication of the viral infectious agent in newly infected honeybee cells (Wiegers 1986, Gliński and Jarosz 1984, 1988). Apart from its role as a vector of bacterial, fungal and viral diseases, the damage caused by infestations even with a few number of mites on the ontogenesis and weight at hatching of the honeybee *A. mellifera* was demonstrated

by Schneider and Drescher (1987). The researchers showed that the loss of weight of a bee infested at the brood stage, compared to non-infested bees, is directly proportional to the number of infesting mites. Infestation of the bee brood with a large number of mites results in the formation of adults with malformed wings, underdeveloped and short abdomen (Hargasm 1973, De Jong et al. 1982, Marcangeli et al. 1992), and underdeveloped hypopharyngeal glands (Schneider and Drescher 1987). It has also been demonstrated that, apart from the lower weight at hatching, honeybees parasitized with Varroa mites in their pupal stages have lower life spans than the unparasitized bees (De Jong et al. 1982, De Jong and De Jong 1983, Schneider and Drescher 1987). In general, bees that emerge from brood infested with higher number of mites are deformed, and incapable of normal life activities (Fig. 2.1).

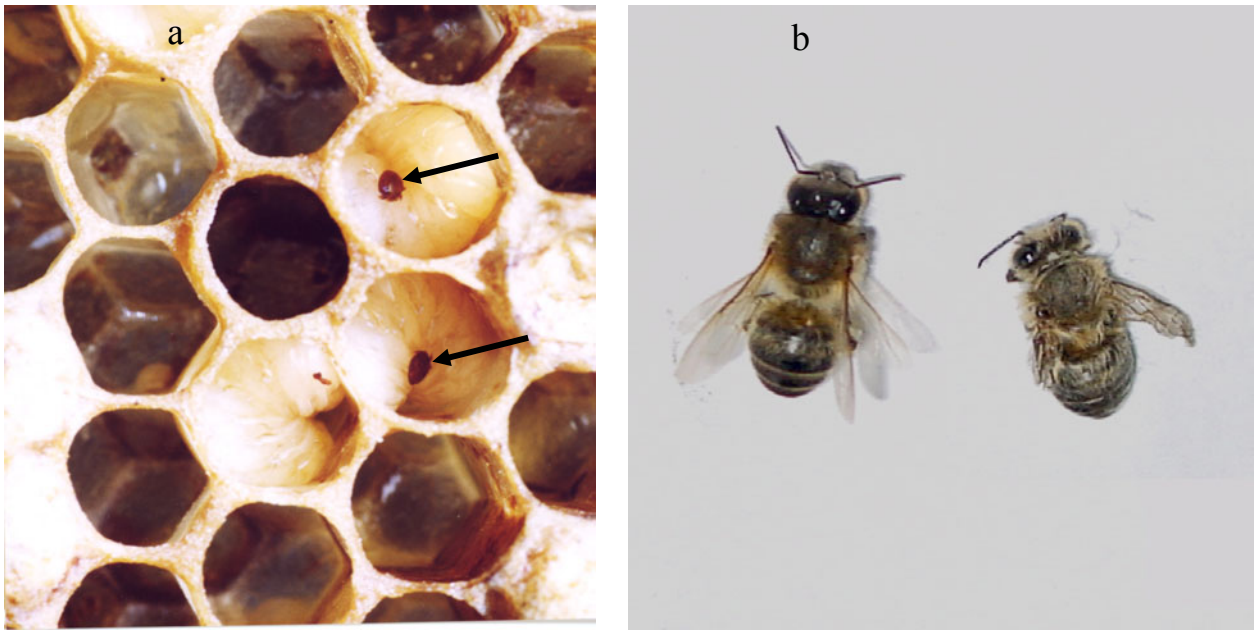

Fig 2.1 Pre-capped worker brood infested with *Varroa destructor* (a), and a deformed adult worker that has been infested with Varroa mites during pupal development (right), and a non-infested healthy one (left) (b).

Investigation of the chemical composition of the hemolymph of pupae infested with Varroa mites displayed lower protein concentration than in the unparasitized ones (De Jong et al. 1982, Weinberg and Madel 1985, Kovac and Crailsheim 1988, Bailey and Ball 1991). The reduced protein concentration in the hemolymph may explain why some organs of the honeybees infested in the brood stage are malformed or underdeveloped, since proteins are generally important in organ formation during ontogenesis. Bees that were not infested with mites at the brood stage but artificially infested immediately after hatching showed less developed hypopharyngeal glands than the non-infested bees (Schneider and Drescher 1987), demonstrating the energy and nutritional demand the parasite imposes on the immature adult host. In turn this

leads to underdevelopment of the bee. Infestation of drone and worker pupae with equal numbers of mites resulted in similar weight losses at hatching, demonstrating that the nutritional value of the hemolymph from worker and drone pupae is the same for the mites (Schneider and Drescher 1987, De Jong et al. 1982).

Apart from the indirect evidence and notion that the mites can be the secondary cause of honeybee colony death, the primary cause being the viral infections transmitted by the mites, no data exists on the energy metabolism of the mites. In this work the energy and nutritional demands of *V. destructor* from various developmental stages and sexes of the honeybee *A. mellifera carnica* shall be demonstrated calorimetrically and gravimetrically at different experimental temperatures. The implications of these demands on the vigour and activities of honeybees will then be evaluated. Furthermore, the rate of utilization of reserve food by the mites during starvation, and also the length of time the mites could survive starvation in the absence of their host shall be examined.

2.3. Materials and Methods

2.3.1 Evaluation of brood infestation level

In order to compare the degree of infestation of worker and drone broods of the honeybee *A. m. carnica* by the mite *V. destructor,* and to subsequently evaluate the energy and nutritional demand of the mites, infested honeycombs were obtained from the research beehives of the Institute of Zoology, Free University of Berlin, Germany in summer 2001. Fifteen honeybee combs containing both types of broods, the worker brood located centrally and drone brood peripherally, were used in the brown and dark skin pupal stages. The combs with brood were collected randomly from the central part of the hives of five colonies, three from each. The experimental colonies were treated with formic acid only at the beginning of autumn of the preceding year. From each comb 200 brood cells (100 from each side) of each sex were selected randomly, opened carefully under a binocular microscope, and all the developmental stages of the female mite were counted

2.3.2 Calorimetric determination of the metabolic rate of capped worker brood

Metabolic rates of the different developmental stages of the capped worker brood of the honeybee *A. m. carnica* were investigated by using isoperibolic twin heat conduction calorimeters. THERMANALYSE (Messgeräte Vertrieb, München, Germany) and Calvet calorimeters (MS 70, Setaram, Lyon, France) of vessel volumes of 15 or 100 cm^3, and sensitivities between 40.65 and 64.34 $\mu V\ mW^{-1}$ were used. Capped worker brood was taken out

of the hive immediately after capping ± 12 h, and incubated further at 35 ± 0.5 °C and 65 ± 5% r.h. A capped brood cell at a certain developmental stage was carefully incised from the rest of the brood, put into a calorimetric chamber, and the heat production rate was recoded for 3 to 5 h. The brood cell was then opened, and the pupa was removed and weighed using a sensitive analytical balance (Type 414/13, Sauter, Ebingen, Germany) of 0.1 mg detection limit. Experiments with each developmental stage were done 12 to 15 times and the mean ± S.D. values were used in the presentation of results.

To avoid the use of Varroa infested and/or diseased brood, the bees were optically examined through the incised transparent brood cell. Brood cells that were infested with Varroa mites and pigmented differently, possibly due to infection, were excluded. Since observation through an old and dark wax is difficult, only brood on new and clear combs were used in the present experiments. In addition to the preliminary observations, brood cells were opened and inspected at the end of each experiment and those with the above mentioned problems were discarded.

2.3.3 Determination of weight change of brood during ontogenesis

In order to determine the rate of weight change and hence reserve food utilization by the capped brood, 15 brood cells that were capped in the last 12 h were sliced carefully from the rest of the brood and weighed. The individual brood cells were then incubated at 35 ± 0.5 °C and 65 ± 5% r.h. in a Petridish. Each of them were weighed every 24 h until hatching. At the end, the weight of the individual empty comb cells from which the adults hatched was determined separately, and subtracted from the previous weightings. The weighing times were maintained as short as possible, < 1 min, in order to avoid the effect of lower room temperature and humidity on pupal development.

2.3.4 Survival of starvation and resource utilization by *Varroa destructor*

In order to indirectly determine the amount of hemolymph the mites suck from their host, and to elucidate the length of time the adult female mites could survive in the absence of their host, mite starvation experiments were carried out. For these tests 130 to 190 mites were collected from brown and dark skin drone pupae (brood older than 20 days), weighed using the analytical balance (Sauter, Ebingen, Germany) and incubated at 35 °C and 60 ± 5% r.h. for 1, 6, 12, 18, 24, 30, or 36 h in a Petridish with or without pupae, approximately 4 to 6 mites per pupa in the former case. At the end of each experiment, the mites were weighed again, and immediately frozen for further analysis. For the determination of the change of body fat and dry

matter composition with starvation time the frozen mites were thawed and dried at 60 °C for 96 h until weight constancy, and the mean of the final three weightings, performed every 12 h, was used in the calculation of percentage dry matter. The dried samples were then homogenised using mortar and pestle. Analysis of the fat composition was done using the Folch method (Folch et al. 1957). Each of these experiments was conducted three times and the mean ± S.D. values were used in the presentation of results.

2.3.5 Calorimetric experiments with *Varroa destructor*

Calorimetric experiments were performed to determine the metabolic rate of *V. destructor* and to evaluate its energy demand from the host. Isoperibolic twin heat conduction calorimeters of types: Biocalorimeter - B.C.P (Messgeräte Vertrieb, München, Germany) with a sensitivity of 44.73 μV mW^{-1} and a vessel volume of 12 cm^3, and a THERMANALYSE calorimeter (Messgeräte Vertrieb, München, Germany) with a sensitivity of 40.65 μV mW^{-1} and a vessel volume of 15 cm^3 were used.

To evaluate differences in the energy metabolism of mites from different sexes and developmental stages of the same sex, mites were collected from adult workers as well as worker and drone brood cells. Mite collection was done from the brood stage by gently opening individual healthy (non-infected) brood cells. During the collection process mites were kept in a Petridish on the corresponding bee pupae in order to avoid starvation. Newly moulted adult mites, identified by their pale colour, and young mites (nymphs), with relatively smaller size and feeble locomotion, were excluded from the experiment. Mites that seemed weak and abnormal were discarded. The collection of mites from adult workers was done by very carefully dislodging them from the surface of the bees with the help of a blunt needle. During the collection of mites from adult workers the former were kept on worker pupae in order to avoid starvation.

25 to 30 mites were weighed and put into the calorimeter and the heat production rate was recorded for 2 to 3 hours. This time interval was chosen for technical reason with the calorimetric experiments, due mainly to the thermal equilibration time needed after opening the calorimeter and placing the mites inside. The thermal equilibration time of the calorimeter was not always the same and varied based on several factors. The little difference in the experimental time interval for the different experiments does not affect the result since the latter is extrapolated to a rate per hour. At the end of each experiment the mites were weighed again for weight change and evaluation of the rate of utilization of reserve food under starving conditions, and consequently to elucidate the amount of hemolymph the mites could utilize from their host

to maintain their weight and physiological condition. The weight change is presented as percentage wet weight change per mite per hour.

All calorimetric and gravimetric experiments with mites from the different groups were conducted at 35 °C. In addition, mites from drone brood were investigated at 25, 30, 35, 40, 45, and 50 °C in order to determine the optimum temperature of mite metabolism and to elucidate the effect of temperature on their metabolic rate and rate of resource utilization. The experimental time with mites at 45 °C and 50 °C lasted 1.5 to 2 h.

2.3.6 Respirometric experiments with *Varroa destructor*

The respiration rate of mites from drone brood was determined at 25, 30, 35, 40, 45, and 50 °C using manometric methods. The tests were run in Warburg vessels of about 12 ml volume. The CO_2 evolved was absorbed by 400 µl of a 4% KOH solution in the side arm of the vessel. To avoid access of the mites into the side arm the opening was blocked with a very thin layer (1 mm thick and 0.8 mm pore size) of porous spongy material that allowed air, but prevented mite entrance. The measurements were started after a temperature equilibration time of 30 min and recording was done in intervals of 30 min for 3 to 5 h (except for 1.5 to 2 h at 45 and 50 °C). The oxygen consumption rates were calculated from the pressure drop in the Warburg vessel with time. Each measurement was carried out five times (but nine times at 45 °C) using 50 to 60 mites per experiment, and the mean ± S.D. values were used in the presentation of results. The latter were compared with those of the calorimetric ones, also at different temperature settings.

Finally, the metabolic rate of the mites obtained from different developmental stages of honeybees was compared with that of the corresponding developmental stages of the host from which the mites were collected. Furthermore, the contribution of the energetic demand of mites on the deterioration and death of infested colonies was evaluated.

2.3.7 Statistical analysis

Results are presented as mean ± S.D. values. Statistical tests were performed using the student's t-test, 1-way ANOVA, and the Tukey's HSD post hoc test. $\alpha = 0.05$ was considered as the critical value.

2.4 Results

2.4.1 Infestation level of brood

Comparison of the infestation level of broods of the same sex located on the two sides of a comb and also from combs of different colonies showed no significant differences. Due to this fact the results were pooled and presented as infestation level of worker brood or drone brood. As can be seen from Fig. 2.2 there is a significant difference in the percentage of infested worker and drone brood cells, the mean values being 5.6 ± 0.8% and 28.4 ± 7.5%, respectively. These results illustrate that drone broods are fivefold more attractive to mites than worker brood (drone brood preference factor). A significant difference between drone and worker brood was also observed in the mean number of female mites counted per 100 brood cells. The 100 drone brood cells opened randomly on each side of the comb and from combs of each colony rendered a mean value of 113.4 ± 13.5 female mites. A corresponding number of worker brood, however, rendered only 7.7 ± 1.4 mites, indicating that the infestation level of the drone brood by female mites is significantly greater than that of the worker brood by 14.8 fold. If we consider individual infested brood cells, a drone brood harbours 3 to 6 female mites with a mean value of 4.1 ± 1.0, whereas a worker brood harbours 1 to 4 female mites with a mean value of 1.4 ± 0.8, indicating that an individual drone brood shelters female mites more than a worker brood by a factor of 2.9.

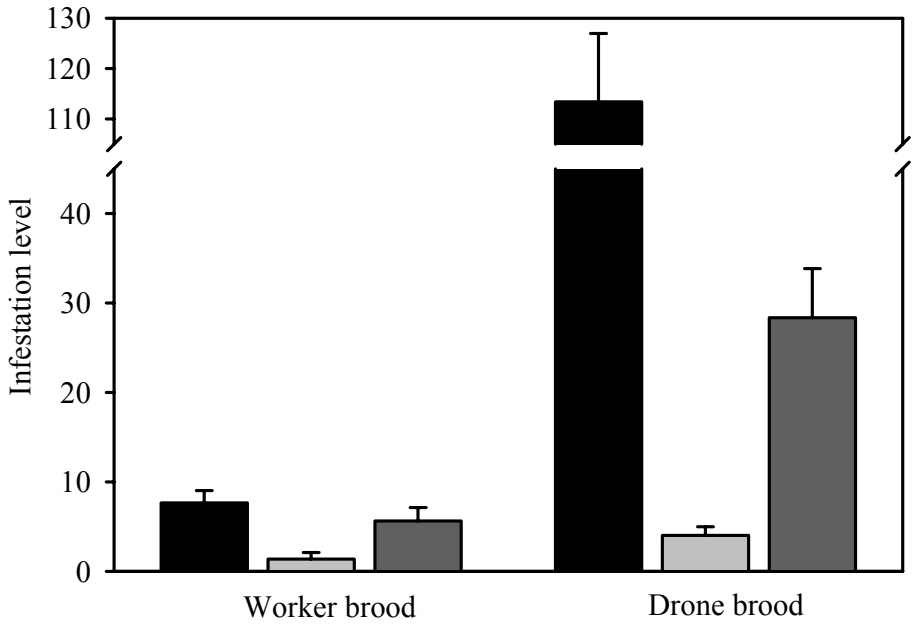

Fig. 2.2 Infestation level of *Apis mellifera carnica* worker and drone brood with *Varroa destructor*. ■ Average number of female mites per 100 brood cells, ▭ Average number of female mites per infested cell, ▭ Percentage of infested brood cells (%).

2.4.2 Metabolic rates and utilization of reserve food under starvation

The starvation experiments with mites from drone brood in the absence of the host showed that no death of mites was observed at least within the first 6 hours. Death ensued with prolongation of the starvation time with about 22% of the mites dying within 12 h, and 50% within 18 h of starvation time. Only 5% of the mites were alive after 36 h starvation time. There was neither death nor loss of weight of mites incubated on drone pupae (control experiment) at least for the 36 h experimental time. The starving mites lost 48.5% of their wet weight within the first six hours of starvation time, which is a loss of 8.1% h^{-1}. The wet weight change of Varroa mites with starvation time during the calorimetric experiments for 2 to 3 hours also displayed a loss of 8.1% h^{-1}. This trend of weight loss did not continue further in the next starvation hours, rather it reduced during the course of incubation time (Fig. 2.3). After 36 h starvation time, when 95% of the mites had already died, the wet weight was reduced by 76%.

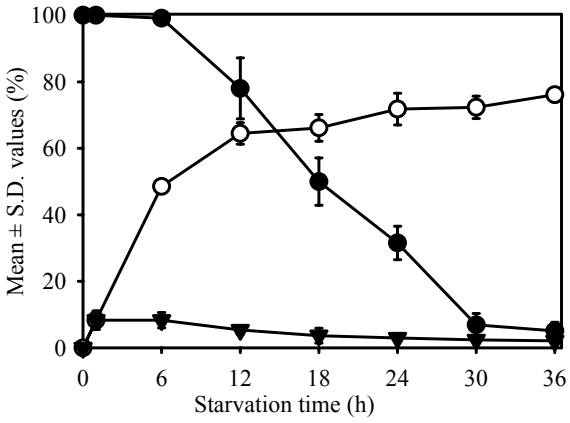

Fig. 2.3 Survival of *Varroa destructor* mites and the rate of reserve food utilization under starvation in the absence of their host, incubating the mites at 35 °C and 60 ± 5% r.h. in a Petri dish. ● Mean percentage of survivor mites (%), ○ Mean percentage loss of wet weight (%), ▼ Mean percentage loss of wet weight per starvation hour (%).

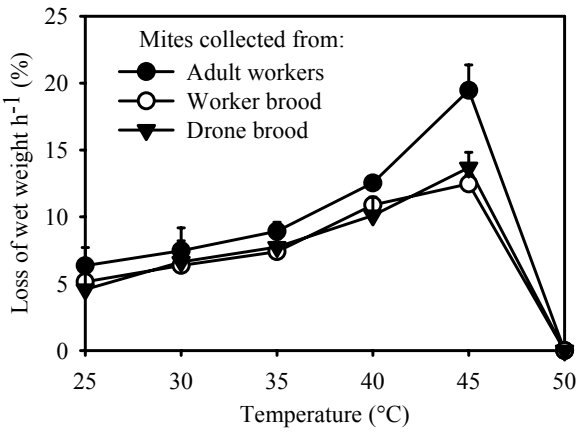

Fig. 2.4 Percentage loss of wet weight (mean ± S.D.) per hour of the mite *Varroa destructor* under starvation and different experimental temperatures, data extrapolated from starvation of the mites for 2 to 3 hours at the corresponding temperature. n = 5, 25 to 30 mites per experiment.

Extrapolation of the wet weight loss of a mite per hour from the weight loss during the 2 to 3 h starvation time in the calorimetric experiments displayed that the percentage weight loss grew with increasing temperature and attained maximum values at 45 °C, amounting to 13.7 ± 1.1%, 12.5 ± 0.6%, and 19.5 ± 1.9% $mite^{-1}$ h^{-1} for mites from drone brood, worker brood and adult workers, respectively. Activity of mites at this temperature lasted a maximum of 3 hours,

and thereafter mites were either dead or highly weakened. The weight change at 50 °C dropped to zero since the mites died immediately. If we consider the weight loss at 35 °C, the temperature prevailing in the beehive environment, mites from drone brood, worker brood and adult workers lost 8.1 ± 0.6%, 7.8 ± 0.9%, 8.9 ± 0.7%, respectively, of their wet weight per hour (Fig. 2.4). A one-way ANOVA showed that there is a significant difference in the loss of weight of mites from the different sources ($p = 0.035$). A pairwise comparison using the Tukey's HSD post hoc test displayed a significant difference of weight loss by the mites from drone brood and adult workers ($p = 0.013$), and worker brood and adult workers ($p = 0.02$), but not by the mites from drone brood and worker brood ($p = 0.25$). The mites incubated at the different experimental temperatures and on the corresponding hosts (controls) did not show any weight change with the incubation time. The weight of mites collected from the three groups of bees in their normal physiological conditions and before starvation did not show any significant difference, even though those from adult workers were slightly lighter, the values being 407 ± 38, 395 ± 43, 361 ± 63 µg for mites from drone brood, worker brood and adult workers, respectively. This could be due to the fact that the brood mites were collected from the late pupal stages (brown and dark skin stages) when there is no much oogenesis, which otherwise would have made the brood mites heavier by up to 30% (Steiner et al. 1995).

The chemical analysis results of starving mites showed that the mean percentage of dry mass remained constant and similar to the control experiments at 68% regardless of the starvation time. In addition, the relative proportion of the lipid to non-lipid components of the dry mass also remained unaffected with the lipid components varying between 11.5% and 14.8% (mean 13.2 ± 1.6%), and the non-lipid components lying between 84.8% and 88.0% (mean 85.3 ± 5.9%) independent of the starvation time. Observation of the inner wall of the transparent calorimetric vessels after each experiment displayed several white spots on the otherwise clean Plexiglas vessel. These white spots are faecal matter of the mites which are considered by Sammataro (et al. 2000) to be mainly composed of guanine.

Calorimetric experimental results at 35°C indicated that mites collected from worker brood and drone brood had comparable (specific) heat production rates, expressed either as µW mg^{-1} or µW $mite^{-1}$. Though 1-way ANOVA showed no significant difference ($p = 0.45$) in the heat production rate per individual mite (µW $mite^{-1}$) between the mites collected from adult workers, drone brood, and worker brood, the same test showed a significant difference ($p = 0.024$) in the specific heat production rates (µW mg^{-1}) of these mites. The Tukey's HSD post hoc test displayed that significant difference exists in the specific heat production rates between

mites from drone brood and adult workers (p = 0.017), and those from worker brood and adult workers (p = 0.023) but not those between worker brood and drone brood (p = 0.35) (Fig. 2.5).

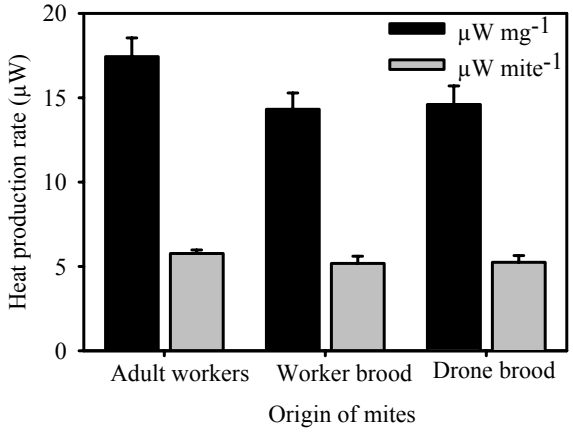

Fig. 2.5 (Specific) heat production rate (µW mg^{-1} or µW $mite^{-1}$) of *Varroa destructor* mites collected from different sexes and developmental stages of the honeybee *A. mellifera carnica* at 35 °C. 25 to 30 mites per experiments, n = 5.

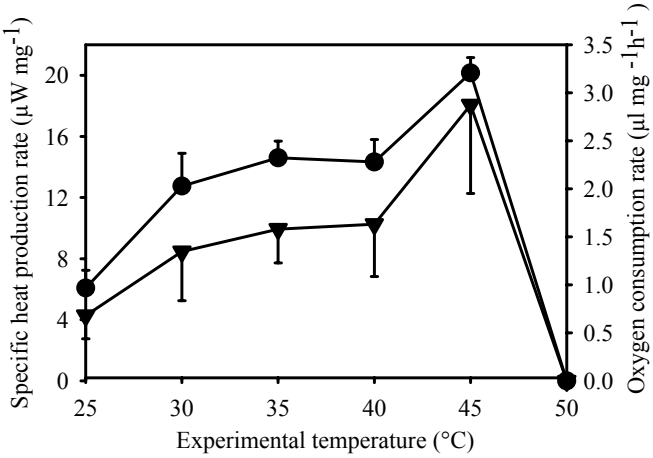

Fig. 2.6 Effect of temperature on the specific heat production rates and oxygen consumption rates of *Varroa destructor* mites from drone brood. 25 to 30 and 50 to 60 mites per experiment for the calorimetric and respirometric experiments, respectively. n = 5 (but n = 9 for the respirometric measurements at 45 °C), mean ± S.D. ● Heat production rate, ▼ Oxygen consumption rate.

Calorimetric and respirometric results of the effect of temperature on the metabolic rate of Varroa mites from drone brood showed identical patterns. Heat production and oxygen consumption rates of mites were small at lower temperatures and rose with increasing temperatures reaching optimum values between 30 °C and 40 °C. In this temperature range both curves attained nearly a plateau phase demonstrating little change of metabolic rate with changing temperature. If we compare the Q_{10} values between 25 °C and 35°C on one hand, where there is a drastic change of heat production rate, and between 30 and 40°C on the other hand, the Q_{10} value of 2.4 for the former is significantly higher than for the latter, which is 1.1 (two-tailed t-test, p = 0.009). Both heat production and oxygen consumption rates increased and achieved maximum values at 45 °C. These elevated metabolic and oxygen consumption rates at 45 °C were, however, maintained only for a maximum of 3 h, after which the mites started dying, and the rates dropped drastically (Fig. 2.6).

2.4.3 Computation of the brood hemolymph robbed by a foundress mite and its offsprings

In order to calculate the energy and nutritional demand of the mites from the brood and to depict the effect of parasitism, the following facts have to be taken into consideration in

combination with data obtained in this work: a foundress mite invades a worker brood on average ca. 20 h and a drone brood 45 h before cell capping, and starts feeding on the brood's reserve food, and later on the brood's hemolymph (Boot et al. 1991). The post-capping period of the brood lasts 12 and 15 days for worker and drone brood, respectively (Moritz 1985, Le Conte and Cornuet 1989). The developmental time of the mite from egg to maturity is 6.9 and 6.2 days for males and females, respectively (Rehm and Ritter 1989). The first egg is laid 60 h after cell capping and develops into a male mite and the rest follows in intervals of 30 h and develops into female mites (Infantidis 1983). Based on these facts the feeding time of the mother mite and its offsprings is tabulated in Table 2.1. The energy and nutritional demand the mites impose on the brood (Table 2.2) is calculated from (i) Table 2.1, (ii) the daily energy demand of the mites (extrapolated from Fig. 2.5) and (iii) the amount of hemolymph the mites suck from the brood (extrapolated from the loss of weight in the absence of the host). If the foundress mites are two instead of one, the energy and nutritional demands of the mites on the brood change, with both values increasing as depicted in Table 2.3.

The metabolic rates of the capped brood recorded calorimetrically, and that of the infesting mites calculated at each instar based on the number of mites and the mean heat production rate of 4.8 $\mu W\ mite^{-1}$ were compared (Fig. 2.7). Though the energy consumption rate of Varroa mites increases with the incubation period of the brood, as seen in Fig. 2.7, it is a non-significant factor to cause damage to the brood since it is about 1.2% to 2.8% of that of the heat production rate of the latter.

The nutritional demand of Varroa infestation was also compared by considering resource utilization of the capped brood and its infesting mites in the capped developmental days. Weight of a worker larva achieved its maximum value at the L6 (the capped 5^{th} instar larva on the 6^{th} day of larval development) with 182 ± 17 mg. The weight achieved at this stage decreased continuously until adult emergence; with a total loss of 77 ± 9 mg for a non-infested brood, which is about 42% of the maximum weight (Fig. 2.8).

The rate of reserve food utilization by the developing brood decreased drastically in the first two days after capping, i.e. during growth from L6 to L7 and from L7 to PP1 (prepupal phase). After this period the rate remained more or less constant. The rate of robbing of brood's reserve food by the mites remained at a lower level during the first five capped developmental days (L6 to P1) and increased from the second to the sixth pupal developmental days (P2 to P6), where most of the progenies become mature. After the fourth pupal developmental day the mites consume up to 65% of the reserve food that the brood could consume Fig. 2.9

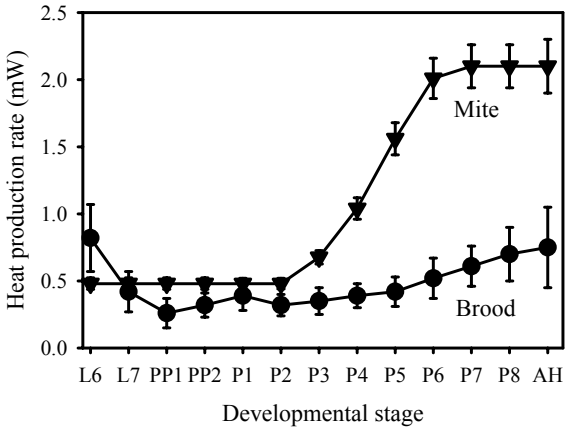

Fig. 2.7 Comparison of the heat production rate of a capped worker brood of the honeybee *Apis mellifera carnica* with an infesting *Varroa destructor* mother mite and its three offsprings during pupal ontogenesis. L = larva, PP = prepupa, P = pupa, AH = adult before hatching. n = 12 to 15 experiments for brood and n = 5 experiments for mites with 20 mites per experiment. The heat production rate of the mites displayed is a multiple of 10^2 of the actual value.

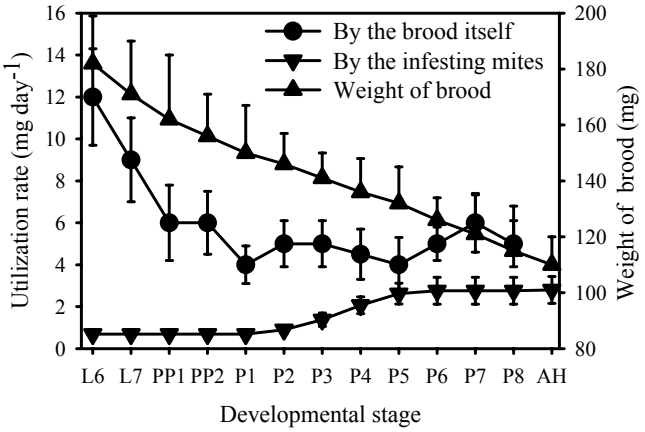

Fig. 2.8 Utilization of the reserve food of the developing capped worker brood of the honeybee *Apis mellifera carnica* by the brood itself and the infesting *Varroa destructor* mother mite with its three offsprings. L = larva, PP = prepupa, P = pupa, AH = adult before hatching. n = 12 to 15 for brood and n = 5 for mites with 20 mites per experiment.

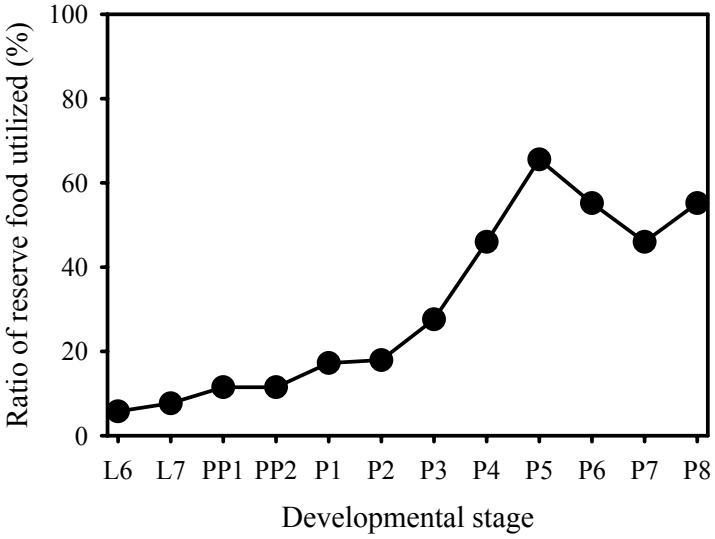

Fig. 2.9 Ratio of reserve food utilization by a mother *Varroa destructor* mite and its three offsprings to that utilized by the worker brood of *Apis mellifera carnica* during development in a capped cell.

Table 2.1 Feeding time of mites in a capped brood
The number of days a mother mite and its offsprings feed on worker and drone broods after invasion of the brood before cell capping. (* - The bee emerges as an adult before the mite completes development to the adult stage). The feeding of the first egg (male) is neglected.

	Egg number	Time (days) after cell capping at which egg is laid	Total time on brood (days)	Feeding time (days) of the mite neglecting the feeding period of the protonymphs i.e. the first 3 days
In a worker brood	Mother mite		12.8	12.8
	1	2.5	-	-
	2	3.8	8.2	5.3
	3	5.0	7.0	4.0
	4	6.3	5.7	2.8*
In a drone brood	Mother mite		17.0	17.0
	1	2.5	-	-
	2	3.8	11.2	8.3
	3	5.0	10.0	7.0
	4	6.3	8.7	5.8
	5	7.5	7.5	4.5
	6	10.0	5.0	2.0*

Table 2.2 Energy and nutritional demand of mites in a capped brood (1 foundress mite)
The total energy needed by a mother mite and its offsprings during the whole developmental time of a worker and a drone brood, obtained by multiplying the total feeding time with the energy demand of a mite per day: 471 mJ (from drone brood) and 491 mJ (from worker brood). The total weight of hemolymph robbed by the mites is a product of the total feeding time of the mites and their weight loss under starvation (extrapolated to hypothetical weight loss per day), which is 0.71 mg (from worker brood) and 0.72 mg (from drone brood). The energy and hemolymph demand of the first offspring (male) is neglected.

	The n^{th} offspring	Total feeding time of the mites (days)	Total energy consumption of a mite (J)	Total weight of hemolymph and brood food consumed (mg)
In worker brood	Mother mite	12.8	6.3	9.1
	1	-	-	-
	2	5.3	2.6	3.7
	3	4.0	2.0	2.8
	4	2.8	1.4	2.0
			Total = 12.3	Total = 17.6
In drone brood	Mother mite	17.0	8.0	12.2
	1	-	-	-
	2	8.3	3.9	5.9
	3	7.0	3.3	5.0
	4	5.8	2.7	4.1
	5	4.5	2.0	3.2
	6	2.0	0.9	1.4
			Total = 20.8 J	Total = 32.8 mg

Table 2.3 Energy and nutritional demand of mites in a capped brood (2 foundress mites)
The amount of hemolymph sucked and the energy needed by two mother mites and their offsprings during the developmental time of a worker and a drone brood considering that both mother mites are fertile. Since the maximum infestation rate in the experimental colony was 4 and 6 mites per worker and drone brood, respectively, the evaluation is limited to the mother mites, and the first female offsprings in the case of worker brood, and the mother mites and the two successive female offsprings of each mother mite in the case of drone brood. FO1-1: first female offspring of mother mite 1, FO1-2: first female offspring of mother mite 2, FO2-1: second female offspring of mother mite1, FO2-2: second female offspring of mother mite 2. Computation was done as in Table 2.2.

	The n^{th} offspring	Total feeding time (days)	Total energy demand of the mite from the brood (J)	Total weight of hemolymph and brood food consumed (mg)
In Worker brood	Mother mite1	12.8	6.3	9.1
	Mother mite2	12.8	6.3	9.1
	FO 1-1	5.3	2.6	3.7
	FO 1-2	5.3	2.6	3.7
			Total = 17.8 J	Total = 25.6 mg
In drone brood	Mother mite 1	17.0	8.0	12.2
	Mother mite 2	17.0	8.0	12.2
	FO1-1	8.3	3.9	5.9
	FO1-2	8.3	3.9	5.9
	FO2-1	7.0	3.3	5.0
	FO 2-2	7.0	3.3	5.0
			Total = 30.4 J	Total = 46.2 mg

2.5 Discussion

Drone brood is highly infested compared to worker brood, and this phenomenon was also confirmed by other researchers (Issa and Gonçalves 1984, Schulz 1984, Rosenkranz 1985, Fuchs 1990). Dividing the percentage of infested drone brood by the percentage of infested worker brood gives a preference factor of 5 for drone brood to worker brood. Several factors could be involved in encouraging the invasion of drone brood rather than worker brood cells. These factors include higher concentrations of mite attractive fatty acid esters in the cuticle of drone brood than in worker brood (Le Conte et al. 1989), higher concentration of aliphatic alcohols and aldehydes in drone cocoons (Donzé et al. 1998), larger volume of the drone brood (Sammataro et al. 2000), and longer Varroa attractive period prior to cell capping by drone brood than worker brood (Fuchs and Müller 1988, Infantidis 1988, Boot et al. 1991). The number of female mites in infested drone brood is higher than in worker brood by a factor of 2.9. This means that a foundress Varroa mite reproduces more in drone brood than in worker brood, mainly because the post capping period in drone brood is longer than that in worker brood by about three days (Moritz 1985, Le Conte and Cornuet 1989). With the mean capped developmental time of 12

days for workers and 15 days for drones (Table 2.1), only the first two female mites mature to adults before the worker hatches, whilst four of the mites could mature before the drone hatches as an adult. Thus, female mites developing in the last eggs mature to adults if the capped brood has a longer developmental time than the average considered. The product of the preference factor of a drone brood to a worker brood and the ratio of infestation level of an individual drone brood to a worker brood gives the infestation ratio of drone brood to worker brood at the colony level, which is 14.8.

Starvation experiments carried out on mites to show how fast they consume their reserve food and the length of time they could survive without their host demonstrated that though the mites were starving in the absence of their host, death was not observed at least during the first six hours. This result illustrates that it is possible to run experiments with Varroa mites for at least six hours in the absence of their host. For this reason the calorimetric experiments were limited to 3 to 6 hours, even though no drop in the level of the curve was observed while recording the heat production rate for 10 continuous hours. As can be seen in Fig. 2.3 the mites utilized their energy reserve at a higher rate (8.1% of their wet weight per hour) for the first six hours, with this rate then declining with starvation time. With prolongation of the starvation time they might have run out of reserve food and decreased its utilization rate. With prolongation of the starvation period their reserve food comes to an end and the mites start dying, as seen with the rapidly declining number of survivor mites. Nearly 95% of the starving mites died within the first 36 hours of starvation time, evidently displaying the extent of dependency on their host. The constant proportion of dry weight to fresh weight after different lengths of starvation time indicates that the loss of weight during incubation is not due to evaporational water loss, but because of the utilization of reserve food. The steady proportion of lipid to non-lipid tissue components, regardless of the starvation time, demonstrates that the mites consume lipid and non-lipid reserve food proportionally.

The enhanced weight loss of Varroa mites with ascending temperature indicates that the metabolic rate, and hence reserve food utilization, augmented with increasing temperature. This feature is typical of ectothermic poikilotherms as their metabolic rate and body temperature follow the ambient temperature. The increased consumption of reserve food and the higher metabolic rate at temperatures higher than that in the normal beehive environment demonstrate that the energy and nutritional demand of the mites increase with overheating of the brood nest and hence the mites could cause more damage than at lower temperatures. The loss of wet weight, the heat production, and oxygen consumption rates achieved maximum values at 45 °C and this activity lasted about three hours, after which the rates dropped drastically. The high rates

at this temperature indicate that the ambient temperature was intolerable for the mites and that they tried to escape, resulting in a higher metabolic rate and increased utilization of reserve food. The plateau phase in the heat production and oxygen consumption rates between 30°C and 40°C is an indication of the optimum temperature range of Varroa metabolism. Similar patterns obtained with heat production and oxygen consumption rates (Fig. 2.6) point to the fact that it may be possible to use the indirect and cheaper respirometric method in Varroa metabolic investigations if calorimeters are not available.

The phoretic mites have relatively higher heat production rates probably due to the fact that they are fully grown up mites and possess a larger proportion of actively metabolising tissue than reserve food, as compared to the mites from the brood stage, contributing to a higher metabolic rate. A further possible explanation could be that as an adaptation to their way of life, actively attaching themselves to flying bees not to fall down, which needs a larger amount of energy, the phoretic mites may use an efficient metabolising system. Though there was no significant difference in the heat production rate per mite and the weight of individual mites between phoretic and brood mites, the specific heat production rate of phoretic mites was significantly higher than that of brood mites. This could be due to the fact that the heat production rate per phoretic mite was a bit higher and their weight smaller, though not significantly, than that of the brood mites.

Though the eight legged protonymphs and male mites feed on the bee brood and could cause damage (Sammataro et al. 2000) the damage during this phase (the first 3 days of development) was neglected, not to overestimate the impact of the mites in general. Since the largest number of mites per brood cell observed in the present experiments was four in worker brood and six in drone brood, these numbers were used as maxima for the corresponding brood in the computations. In order to compare the heat generation and reserve food consumption of Varroa mites with that of drone and worker brood during ontogenesis the following facts are to be considered: the heat production rate of a drone pupa during the capped developmental stage ranges from 0.7 to 1.9 mW $pupa^{-1}$ with the calculated mean heat production rate and the energy use per day being 1.03 mW $pupa^{-1}$ and 89 J $pupa^{-1}$ respectively. During the whole ontogenetic phase a drone pupa releases 1.34 kJ of energy. The heat production rate of a worker pupa during the capped developmental stage lies between 0.3 and 0.8 mW $pupa^{-1}$ with the calculated mean heat production rate of 0.5 mW. The mean energy use of a worker pupa per day and during the whole ontogenetic phase amount to 43.2 J and 518 J, respectively. The percentage of energy dissipated by Varroa mites as compared to that released by drone pupa ranges from 1.2% (only 3 infesting mites) to 1.6% (6 infesting mites), with the mean value being about 1.3% (4 invading

mites). The percentage of energy released by the parasitic mites ranges from 1.2% of that of worker pupa (with only 1 infesting mite) to 2.4% (with 4 infesting mites) with a mean value of 1.4% (with 1.4 mites infesting). As can be seen clearly, the energy demand demonstrated by the heat dissipation rate of a mite, is insignificant even at maximum infestation levels.

The pupal stage of honeybees does not feed and hence depends for the entire ontogenetic process on the reserve food accumulated in the tissue during the larval stage. A non-infested brood achieves a maximum weight during the L6 (182 ± 17 mg for worker brood) and L7 stages (402 ± 19 mg for drone brood). L6 and L7 represent the capped larval stages at the 6^{th} and 7^{th} days of larval development, both at the fifth larval instar. The pupa consumes the reserve food and a freshly hatched worker weighs 105 ± 15 mg and a drone weighs 256 ± 13 mg. Thus, a worker brood and a drone brood utilize 70 mg and 146 mg, respectively, during the entire capped developmental stages. If one compares the amount of reserve food consumed by the developing brood (pupa) during ontogenesis with the amount of hemolymph robbed by Varroa mites subsequently indicating the amount of weight loss by the bee, the mites consume 13% (9.1 mg) (only one infesting mite) to 25% (17.5 mg) (four infesting mites) of the reserve food of the worker brood with a mean value of 15% (10.5 mg) (1.4 infesting mites). Considering the case of drone brood, the infesting Varroa mites rob 16% (23.4 mg) (three infesting mites) to 22% (32.1 mg) (six infesting mites) with a mean value of 19% (27.7 mg) (four infesting mites) of the reserve food of the non-feeding drone pupa.

Energy density analysis of the pupal tissue using bomb calorimetric experiments (Kösece 1998, Contzen et al. 2003) showed that worker and drone pupae possess 26.4 J mg^{-1} and 30.1 J mg^{-1}, respectively. Using these values and the total hemolymph robbed by the mites and comparing it with the total energy dissipated by the them during the capped developmental phase of the pupa, we come up with the result that the mites dissipate only 2.2% of the hemolymph energy they suck from the brood. This result shows that Varroa mites have a very inefficient metabolic machinery and have to feed continuously to fulfil their energy demand. This continuous robbing of the host's reserve food could weaken the colony. Apart from its direct impact, resulting in the formation of underdeveloped and malformed bees, nutritional shortage can also have secondary consequences. These include making the bees vulnerable to viral and bacterial infections, which may otherwise be non-infectious or latent under non-parasitized situations since the immune system of the non-parasitized host could suppress such infections.

The weight loss of workers and drones due to Varroa infestation obtained by the back calculation from the resource utilization of the infesting mites during starvation, and also by the direct weighing of adults immediately before hatching shown in the present results, agrees very

well with the results of Schneider and Drescher (1987) obtained by directly weighing the bees immediately after hatching. The authors tabulated their results showing that a worker bee parasitized with 1 to 3 and > 3 mites during brood developmental stage lost 9.6% and 21.6%, respectively, of its unparasitized wet weight. Considering the weight of freshly hatched drones parasitized during the brood stage, Schneider and Drescher (1987) gave a weight loss of 14.1% due to infestation with > 3 mites, in good agreement with the present results of weight loss of 19% when a drone was infested with four mites during its brood developmental stages.

It can be concluded here that the Varroa mites rob a tremendous amount of the reserve food and hemolymph of the brood, contributing to malformations and improper development of wings, abdomen, legs, and the hypopharyngeal glands (Hargasm 1973, De Jong et al. 1982, Schneider and Drescher 1987, Marcangeli et al. 1992). This leads to the development of weak and incapable bees since the reserve food and hemolymph protein are important for ontogenesis and the proper development of the different body parts (Maurizio 1954, Knox et al. 1971). It can not, however, be excluded that factors other than Varroa infestation could play roles in the malformation of bees infested with Varroa mites.