



Hypoxia-Controlled Winter Metabolism in Honeybees (*Apis mellifera*)

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ABSTRACT. Experiments during three winters have revealed a metabolism controlling function of bee-induced hypoxia in the winter cluster. Permanent low oxygen levels around 15% were found in its core. This hypoxia was actively controlled, probably via indirect mechanisms. Varying ambient oxygen levels demonstrated a causal relationship between lowered oxygen and reduced metabolic rate (MR). Under deeper ambient hypoxia the bees switched to ultra low MR (ULMR), optional-occasional at 15% oxygen, obligatory at 7.5% oxygen. This dormancy status resembled deep diapause in insects. It stayed reversible after at least several days, and was terminated under normal oxygen at 15°C. Reduced MR via core-hypoxia is essential in water conserving thermoregulation of the wintering cluster. It allows bees to reconcile warm wintering in alert state—for defence of stores—with energy saving and longevity. Two further hypotheses discussed are that winter MR of bees might be related to insect diapause, and that in-body hypoxia might be functional in insect diapause. COMP BIOCHEM PHYSIOL 117A;4:445–455, 1997. © 1997 Elsevier Science Inc.

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INTRODUCTION

Honeybees (*Apis mellifera*) are social insects living in a well-organised colony. In summer it consists of up to 70,000 (7 kg) short living individuals (life span around 30 days). A hibernating colony numbers 10,000 to 20,000 long living adults (life span several months), which cluster in the comb streets inside the hive. Very small colonies down to, e.g. 1000 bees, as used in queen rearing, can thrive and increase in summer but hardly have any chance to survive winter. Less undersized colonies, under about 1 kg and curiously oversized colonies as well, are subject to increased winter mortality (8,14). Successful wintering of small colonies is sought for as an instrument in pollination of different crops (6).

Unlike other insects *Apis mellifera* displays “warm wintering” in its cluster, where temperatures range from 8–12°C in the mantle to 13–35°C in the core (15). It often is believed in apicultural practise that excessive heat loss explains the winter handicap of undersized colonies. However Southwick (40) found a bee mass as low as 100 g as a threshold under which mass-specific heat loss rises sharply. The true factors responsible for these size-related problems have as yet not been determined.

Unlike other insects *A. mellifera* hibernates in the presence of large food stores. They are partly used for heat production but their true destination seems to be the quick breeding of a new large population in the next spring (36), followed by swarming for reproduction and gathering of new stores. Warm wintering despite its energy cost, is beneficial since it conditions the defence of food stores (28). However, higher temperatures may be detrimental to longevity. Higher metabolic rate is indeed amongst factors shortening life span of worker bees (24,25,29). In view of the Q_{10} law (i.e. the factor by which MR increases for a 10°C temperature rise), warm wintering thus should not be beneficial for longevity.

Warm wintering is made possible by clustering as a collective behaviour. It has been viewed as an alternative to anti-freeze (41). However, what can be the purpose of a cluster core temperature up to 35°C? A widespread view holds that a “heating core” keeps temperature of the insulating cluster shell above 8–10°C, the “chill coma” temperature of bees (10). On the other hand the existence of “warm resp. cold clusters” has been reported (20); we observed core temperatures down to 13.5°C in a healthy small (300 g) colony. Are high temperatures functional or are they merely a side effect?

Extensive experiments by Heinrich (12) indeed demonstrated a large virtual heat excess in cluster cores, on the basis of resting MR. He concluded thermoregulation to be achieved in the outer cluster layers. This raises the question:

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can the cluster core reduce its MR below levels dictated by Q_{10} law?

Our program comprised research on MR, thermoregulation and water balance. This paper reports on results with reference to oxygen-MR relations. It explains for the first time how bees can achieve a degree of control of resting MR, why they need it for thermoregulation, and how they manage to combine warm wintering with limited energy consumption and unimpaired longevity.

MATERIALS AND METHODS

Methods for our study on hibernating strategy of bees intended to obtain MR and related data, on fully undisturbed small colonies, over long experimental periods (weeks to months). Small colonies were preferred in order to obtain insights in their handicaps. They were raised in small hives in June–July, and remained undisturbed until experiments in winter.

The hives were of an oblong type ($45 \times 12.5 \times 16$ cm inner space), with lateral and top insulation of 5 cm polystyrene foam. They hold 3 Dadant-Blatt super frames. Entrance is located in the middle of the front side and a feeder hole in the back side (normally closed), both having a diameter of 3 cm. A first plastic cover on the frames allows visual observation of the bee mass after removal of the insulating cover. Previous experiments (48) had shown this flat narrow hive type to be well suited for wintering of small colonies. The cluster in it stays constantly in the front part about 5 cm behind the entrance, clinging to the insulating cover in a half-ellipsoid shape. Under stable conditions it is not moving around as observed elsewhere (43). This peculiarity eases observations. Temporary movements for food intake are described below.

Bee mass was not weighed prior to experiments (no disturbance). Experimental MR data are therefore not expressed as weight-specific MR. Bee mass was estimated—by comparison with two weighed colonies—in order to obtain estimated weight-specific MR values. Also two weighings of bee masses (shaken in plastic bag) were made after experiments. Colonies after ULMR were left undisturbed for observation of spring development.

Colonies (in hives) were weighed before and after a “session” of experiments. Weight loss was compared with similar data for the lot of small colonies. The overall MR level known via weight loss, for our small colonies around 300 g bee mass, was 5.7 w/kg in broodless winter months (mean temperature Dec.—Feb. 3°C). No marked impact of experimental conditions on MR was observed.

For temperature recording, probes (Wilmo RTD PT100, $0^{\circ}\text{C} = 100$ Ohm, non-linearity IC compensated) were at equal distances positioned 6 cm deep inside the comb streets and connected to a computer, recording on line. In the flat narrow hive type all movements of the cluster were reflected in changing temperature on the spot; visual checking (cover

transparent) confirmed this. For detection of cycles under natural conditions colonies stayed outdoors, wiring passed through a wall. Combined recording of temperature and oxygen consumption was made in indoor experiments.

For slow reliable humidity recording, avoiding disturbance, removable Pasteur pipettes were through the hive cover stuck in the comb streets. The pipettes were filled with dry granulated silicium gel (Ucegel: SiO_2 99.7%; RH max. 1% uptake water 30 mg/g at 20°C) and connected via 0.5 mm tubes to a mechanical device designed to repeatedly (period 2 min) eject and aspirate samples of exactly 2 ml of cluster air over the pellets to absorb the water. Unconnected pipettes at same location made it possible to evaluate natural diffusion of water vapour. Pipettes were weighed “before and after.” Dead space in the pipette tips was ignored (8 mm³, partly equilibrated by diffusion). The method was tested via air over oversaturated salt solutions.

The absolute humidity of the air inside the cluster was calculated as follows: g of water absorbed by the silicagel tubes under periodic air flow, minus g of water absorbed by the silicagel tubes without air flow, divided by the aspired volume in m³. Relative humidity (%) was deduced from absolute values via temperature and Molier diagram (humidity- t° relations of air; modified in ASHRAE guide) (1). The signification of RH in the cluster with regard to water balance, respiration modus and MR is elucidated below in Discussion 7.

Air samples for oxygen and CO_2 analysis, could be collected “disturbance-free” inside the cluster, in syringes via needles (prolonged with 5 cm flexible tube) permanently fixed through the hive cover. Prior to the real samples 0.5 ml was aspired and rejected. Analysis of CO_2 and/or O_2 was made by gas chromatograph (HP 5700A; column Poropak R 3m 1/8” ss; gas H_2 ; t° 60°C ; detector TCD). Hundreds of measurements were made in this way over three seasons. They showed i.a. the slow building up of CO_2 and hypoxia in the cluster core, from 0.5–2% in October to a “winter level” around 6% from November on.

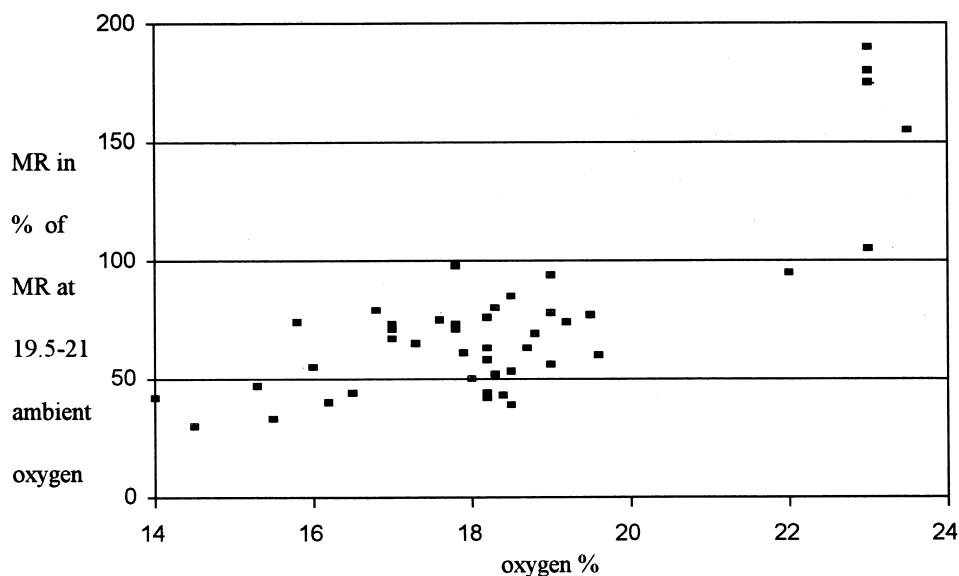
Values of in-cluster hypoxia (% below normal “about 21%”) are expressed in graphs via % CO_2 . Both gases were found to be in the cluster perfectly complementary, despite differing theoretical coefficients for diffusion velocity (relating for O_2/CO_2 as 0.178/0.139) (3). This equality of hypoxia- CO_2 levels under “cluster conditions” was observed on a sample of 180 “twin” readings for oxygen and CO_2 . Calculated regression equation is:

$$\text{O}_2\% = 20.69 - \text{CO}_2\% \times 0.98; R = 0.95 \text{ for } n = 180.$$

Since the slight atmospheric variation in oxygen levels does not impact CO_2 levels, it was preferred to express in-cluster hypoxia via CO_2 as its indicator.

For experiments with continuous MR and temperature recording, the hive was placed in an airtight polyacrylate box ($31 \times 60 \times 42$ cm) with a volume of 78 l. To obtain well mixed air and simulate outdoor diffusion conditions, a

FIG. 1. Metabolic rate (in % of mean MR at 19.5–21% oxygen) in function of oxygen in experimental chamber, observed on 2 colonies over 3 winter months.



small electric fan was installed inside the box. Wiring (and 40 cm 0.5 mm tubing if gas sampling in cluster) passed through an oil-filled lock. The box was connected to an air pump in series with an oxygen meter Servomex 570A (oxygen expressed in %; accuracy 0.1%). Extra O₂, N₂, or CO₂ could be supplied in known exact volumes, first via pump and timer, later (Fig. 5 and foll.) in automated way. Finally the whole device was elastically suspended in a container imposing temperature regimes as wanted. Experiments of this type totalled 18 colony-weeks prior to this article.

The equipment made it possible to dose an airflow throughout the box ("chamber"), and to measure O₂ consumption in a closed or open air circuit. This "through chamber flow" was adopted deliberately, (and "through hive flow" rejected) in order to respect: i) the "still air condition" inside the hive, and ii) the normal "doorway gradients" of metabolic gases at its entrance. Still air in comb streets within a closed cavity indeed is a basis of the bees insulating system; their natural nest is draughtfree (35); they plug the tiniest crack in the upper hive parts. It was preferred therefore to avoid an artificial draught, as is "through hive flow" (see Discussion 9 and 10).

The "through chamber flow" respects the natural gradient of gas levels in the hive, and is subject to a short time lag between changing gas levels in/out hive. Another time lag (fraction of 1 hr) between values in chamber and values recorded depends on volume of chamber, value of MR and sensitivity of recorder. In the "pictures" of metabolic data this did not affect either the general picture of hourly means or the amplitudes of variations on the scale of hours.

The air flow entering under open circuit was not dried. Via water balance of the bees this "artifice" might, or might not, impact winter MR, nonetheless, humidity impacts bee

life (17,47). The flow for oxygen recording was dried via silicium gel.

To calculate the metabolic rate in w/kg in the open circuit the following formula was used:

$$\text{Watt} = [(0.21 - \bar{X}_i) * E * T + (\bar{X}_{i-1} - \bar{X}_i) * 78] * 5.9$$

where: 0.21 = vol.-% oxygen in air; E = exit flow in lit/h, $\bar{X}_i$ = mean O₂% at time I; $\bar{X}_{i-1}$ = mean O₂% at hour I-1; 5.9 = conversion coefficient lit. O₂ into watt; 78 = box volume in lit.; T = 1 hr. For calculation of w in a closed circuit E = 0.

RESULTS

1. Body Exterior Oxygen Factor of MR

The program started with research on MR of small wintering colonies under different ambient conditions. In experiments with varying ambient oxygen levels (outside hive), a MR-oxygen relation was observed, first within the 21–15% oxygen range. In experiments on a broader 24–13% range, this causal effect of oxygen levels on MR was clearly confirmed. Oxygen to MR relations, observed on 2 colonies over 3 winter months, are displayed in Fig. 1: a point represents stable MR over periods ranging from 2 to 12 hr (mean records in 21–19.5% zone represent the reference value). No effect on MR was detected in experiments with varying CO₂ levels up to 6%, under oxygen 21–19.5%. In experiments where oxygen was lowered via increased proportion of nitrogen, the oxygen–MR relation was confirmed. All this revealed hypoxia as the active factor.

The data probably do not express the full physiological impact of hypoxia on MR, since the artificial oxygen levels do not (fully) affect the core bees (Fig. 5). To a large degree these can indeed control their own oxygen level (for this reason no regression is calculated for Fig. 1). Given this

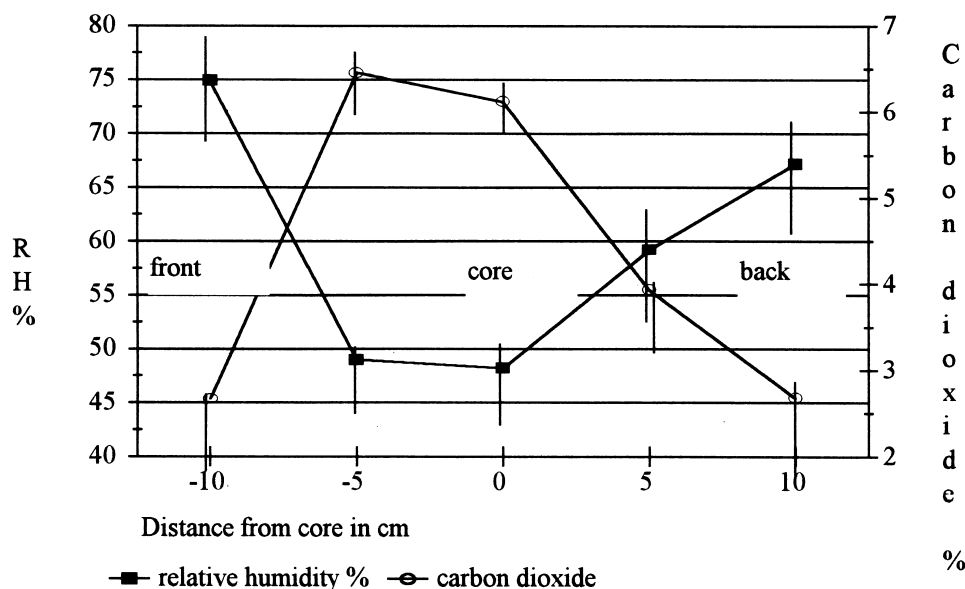


FIG. 2. RH and carbon dioxide (vol. %) in a bee cluster of 400 g at 0°C in Nov. RH data are means of 10 runs of 24 hr. Carbon dioxide data are means of 10 daily records at 8 a.m. See in Methods: carbon dioxide as indicator of hypoxia.

fact, a relationship between hypoxia effects and cluster mass seems possible. Our colonies were small: after experiments (Fig. 1), one weighed 226 g; the other was estimated at 300 g.

2. Observations on Metabolic Gases in the Winter Cluster

Research on metabolic gases in the winter cluster was the next step in the program. Observations outdoors on CO₂ and oxygen in colonies after summer revealed increasing in cluster levels of CO₂ (and hypoxia), from 0.5–2% towards 5–6% as colony activity dropped to winter level. This high level was maintained uninterrupted throughout the winter.

The CO₂ levels were found to be reliable indicators of hypoxia (see Methods).

For more comprehensive information, experiments on oxygen and CO₂ were made via the equipment as described. In Fig. 2 typical gas levels over a 10-day period early in winter are shown: for 0°C; sampling at 8 A.M.; 5 locations in the hive (50 twin analyses); RH values are 24-hr means. The levels of CO₂—and concomitant hypoxia—are highest in the core, whereas RH there is lowest. For the signification of this combination of gas levels see Discussion 7.

Figure 3 shows the course of gas levels during the day. Substantial changes in CO₂ and oxygen expressed in this period diurnal MR rhythms: concentrations, particularly in the core, were always lowest (oxygen highest) around noon.

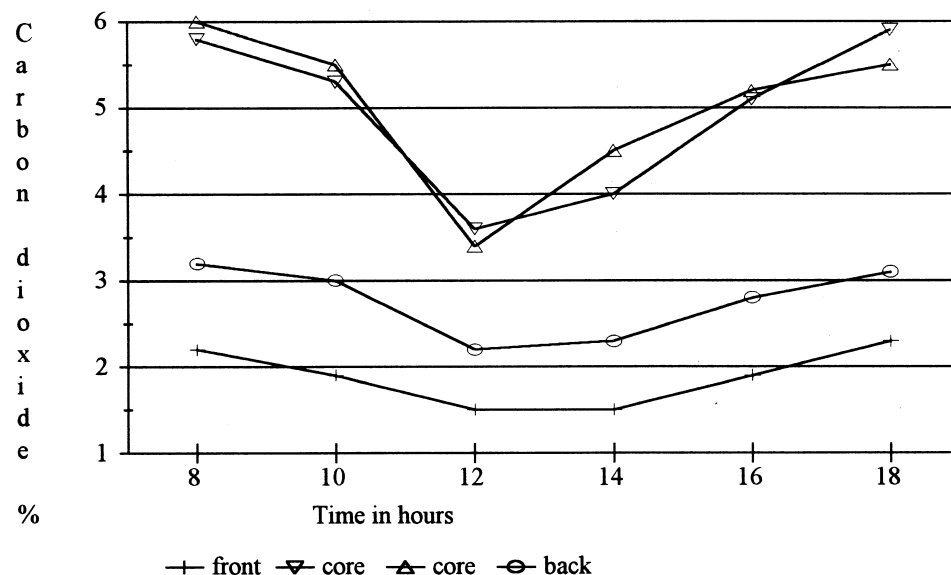
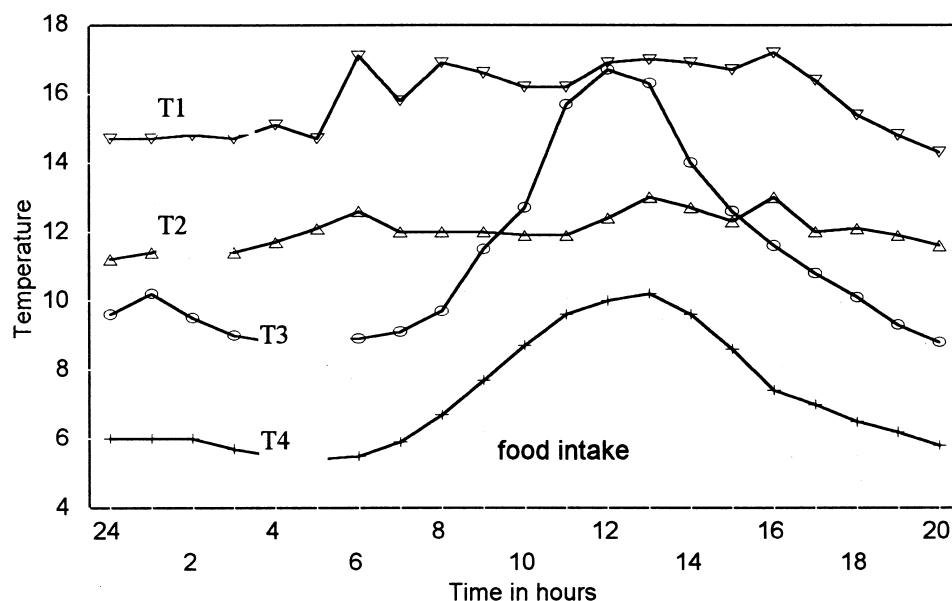


FIG. 3. Example of a course of carbon dioxide concentration (vol. %) from 8 a.m. until 6 p.m. at 4 locations in a cluster of 400 g in a freezer at 0°C. Low levels around noon express cluster movement.

FIG. 4. Course of temperature at four locations in cluster and hive. Rising temperature of back sensors reveals cluster movement (to be compared with Fig. 3). Data of Nov. 20, 1994. Limit of sealed food area was situated between T3 and T4.



This low coincides with a cluster movement, as indicated by the warming up of a back sensor up to core temperature (Fig. 4). The movements expressed, always or at intervals, food intake operations: the cluster indeed stayed on the front side, and stores were in the back half of the hive. Bees prefer not to hibernate on honey filled combs, as empty combs are better insulators due to the air trapped inside. Stable temperatures on the front side indicated that, rather than shifting position, the cluster stretched till food was reached. Afterwards, the cluster resumed its initial shape and high CO_2 -hypoxia was restored. Rhythms involving cluster movement were not strictly diurnal: later in the winter more day periods were prevailing.

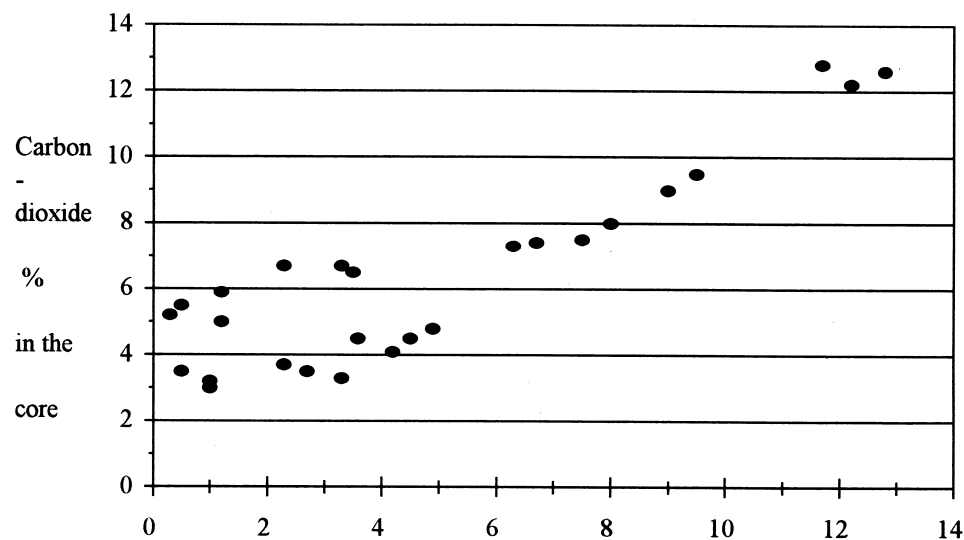
In another series of experiments the effect of changing hypoxia (outside hive) on in-cluster gas levels was investi-

gated. The bees tended to reduce O_2 in core to a 15% level. Below 15% ambient oxygen no real differences between in-core and ambient oxygen concentrations were observed: Fig. 5 (expressed in CO_2 as indicator of hypoxia as well: see Methods). This indicated that a "winter level" of metabolic gases in the wintering cluster is actively controlled by the bees. For the controlling mechanism: see Discussion 4.

3. Cycles in MR During Winter

Two types of cycles were observed under winter conditions: those simply reacting to changes in ambient temperature, and those reflecting cluster movements. The last were both exo- and endogenous. When possible the bees obviously took advantage of warmer hours, or a warmer day after a

FIG. 5. Carbon dioxide concentrations (vol. %) in the core in relation to ambient concentrations (carbon dioxide indicates "hypoxia % below 21%" as well).



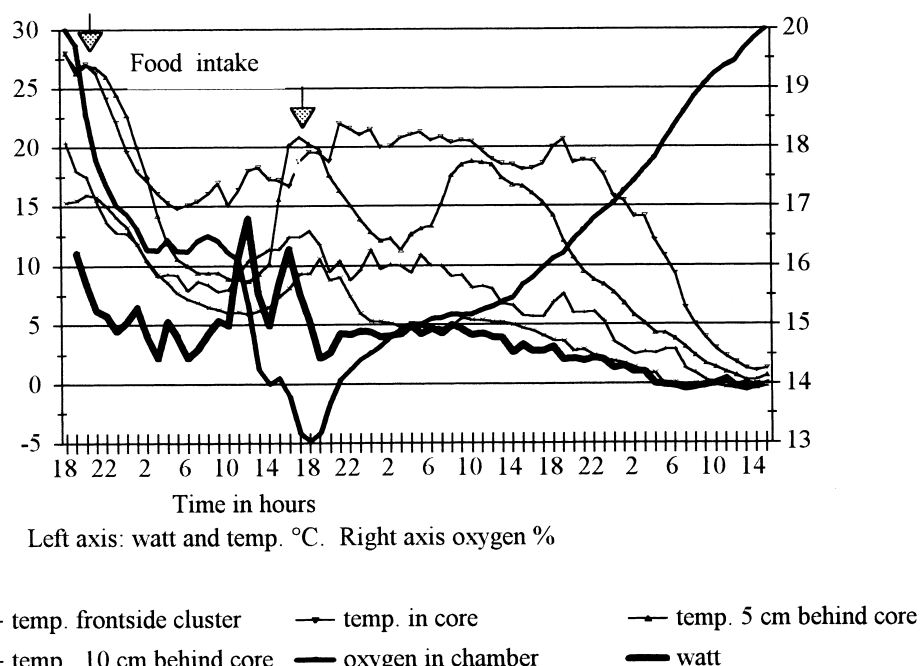


FIG. 6. Course of temperatures and MR under lowered oxygen. The cluster eventually switches to ultra-low MR around 15% oxygen in chamber; oxygen then rises to normal level. Airflow was 250 ml/min.

cold spell, for an “expedition.” In the winter periods of several days (often 2 days; up to 6 days) were prevailing. Substantial rising MR was limited to this type of cycles. Rhythms or changes in ambient temperature—whether diurnal or not—were reflected in cluster-temperatures, until brood production started. As a rule the reaction was passive (ectothermic) at ambient temperatures above 5°C. No “aimless” MR cycles (= phases of lowered hypoxia) were observed in continuous detailed recordings during over 20 colony-weeks in winter.

4. Observations on Ultra-low MR

The experiment displayed in Fig. 6 was made at an ambient temperature of 0°C and (through the box) a 250 ml/min airflow (21% O₂). The O₂ concentration was rapidly reduced during a MR peak. Lowered MR followed at 16.5% oxygen for 10 hr. Daily cluster movements, probably food intake excursions, were revealed by three temperature increases of the back sensor. After the third excursion (for unclear reasons) the bees switched to ultra-low metabolic rate (ULMR), as indicated by the general temperature decrease towards ambient temperature, cluster core last, and by O₂ rising towards 21%.

Collective ULMR when ambient oxygen was between 15–13%, was observed twice in previous experiments (over 3 colony-months), but not documented with temperature data. Conditions triggering, at this oxygen level, this collective ULMR are not yet clear.

Conditions of forced ULMR were investigated in experiments under quickly dropping oxygen in a closed air circuit.

An example of results is displayed in Fig. 7. Since no air was supplied, O₂ was rapidly depleted by respiration. A sudden drop of the metabolic rate to an ultra-low level was observed at 7.5% O₂. As a result the temperature of the cluster decreased sharply to ambient level (set at 0°).

The states of ULMR, as displayed in Figs. 6 and 7, were fully reversible under specific conditions. When O₂ concentration was increased at low ambient temperatures, the cluster remained in ULMR. When at normal O₂ the ambient temperature was increased, cluster temperatures followed passively until 14–15°C was reached. At this level the clus-

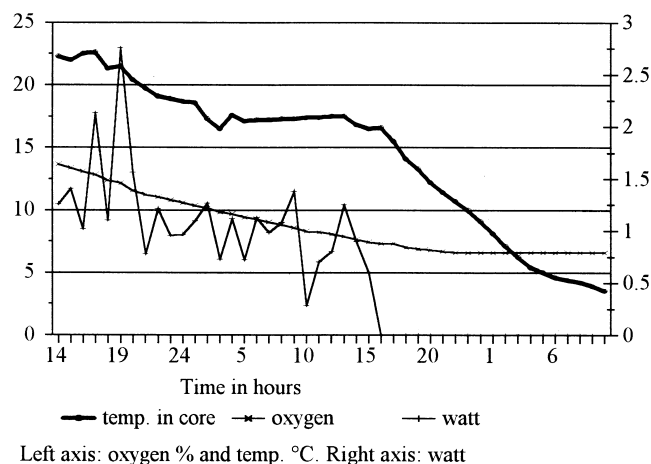
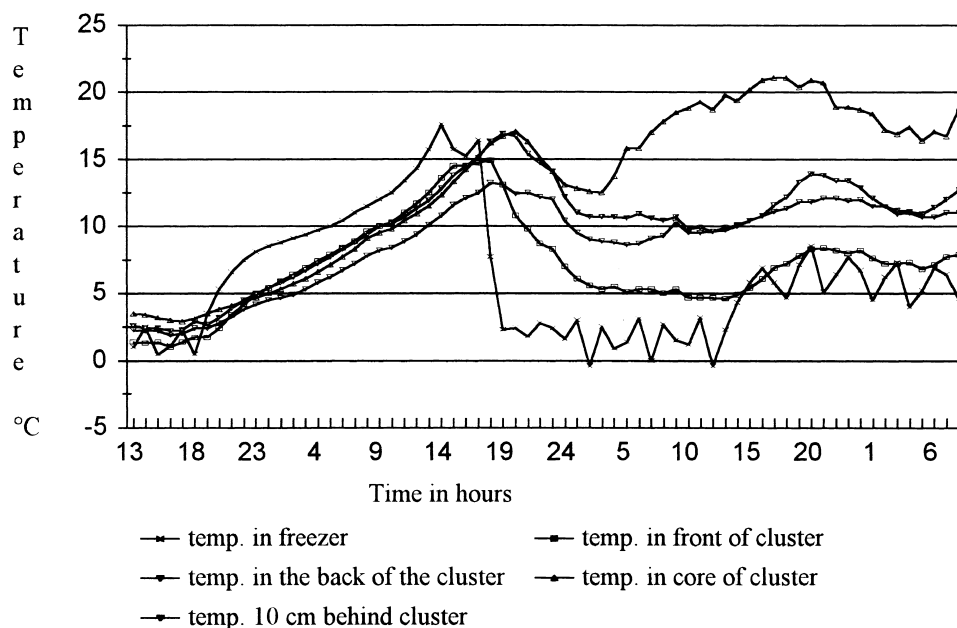


FIG. 7. Respiration in watt, oxygen % in chamber and core temperature of a cluster submitted to dropping oxygen level. Run without air intake. At 7.5% oxygen, respiration was no more detectable with the same equipment.

FIG. 8. Termination of ultra-low MR under 21% oxygen and rising ambient temperature. At 15°C the cluster resumed autonomous thermoregulation.



ter resumed autonomous thermoregulation, and continued it at lower ambient temperatures (example in Fig. 8). The described procedure was repeated five times with a duration of ULMR of 3 days without visible damage to the colonies. The 14–15°C level apparently was essential for “revival” (see Fig. 9).

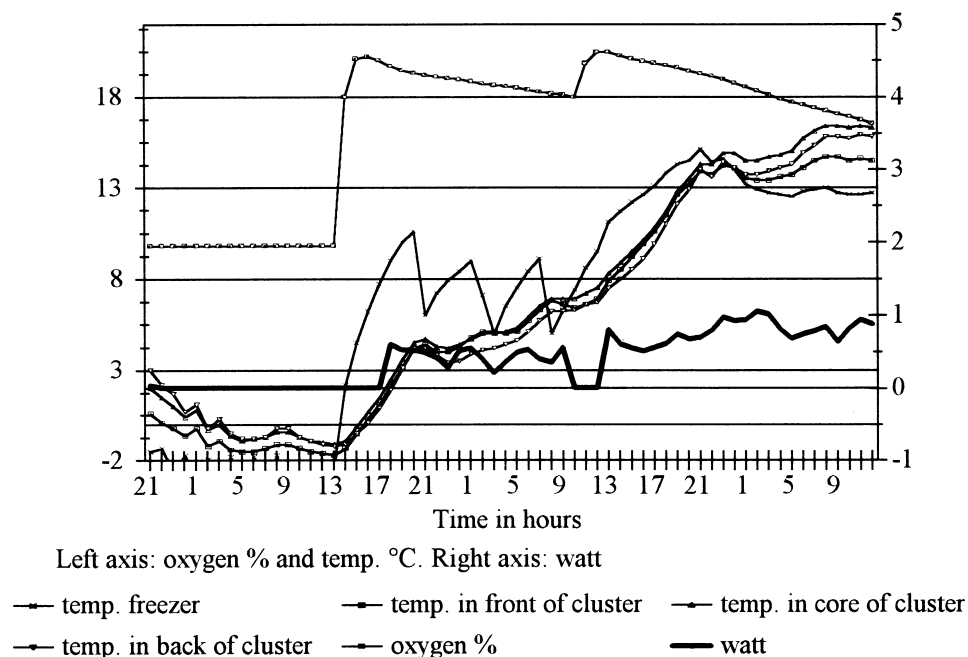
The conditions of the experiment suggest that under normal “in-cluster conditions,” individuals and groups of bees are capable of switching to reversible ULMR; whether this is “common practice” remains as yet unknown. Further as-

pects of this hypoxia-induced ULMR are currently under investigation.

DISCUSSION

1. The data and their significance can be summarized as follows: i) hypoxia is permanent and actively achieved in the winter cluster; ii) it is a factor of MR control; iii) acting as a temperature independent factor of resting MR, hypoxia is essential for cluster thermoregulation, as discussed below;

FIG. 9. Ultra-low MR (at 9.7% oxygen 1–2°C) and its termination under risen oxygen and rising temp. At 15°C the cluster resumed autonomous thermoregulation. Experiment in closed circuit opened temporarily for air intake.



iv) under hypoxia bees can switch to ultra-low MR (ULMR), a state resembling deep diapause in insects.

2. The results presented demonstrate that the hibernating bee cluster achieves and controls in-cluster hypoxia and can use it as a MR controlling factor. Bees, as a colony, thus do not simply have to undergo the Q_{10} law. On the contrary, they have developed a highly flexible mechanism based on body exterior hypoxia, created by clustering and rest, suited to control at least partially their resting metabolism independently of temperature.

High CO_2 values were reported in literature. Their permanent character, signification as hypoxia, and their function appear in the results presented here. A level of 9% CO_2 in the hive, and slightly more in cluster, is the highest we found reported (11). The ambient atmosphere here was man-made (wintering in cellar). The observed low gradient (ambient to in-cluster) seems to be consistent with bee-controlled in-cluster gas levels, as illustrated in Fig. 5.

Since originated by metabolism, high concentrations of CO_2 might be expected to be related to high MR. In fact, they indicate the combination of low activity, clustering, choked diffusion, and hypoxia.

3. To our knowledge no experiments on functional body-exterior hypoxia in insects were yet reported on. However Mikhailov and Taranov (26) suggested a relation between high CO_2 (= hypoxia) and low food consumption in cellar-wintering of bees. For one clustering tropical diapauser, a beetle in Panama (*Stenotharsus rotundus*) (43), a lowering effect of clustering on MR has been reported but not explained. The mechanism might be similar to what we found in bees. A relation between reduced atmospheric pressure (= oxygen tension) and reduced respiration was found in honeybees by Woodworth (49). It was considered a possible effect of altitude on bees, and received no further attention.

4. Bees control the level of hypoxia in the cluster de facto. At ambient O_2 concentrations over 15%, the bees tended to maintain the concentration in the cluster core at around 15%, while at lower ambient O_2 (higher CO_2) no further decrease was observed (Fig. 5).

Self-controlled in-cluster hypoxia implies that artificial ambient hypoxia in our experiments (Fig. 1) only affected the bees in the cluster mantle. It thus revealed an apparent degree of oxygen-MR relation: the real relation, and its functional value, might be stronger.

The controlling mechanism remains unclear. A rough capacity to sense CO_2 levels, very high tolerance of it (up to 60%), and absence of perception of oxygen level, were demonstrated by Lacher (19) and Seeley (34). These data suggest that control of in-cluster oxygen proceeds indirectly. Controlled CO_2 level, which we found to express hypoxia, might be a mechanism. Water conservation imposes unventilated clustering: since this results in hypoxia, and vice versa, a two-way partly self-regulating process might be playing.

5. The hypoxia principle seems to be essential for the

bee cluster on account of thermodynamic principles. Virtual oversupply of heat in the cluster core is an obvious theoretical problem. In the thermogenic strongly self-insulating hairy bee cluster (12), four factors would be expected to conspire towards a non-linear temperature gradient:

a) heat generated in the core and drained through a passive insulating mantle would create a linear temperature gradient;

b) however, the insulating mass is not passive but thermogenic, therefore the temperature gradient must be steeper than linear;

c) heat production per unit mass is an exponential function of temperature (Q_{10} law), making the temperature gradient even steeper;

d) insulating combs prevent free lateral heat transfer.

On the basis of resting metabolism an oversupply of heat in the core thus has to be expected. In other words, biological and thermodynamic laws predict temperature isotherms tightening from edge to core of a bee cluster. This is particularly valid at low temperatures, when the cluster density is maximised. In the real bee cluster isotherms widen from edge to core (12,40,50).

Mathematical models (22,30) claim heat production decreasing exponentially from the periphery to the centre of the cluster. This conflicts with the Q_{10} law. They predict heat excess in core, unless assumptions are made, such as non-insulating effect of combs [contradicted in Southwick (39)], constant low bee density in core (1.25 bee/ml), or an unspecified Q_{10} countering factor. A normal-sized "cold cluster" (20) would be "mathematically impossible."

The very problem for thermoregulation in the bee cluster, considered as a "homeothermic superorganism" (38), is the absence of heat transport via a bloodstream: water resp. air (at 50% RH) can transport heat per volume-unit in a relation as 2500 to 1. Moreover, water conservation forbids thermoregulation of the winter cluster by substantial ventilation.

6. These thermodynamic problems are playing in the real bee cluster; the hypoxia principle can explain how bees manage them.

Precise experiments on "fractional MR" (MR of concentric partial cluster masses) were made by Southwick (40). The sum of fractional MR (based on observed resting MR at distinct in-cluster temperatures) was 3-fold the observed lowest MR of the real cluster (11.42 versus 3.76 w/kg.).

The extensive experiments on swarms by Heinrich (12) raise the same question of virtual heat excess in the cluster core. A major conclusion was: "The active thermoregulatory metabolism (in large tight swarms at 5°C and lower) was due to the bees on the swarm mantle. The resting MR in the swarm core was more than sufficient to maintain core t° at 35°C or above."

From Heinrich's experiments on dead swarms virtual heat excess can be quantified via heat output (= MR) required to compensate for passive heat loss of the core. Cooling rate

(at gradient 24°C core to ambient) for the core in a 30,000 bees cluster was 0.078°C/hr; to compensate for this heat loss a MR of 0.073 w/kg would be required (assuming specific heat 0.81 Kal/kg/1°C for a bee mass at 75% water content, and converting 1 Kal/hr in 1.16 w). The corresponding value for a 1000 bees mass was 1.87 w/kg (since both cooling rate and compensating MR are weight related, these data are valid for a range of bee densities in the core). For a normal unventilated cluster thus heat loss of the core is less than the lowest MR values hitherto measured (see par. below). The open question then is: can bees metabolise “below resting MR” as “imposed” by Q_{10} law, and if so, by what mechanism?

Hypoxia-controlled MR can explain these open questions. It offers a “missing tool.”

Experimenting on individual bees, via new techniques indicating “true resting MR,” Rothe and Nachtigall (33) found low MR values. Values down to 2 w/kg (lowest 20% of values in graph p. 741) were observed in the 20–30°C range, and much lower values below this level (see 7 and 13 below as well). Under the assumption that hypoxia helps in reducing a 2 w/kg level (observed at normal oxygen), the “heat excess in core” question gets a full answer.

7. Gas levels in the winter cluster stay also in a complex relation to the water balance and modus of respiration. A major problem for resting insects is water loss via exhaled air, since this is water vapour saturated. Discontinuous respiration, deeply extracting oxygen out of inhaled air, is one of the principles minimising this loss (48). High CO₂ levels in the bee cluster first indicate choked diffusion, thus choked water vapour loss as well. Second, the coincidence of high CO₂ (= hypoxia) and low RH also indicates deep extraction of oxygen out of low-oxygen in cluster air. Dry air under choked diffusion in the bee cluster indeed implies CO₂ output (and oxygen intake) per breath to be a multifold of water output. Moreover Rothe and Nachtigall (33) found discontinuous respiration in “truly resting” bees and implications of “exhaustive respiration” in wintering bees have been formerly reported (46).

The “dormancy threshold” we found around 7.5% ambient oxygen may provide supplementary information on this process. This threshold seems to indicate a critical bottom value for the range of oxygen levels honey bees can exploit, while staying operative as a colony. Extracting in body a (supposed) 15–7.5% oxygen range might offer other physiological opportunities, as compared with a “one step” extraction of, e.g. a 21–7.5% range. The “two-step hypoxia” of bees thus might be functionally important.

8. A temperature-independent controlling factor of resting MR may answer evolutionary questions. Cluster core temperatures over 30°C are common, but 20° and less exists (“cold and warm clusters”) (20) and works. Winter brood is not a general rule, but it is not uncommon (2,27); it imposes a constant temperature of 32–34°C. It is not easy to find evolutionary sense in both the existence and variance

of this behaviour, believed to be both energy wasting and life shortening. Hypoxia-controlled MR, loosening the link between temperature and resting MR, explains low or absent evolutionary pressure against high core temperature and winter brood.

Cycli in MR are well known. High amplitudes were observed, e.g. MR 40 to 160 w/kg (18), or 6 to 30 w/kg (37). The function of these marked cycles was as yet unclear. In our experiments substantial endogenous cyclic rising of MR was related to cluster movements, to be interpreted as food intake operations. Their period ranged from 1 day, over 2 days (most frequently), to a 6-day maximum. No “aimless” cycles, and thus no needless breaking of hypoxia nor senseless wasting of energy, were observed.

9. A “heating core–insulating mantle” view on the winter cluster has long been prevalent. It was based on the observed fact of temperatures rising in cluster when dropping outside; changing densities can explain this. Objections to the view were analysed under 5 and 6 above. Testing the view in a model, Omholt (34) found 100°C to be required in cluster core to ensure 10° on its skin at –20° outside. However “heating core” must be partially correct: anyway “skin bees” take in heat (some) on their “cluster side” and lose (mostly much more) on their outer side.

Current core temperatures in our experiments were “low”: between 16° and 25°C. The lowest gradient (healthy small colony 350 g) was 13.5°C in core at 8.5° hive-exterior temperature. This contrasts with the often reported 30–35° level (15). Facts thus suggest that core temperature is not functionally important, rather a side-effect; cluster size is a probable factor. If resting MR is not rigidly associated with temperature, as indicated by our results, cluster core temperature would indeed not have a decisive impact either on MR or on survival chances.

Movements of the winter cluster have often been reported and were object of study (42,43). Their purpose remained unclear. In our experiments, in view of hive shape, of permanent cluster location in its front, and of location of food in the back, food intake excursions had to be considered as one function, probably the only one. The hypoxia principle helps to explain why movements, by reducing hypoxia, are accompanied by remarkably high MR peaks.

10. The hypoxia principle throws light on puzzling facts. A first one is the higher winter mortality in both under- and oversized colonies (14). Deficient hypoxia seems to be a probable factor of small-size handicap, explaining it better than the heat loss hypothesis (see Introduction). In oversized colonies a vicious cycle might be playing: heat excess, ventilation, more heat via activity and less hypoxia, water loss. Möbus (27) indeed reported massive mortality in experimental giant colonies (over 3 kg), due to flying out of thirsty bees in the cold.

Disturbance of wintering colonies, even slight, is in apiculture known to be detrimental to a surprising degree. Wind, noise such as a slamming door of the bee house, mice

or bird visits, etc. can even be fatal. Hypoxia might explain this: it indeed relies on efficiently choked diffusion in a skin-less clump of loose bees, *i.e.* on absolute rest.

Troubling divergence of values found in experimental data on colony-MR might be better understood. The "through hive flow" method (airflow for gas analysis drawn through hive) yielded high values: *e.g.* 12 w/kg at 0°C and 4–30 w/kg at 10°C (37). The "through chamber flow" (flow around hive) method yielded the lower values: *e.g.* 7.08 w/kg at 20°C and 2.06 w/kg at 10°C (50). The latter values are consistent with "natural" winter MR levels, based on long-term data on food consumption, as 4 to 5 w/kg at 0–5°C (13). A slow airflow for gas analysis is a poor heat transporter, but it is a draught and bees abhor this. It destroys the insulating still-air situation and might cause unrest undoing hypoxia.

11. Ultra-low MR (ULMR) in honey bees was observed in our experiments under artificially reduced ambient oxygen. The state resembled deep diapause in insects: absolute immobility, no temperature control. The switch seemed to be optional (and exceptional for a total cluster) at 15% oxygen, while obligatory at 7.5%. This suggests the existence of critical thresholds. The 15% level has been observed in other (not dormant) insects as a level under which MR becomes oxygen limited (31). An oxygen level around 7% has been observed in more insects as a critical level, under which metabolism suddenly drops (7). As true insect diapause, the state of deep dormancy in bees could be terminated under specific conditions: in our experiments when 15°C was reached under normal oxygen or close to this level.

Chill coma of bees is well known. It was studied by (10): mortality at 0°C was 20% and 96% after resp. 50 and 80 hr. This suggests chill coma and hypoxia-related reversible ULMR to be different states.

12. Apparently bees maintained a faculty to enter deep diapause, and seemingly kept it unused for millions of years. This would be surprising unless bee hibernation might be considered a mild form of diapause, based on similar mechanisms as "true diapause." Since optional collective ULMR was observed at 15% oxygen and its reversal at 15°C, periodic individual transitions by core bees to this state seem possible.

13. Conversely, it might be worthwhile to investigate whether oxygen plays a role in insect diapause. Insect diapause is an extremely flexible and multiform (in duration, degree of dormancy, *etc.*, even within species) "rest phase" enabling survival in adverse seasons. It is known to be based on a diversity of cellular and hormonal processes (4,21,23,45). However, it might be that the potential to achieve low in-body oxygen levels belongs to the instrumentarium. Ultra-low loss of water vapour is indeed a prerequisite for successful diapause; it requires spiracles to be efficiently closed. The widespread discontinuous respiration (down to 1 exchange/24 hr) (32) of diapausing insects is considered a wa-

ter-saving strategy (48); however, it is keeping in body oxygen necessarily low. Rothe and Nachtigall (33) observed this discontinuous respiration on bees in "true resting MR."

14. Basic metabolism of ectothermic animals is believed to be determined by two major laws: the body mass to MR relation, and the Q_{10} temperature law (16). Controlled hypoxia apparently is a third principle exploited by hibernating bees. It enables them to reconcile warm wintering in an alert state, with water and energy saving and prolonged life span. Bees this way exploit the opportunities of a double—both ecto and endothermic—behaviour.

Oxygen tension as a MR factor in insects is not fully unknown: a few casual oxygen-MR relations are reported in literature. However, oxygen might have been overlooked as a more general MR controlling factor in insects and beyond. It also may be remembered that modern fruit conservation is largely based on low oxygen. Evidently self-achieved body exterior hypoxia, and *a fortiori* its controlled character, only can be extremely exceptional in higher organisms.

15. Practical apicultural applications of the findings are not available as yet, but *e.g.* wintering under artificial hypoxia is no longer an unrealistic prospect.

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References

1. Asrae Guide and Data Book. New York, NY: Am. Soc. of Heating, Refrigerating and Air Conditioning Engineers Inc.; 1965.
2. Avitabile, A. Brood rearing in honey bee colonies from late autumn to early spring. *J. Apic. Res.* 17:69–73;1978.
3. CRC Handbook of Chemistry and Physics, 53rd ed.
4. Denlinger, D.L. Hormonal control of diapause. In: Kerkut, G.A.; Gilbert, L.I. (eds). *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Oxford: Pergamon; 1981.
5. Denlinger, D.L. Dormancy in tropical insects. *Ann. Rev. Entom.* 31:239–264;1986.
6. De Ruyter, A.; Van Den Eynde, J.; Van Der Steen, J. Ambrosiushoeve reports; Min. Agric. (The Netherlands) 1991.
7. Fraenkel, G.; Herford, G.V.B. The respiration of insects through the skin. *J. Exp. Biol.* 15:266–280;1938.
8. Free, J.B.; Racey, P.A. The effect of the size of honeybee colonies on food consumption, brood rearing and the longevity of the bees during winter. *Entom. Exp. Appl.* 11:241–249;1968.
9. Free, J.B.; Simpson, J. The respiratory metabolism of honeybee colonies at low temperatures. *Ent. Exp. Appl.* 6:234–238; 1963.
10. Free, J.B.; Spencer-Booth, Y. Chill coma and cold death temperatures of *Apis mellifera*. *Entom. Exp. Appl.* 3:222–230; 1960.
11. Hallund, V. Carbon dioxide concentration in the hive in winter. *Nord. Bitidskr.* 8:78–82;1956.
12. Heinrich, B. The mechanisms and energies of honeybee swarm temperature regulation. *J. Exp. Biol.* 91:25–55;1981.
13. Jebsen, W. Das Bienenleben in Zahlen. *Archiv f. Bienenkunde* 29:17–31;1952.
14. Jeffree, E.P.; Allen, D. The influence of colony size and of

- nosema on population loss in honey bee colonies in winter. *J. Econ. Entom.* 49:831;1956.
15. Johansson, T.S.K.; Johansson, M.P. The honeybee colony in winter (Review). *Bee World* 60:155–170;1979.
 16. Jones, D.J. Comparative physiology of respiration. Arnold, E. (ed). London: 1972.
 17. Kellner, A.; Jacobs F.J. Symp. on bee flight rooms. *Insectes Sociaux* 24:297;1980.
 18. Kronenberg, F.; Craigh Heller, C. Colonial thermoregulation in honey bees. *J. Comp. Physiol.* 148:65–76;1982.
 19. Lacher, V. Verhaltensreaktionen der Bienenarbeiterin bei dressur auf Kohlendioxid. *Zeits. f. Vergleich. Physiol.* 54:75–84;1967.
 20. Lavie, P. L'enregistrement thermique continu au cours de l'hivernage d'*Apis mellifera*. *Ins. Soc.* 1:1954;1954.
 21. Lees, A.D. Physiology of Diapause in Arthropods. Cambridge Univ. Press; 1955.
 22. Lemke, M.; Lamprecht I. A model for heat production and thermoregulation in winter clusters of honey bees using differential heat conduction equations. *J. Theo. Biol.* 142:261–273; 1990.
 23. Masaki, S. Summer diapause. *Ann. Rev. Entomol.* 25:1–25; 1980
 24. Maurizio, A. The influence of pollen feeding and brood rearing on the length of life of the honey bee. *Bee World* 1950: 9–12;1950.
 25. Merz, R.; Gerig, L.; Wille, H.; Leuthold, R. The problem of short- and long living bees. *Rev. Suisse Zool.* 86:663–671; 1979.
 26. Mikhailov, K.I.; Taranov, G.F. Kulsyre kontsentsajonen I bienes vinterklase. *Nord. Bitidsk.* 18:3–7;1966. (First publ. in Russian 1961).
 27. Mobus, B. The winter cluster of honey bees (in German). *Bienenwelt* 1978:170–227;1978.
 28. Morse, R.A. Honey bee colony defense at low temperatures. *J. Econ. Entom.* 99:1091–1093;1966.
 29. Neukirch, A. Dependence of the lifespan of the honeybee (*Apis mellifera*) upon flight performance and energy consumption. *J. Comp. Physiol.* 146:35–40;1982.
 30. Omholt, S.W. Thermoregulation in the winter cluster of the honey bee. *J. Theo. Biol.* 128:219–231;1987.
 31. Park, H.D.; Buck, J. The relation of oxygen consumption to ambient oxygen concentration during metamorphosis of the blowfly, *Phormia regina*. *J. Insect. Physiol.* 4:220–228;1960.
 32. Punt, A. The respiration of insects. *Phys. Compar. Oecol.* 2: 59–63;1950.
 33. Rothe U.; Nachtigall W. Respiratory quotients and MR during sitting, walking and flying of honey bees. *J. Comp. Physiol. B* 158:739–749;1989.
 34. Seeley, T.D. Atmospheric carbon dioxide regulation in honey bee colonies. *J. Insect Physiol.* 20:2301–2305;1974.
 35. Seeley, T.D.; Morse, R.A. The nest of the honey bee. *Insect. Soc.* 23:495–512;1976.
 36. Seeley, T.D. Honeybee Ecology. Princeton Univ. Press; 1985.
 37. Southwick, E.E. Metabolic energy of intact honey bee colonies. *Comp. Biochem. Physiol.* 71A:277–281;1982.
 38. Southwick, E.E. The honey bee cluster as a homeothermic superorganism. *Comp. Biochem. Physiol.* 75A:641–645;1983.
 39. Southwick, E.E. Thermal conductivity of wax comb and its effect on heat balance in colonial honey bees. *Experientia (Basel)* 41:1486–1487;1984.
 40. Southwick, E.E. Allometric relations, metabolism and heat conductance in clusters of honey bees. *J. Comp. Physiol. B* 156:143–149;1985.
 41. Southwick, E.E. Cooperative metabolism of honey bees: An alternative to antifreeze and hibernation. *J. Therm. Biol.* 12: 155–158;1987.
 42. Szabo, T.I. The thermology of wintering honey bee colonies. *J. Apic. Res.* 24:28–31;1985.
 43. Szabo, T.I. Honey bee induced hive entrance defrosting. *J. Apic. Res.* 27:115–121;1988.
 44. Tanaka, S.; Wolda, H.; Denlinger, D. Group size affects the metabolic rate of a tropical beetle. *Physiol. Entom.* 13:239–241;1988.
 45. Tauber, M.J.; Tauber, C.A. Insect seasonality: diapause maintenance, termination and postdiapause development. *Ann. Rev. Entomol.* 21:81–107;1976.
 46. Van Nerum, K.; Buelens, H. Low oxygen in nest and body controls metabolic rate and lifespan in overwintering *Apis mellifera*. In: Benelux Zool. Congr., Leuven Univ. 1994. *Belgian J. Zool.* 114:1;1996.
 47. Van Praagh, J.P. The atmospheric humidity and brood rearing in a flight room. *Apidologie* 6:283–293;1975.
 48. Wharton, G.W. Water balance of insects. In: Kerkut, G. (ed). *Comprehensive Insect Physiology Biochemistry and Pharmacology*. Pergamon: Oxford; 1985.
 49. Woodworth, C.E. Some effects of reduced atmospheric pressure upon honey bee respiration. *J. Econ. Entom.* 25:1036–1042;1932.
 50. Worswick, P.V. Comparative study of colony thermoregulation in the African honey bee, *Apis Adansonii mellifera* Latreille and the Cape honeybee, *Apis mellifera capensis* Escholtz. *Comp. Biochem. Physiol.* 86A:95–102;1987.

