

Flight of the honey bee

IV. Respiratory quotients and metabolic rates during sitting, walking and flying

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Summary. Using manometric and gas analytical methods oxygen consumption \dot{V}_{O_2} , carbon dioxide production \dot{V}_{CO_2} , respiratory quotient RQ , (Fig. 1A–C) and thorax surface temperature difference ΔT_{ts} (Fig. 3) were determined in single bees. The animals were either sitting in respiratory chambers or were suspended by the scutum, in which case they were resting, ‘walking’ (turning a small polystyrene ball) or flying in a closed miniature wind tunnel.

During resting (sitting in Warburg vessels) at an ambient temperature $T_a = 10^\circ\text{C}$, RQ was 1.01 ± 0.2 ($n = 905$) with variations due to method (Fig. 1D, E). RQ values during walking were determined in single cases. In no case were they significantly different from 1.00. After the first 10 min of flight mean RQ was 1.00 ± 0.04 . It was significantly smaller than 1.00 ($RQ = 0.97$) only during the last 5% of long-time flights (mean flight duration 58.8 ± 28.8 min). With the exception of near-exhaustion conditions no signs of fuels other than carbohydrates were found.

Metabolic rate P_m was 19.71 ± 21.38 mW g^{-1} during ‘resting’ at $20^\circ\text{C} \leq T_a \leq 30^\circ\text{C}$ indicating that many resting bees actively thermoregulate at higher T_a . After excluding bees which were actively thermoregulating, by an approximation P_m was 5.65 ± 2.44 mW g^{-1} at $20^\circ\text{C} \leq T_a \leq 30^\circ\text{C}$. ‘True resting metabolic rate’ for sitting bees at $T_a = 10^\circ\text{C}$ was 1.31 ± 0.53 mW g^{-1} (Fig. 2A, B).

A significant negative correlation was found between relative (specific) oxygen consumption $\dot{V}_{O_2,rel}$ and body mass M_b at $85 \text{ mg} \leq M_b \leq 150 \text{ mg}$.

At $0^\circ\text{C} \leq T_{ts} \leq 16.5^\circ\text{C}$ a significant ($\alpha = 0.01$) positive correlation was found between \dot{V}_{O_2} and ΔT_{ts} in single resting bees: $\dot{V}_{O_2} = 0.229 \Delta T_{ts} + 0.099$, or between P_m and ΔT_{ts} : $P_m = 1.343 \Delta T_{ts} + 0.581$ (Fig. 3D) (\dot{V}_{O_2} in ml h^{-1} , P_m in mW, T in $^\circ\text{C}$).

During walking (duration 13.15 ± 5.71 min, $n = 13$) at $12.5^\circ\text{C} \leq T_a \leq 21^\circ\text{C}$ a stable ΔT_{ts} of $11.41 \pm 3.37^\circ\text{C}$, corresponding to 167 mW g^{-1} , was reached for 80 to 90% of the walking time (Fig. 4B).

During wind tunnel flights of tethered animals the minimal metabolic power measured in exhaustion experiments was 240 mW g^{-1} . Calculation of factors of increase in P_m is of limited value in poikilotherms, in which true resting conditions are not exactly defined.

Introduction

Determination of resting metabolic rate (metabolic power) is important for comparison with working metabolic rate and for calculating the factor of increase, although ‘resting’ is not easily defined in animal physiology. This may be the main reason why resting metabolic rates reported in the literature vary so greatly (Table 1). Measuring is often complicated and prone to error so that a more easily measured parameter, proportional to the resting metabolic rate, would be of considerable interest. In a working bee walking should be less expensive than flying, but very little data are available on the energetic demands of the former locomotory activity. Thus, it is important to redetermine resting metabolic rates and to remeasure walking metabolic rates on a broader scale and under defined conditions, in order to determine an easily measured substitute for oxygen consumption and to compare resting to locomotory metabolic rates.

Methods

Animals were reared at the institute in a flight room (Rothe and Nachtigall 1980, 1982). Metabolic rates and thorax temperatures were determined in the following way. Resting: Warburg methods and thermistors glued to the scutum with wax were used (Feller and Nachtigall 1989). Walking: A vessel was used, the air content of which was pumped via a secondary circuit through an oxygen- and carbon dioxide analysing circuit. Flying: A specially built, gas-tight, closed, miniature wind tunnel, using the same gas analysis circuit, was used. Further methods were exhaustion flights and thorax cooling analysis (Nachtigall et al. 1989). Details of measuring procedures and calibrations will be published elsewhere.

Results

Metabolic parameters during resting

Definition of resting. The following parameters were used: no visible signs of activity; the animal sits totally motionless on the bottom of the Warburg vessel. In a true resting bee the thorax surface temperature T_{ts} almost equals ambient temperature T_a . During passive heating after cooling, and vice versa, T_{ts} is exponentially approximating T_a .

Respiratory quotient $RQ = \dot{V}_{CO_2} / \dot{V}_{O_2}$. Taking into account an adaptive phase of several minutes, the respiratory quotient was determined for successive 4-min intervals from plots of carbon dioxide gain and oxygen loss in manometer vessels at $T_a = 10^\circ\text{C}$. Three examples showing typical gradients are plotted in Fig. 1A–C. Fig. 1D shows the RQ distribution plot for 905 measurements. Since the difference between mean value, \bar{x} , and density mean, d , is smaller than the accompanying double error $2\sqrt{3s/2N}$ (Sachs 1974), distribution is taken as symmetrical. Mean value and standard deviation are 1.01 ± 0.2 . Thus, a respiratory quotient of 1 can be accepted, indicating pure carbohydrate combustion under test conditions (correlated caloric equivalent of oxygen $21.117 \text{ kJ l}^{-1} \text{ O}_2$). For $RQ \leq 1$ see discussion.

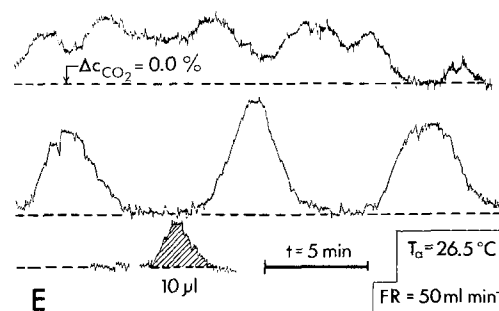
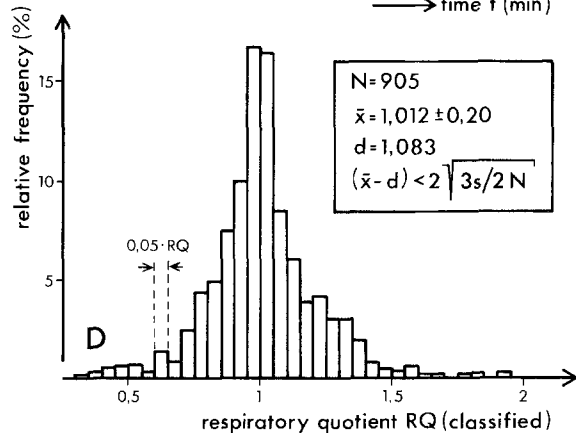
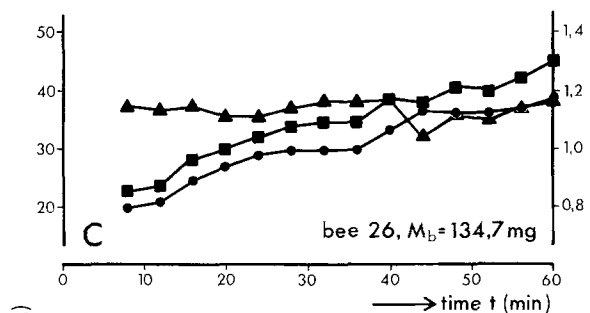
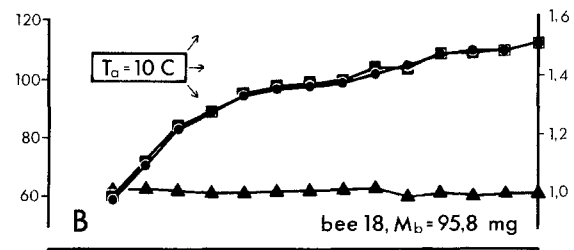
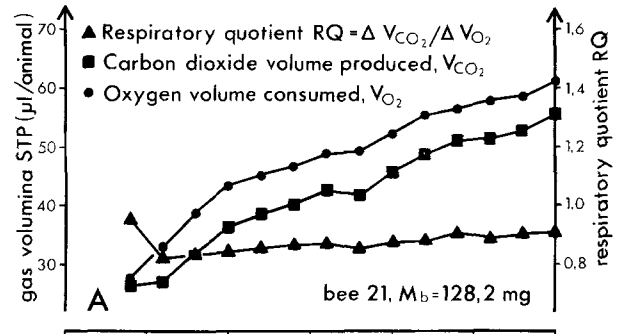


Fig. 1A–E. Respiratory gas exchange and respiratory quotients in resting, walking and flying bees. A–C Examples of time functions of carbon dioxide gain and oxygen loss in 1 h Warburg experiments at $T_a = 10^\circ\text{C}$. D Distribution of the respiratory quotients measured by Warburg methods in ‘resting’ bees. For parameters d and $(\bar{x}-d)$ see text. E Example of discontinuous CO_2 exchange. Oscillating CO_2 release in a bee resting in a respiration vessel. FR flow rate, T_a ambient temperature. A $10 \mu\text{l}$ calibration area for CO_2 is indicated. Open-circuit gas-analytic measurement

Relative metabolic power $P_{m\ rel}$ of resting animals as a function of ambient temperature T_a . Measurements were made on 356 apparently resting bees. The relative metabolic power was calculated from O_2 measurements at 6 ambient temperatures, without taking into account thorax temperatures, and plotted as histograms (Fig. 2A). Besides extremely low and high ambient temperatures ($T_a=10^\circ\text{C}$ and $T_a=35^\circ\text{C}$) histograms all are 'broad', showing a wide scatter of data. For example, at $T_a=30^\circ\text{C}$, 31% of all values were in class nos. 1 and 2 (up to 4-fold the minimum observed value), 17.4% in class no. 5 (up to 32-fold). Standard deviations are so high (Fig. 2B, dashed lines) that there is no statistical difference in the $P_{m\ rel}$ values at all temperatures, except between $T_a=10^\circ\text{C}$ and $T_a=35^\circ\text{C}$. Both aspects indicate that seemingly resting animals were in fact often not under true resting conditions; internal thermoregulatory activity e.g. shivering thermogenesis seemed to occur frequently. Thus, it is not possible to determine whether a bee is truly resting or not without additional information.

Relative metabolic power $P_{m\ rel}$ of truly resting animals as a function of ambient temperature T_a . From synchronous measurements of oxygen consumption and thorax temperature (see below) it seems safe to restrict 'true resting conditions' to metabolic rates which are up to five times as high as the minimum value observed, and which barely enhance the thorax temperature. This was the case in 241 out of 356 experiments. There is a significant linear correlation between $P_{m\ rel}$ and T_a (Fig. 2B, filled circles). For the equation and significance matrix see insets in Fig. 2B.

Relative oxygen consumption $\dot{V}_{O_2\ rel}$ as a function of body mass M_b . In the mass range $85\text{ mg} \leq M_b \leq 150\text{ mg}$ a significant negative linear correlation $\dot{V}_{O_2} = -2.94 M_b + 589.46$ (\dot{V}_{O_2} in $\mu\text{l STP g}^{-1}\text{ h}^{-1}$, M_b in mg; $R = -0.53$, $\alpha = 0.001$) was found, confirming the trend to higher specific metabolic power in smaller animals. The exponents of the power function of this small mass range were not calculated.

Oxygen consumption \dot{V}_{O_2} and surface thorax temperature difference $\Delta T_{ts} = T_{ts} - T_a$. In seemingly resting animals, \dot{V}_{O_2} , T_{ts} and T_a were registered simultaneously and usually for more than 2 h. It could be demonstrated that metabolic power in these animals is positively correlated to thorax temperature. Three examples are plotted in Fig. 3A-C. Bees with thorax temperatures near

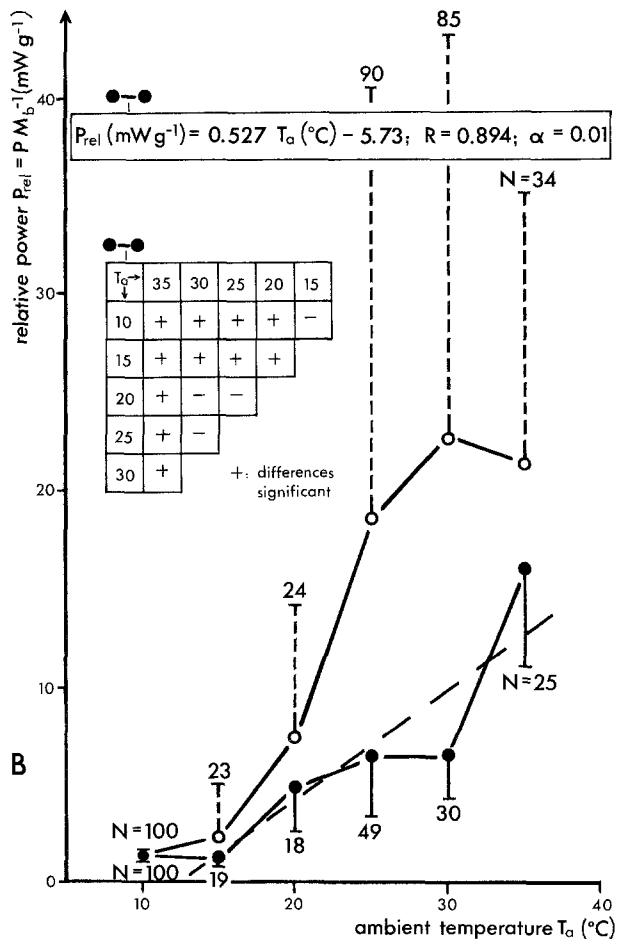
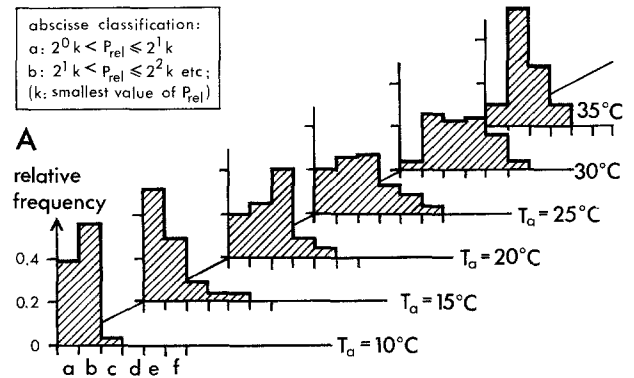


Fig. 2A, B. Relative metabolic power (P_{rel}) at different ambient temperatures (T_a). A Classified P_{rel} of bees seemingly resting at different T_a . Inset: classification principle. B Plots of the P_{rel} values corresponding to the mean values of the histograms in A (open circles) and of the P_{rel} values corresponding to the mean values of $<5\text{ k}$ (solid circles; see inset in Fig. 2A and the text). Standard deviations and numbers of measurements, N , are indicated. The equation and the significance matrix belong to the plot characterized by solid circles

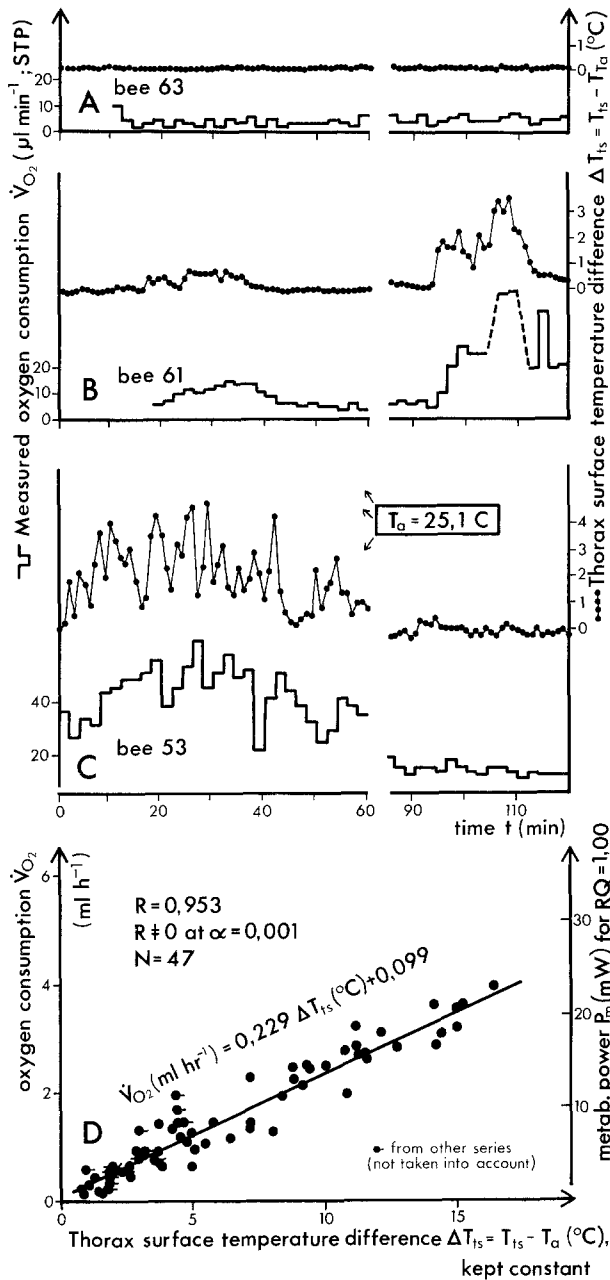


Fig. 3A–D. Oxygen consumption (\dot{V}_{O_2}) at different ambient temperatures (T_a), and thorax surface temperature differences (ΔT_{ts}). **A** Synchronous registration of \dot{V}_{O_2} (steps) and T_{ts} (points) of a truly resting bee. **B, C** same registrations in seemingly resting, but partially actively thermoregulating bees. **D** Correlation of oxygen consumption and related metabolic power necessary to sustain certain ΔT_{ts} values in a single bee. To convert P_m (mW) to $P_{m \text{ rel}}$ (mW g^{-1}) multiply by 10 (100 mg bees)

ambient temperature show the lowest oxygen consumption i.e. metabolic power (Fig. 3A) compared to animals with higher thorax temperatures (Fig. 3B, C).

Oxygen consumption \dot{V}_{O_2} and metabolic power P_m as a function of sustained thorax surface temperature difference $\Delta T_{ts} = T_{ts} - T_a$. Heat loss in bees with occasionally enhanced thorax temperatures ($0^{\circ}\text{C} \leq \Delta T_{ts} \leq 16.5^{\circ}\text{C}$ was calculated using Newton's law of cooling (details in Nachtigall et al. 1989). Only regressions with $R = 0.95 \pm 0$ ($\alpha = 0.01$) were used to illustrate passive cooling. Using values of $3.3 \text{ J g}^{-1} \text{ }^{\circ}\text{C}^{-1}$ and $21.117 \text{ kJ l}^{-1} \text{ O}_2$ for specific heat and caloric equivalent of oxygen, respectively, \dot{V}_{O_2} and P_m were calculated in order to interpret the ΔT_{ts} values measured. A highly significant linear correlation $\dot{V}_{O_2} = 0.229 \Delta T_{ts} + 0.099$ or, since $1 \text{ ml O}_2 \text{ h}^{-1} \hat{=} 5.866 \text{ mW}$ at $RQ = 1.00$, $P_m = 1.343 \Delta T_{ts} + 0.581$ was found (\dot{V}_{O_2} in ml h^{-1} , T in $^{\circ}\text{C}$, P_m in mW) (Fig. 3D). The heat prior to cooling was produced by shivering thermogenesis of the flight muscles; the degree of efficiency of insect flight muscles during flight is not higher than 0.2 (Weis-Fogh 1972). Assuming that the same efficiency holds for shivering movements and that, compared to shivering, other branches of metabolic power are negligible, the total metabolic power used is $1/(1 - 0.2) = 1.25$ times higher.

Metabolic parameters during walking

Definition of walking. Instead of moving forwards a small, lightweight, half black and half white coloured polystyrene ball was turned by the moving legs of the suspended bee. The output of a photoelement, monitoring the light-dark-alternations of the ball, was used as a qualitative indicator of walking activity (Figs. 4, 5).

Thorax surface temperature difference $\Delta T_{ts} = T_{ts} - T_a$ during walking (Fig. 4A, B, Fig. 5A). As soon as walking activity began, ΔT_{ts} rose more or less linearly (Fig. 4A, B) from very small values during resting and reached more or less constant values during prolonged regular 'walking' (Fig. 4B). Mean temperature difference was near 12°C (Fig. 4B).

During a cycle of activity ΔT_{ts} increased characteristically before intensive walking activity took place. In 45% of the experiments (31 walks of duration t_w longer than 4 min; $t_{w \text{ mean}} = 10.81 \pm 5.33$ min; $t_{w \text{ max}} = 27.0$ min) temperature stability was attained at the thorax surface (13 walks; $t_{w \text{ mean}} = 13.15 \pm 5.71$ min; $\Delta T_{ts \text{ mean}} = 11.41 \pm 3.37^{\circ}\text{C}$). If stability was not attained, cooling and heating phases followed each other. In some cases ΔT_{ts}

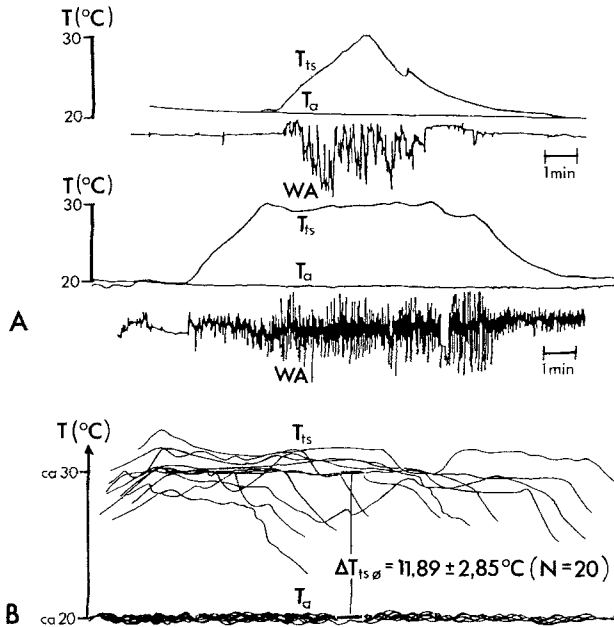


Fig. 4A, B. Thermic parameters during 'walking' (a bee fixed at the scutum turns a polystyrene ball). **A** Two examples of the course of thorax surface temperature T_{ts} and ambient temperature T_a showing shorter and longer walking activity (WA); $T_a = 20^\circ\text{C}$. **B** Registration examples for the course of T_{ts} relative to the normalized course of T_a ($18^\circ\text{C} \leq T_a \leq 23.5^\circ\text{C}$; absolute T_{ts} values unquotable)

slowly decreased during the last minutes of activity. Once walking had ceased, exponential passive cooling to values near T_a took place (Fig. 4A, B). In the temperature range $17^\circ\text{C} \leq T_a \leq 23^\circ\text{C}$, T_{ts} was negatively correlated to T_a ($T_{ts} = 88 \cdot e^{-0.103 T_a}$; T in $^\circ\text{C}$; $R = 0.506 \pm 0$; $\alpha = 0.05$).

Activity and ambient temperature T_a . All of the bees from $n = 54$ walked for more than 4 min over the temperature range $19^\circ\text{C} \leq T_a \leq 21^\circ\text{C}$, 57% from $n = 14$ over the temperature range $12.5^\circ\text{C} \leq T_a \leq 15^\circ\text{C}$ (all animals not walking at these low temperatures walked at $T_a = 21^\circ\text{C}$) but none from $n = 15$ walked at $T_a = 7^\circ\text{C}$.

Respiratory parameters (Fig. 5A, B). Before walking a pronounced increase in oxygen consumption (\dot{V}_{O_2}) was apparent. This was followed after approximately 1.5 min by a corresponding increase in thorax temperature (T_{ts}), and after another 1.5 min by carbon dioxide release (\dot{V}_{CO_2}) (Fig. 5A). With these characteristic delays all curves followed walking activity (Fig. 5B). Rates of gas exchange (\dot{V}_{O_2} and \dot{V}_{CO_2}) were significantly ($R = 0.9 \pm 0$ at $\alpha = 0.001$) correlated both to ΔT_{ts} (Fig. 5A, a, c)

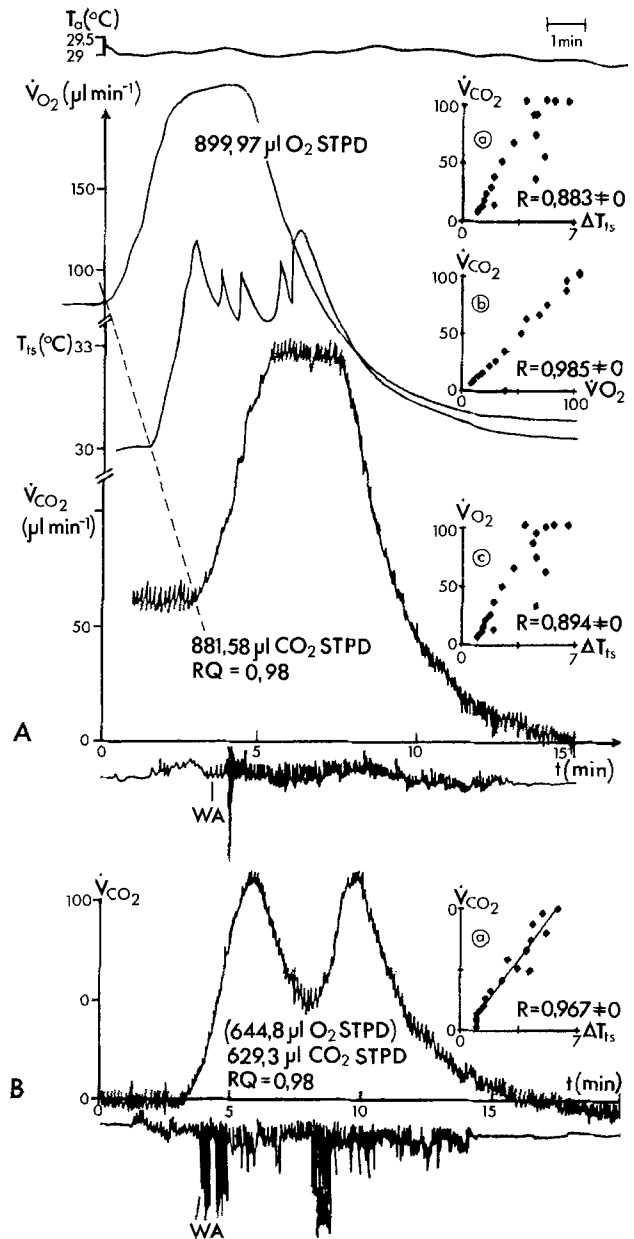


Fig. 5A, B. Respiratory parameters during 'walking' (compare Fig. 4). **A** Example of a continuous phase of walking activity, corrected for instrumental delays. See time shifts in the curves $\dot{V}_{O_2} \rightarrow T_{ts} \rightarrow \dot{V}_{CO_2}$. Inserts: correlation plots. For discussion and for the numbers indicated see text. **B** \dot{V}_{CO_2} plot of an example of a 'biphasic' walking activity. The course of the \dot{V}_{O_2} and T_{ts} curves are similar to the \dot{V}_{CO_2} curve; time shifts similar to **A**. $\Delta T_{ts} = T_t - T_a$ thorax surface temperature difference, R correlation coefficient, WA walking activity, RQ respiratory quotient $\dot{V}_{CO_2}/\dot{V}_{O_2}$, \dot{V}_{O_2} oxygen consumption, \dot{V}_{CO_2} carbon dioxide production

and to each other (b). From $\dot{V}_{CO_2 \text{ total}}$ and $\dot{V}_{O_2 \text{ total}}$ during the activity phases (see examples in Figs. 5A, B), a respiratory quotient of 0.98 was calculated which is not significantly different from 1.0.

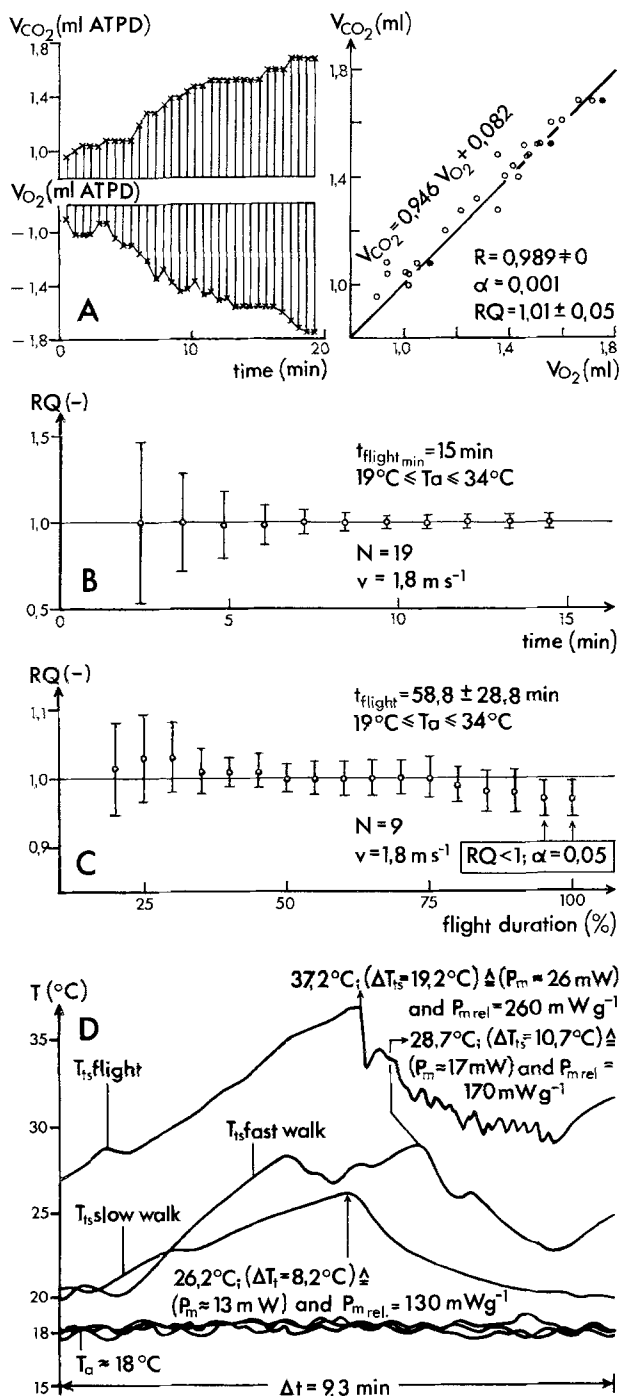


Fig. 6. A Example of \dot{V}_{CO_2} gain and \dot{V}_{O_2} loss in the air volume of the closed miniature wind tunnel (Nachtigall et al. 1989) due to gas exchange of a single, flying bee (left), correlation of \dot{V}_{CO_2} to \dot{V}_{O_2} and RQ and reduction of standard deviations during the first 15 min of wind tunnel flights (right). B Mean values of RQ and reduction of standard deviations during the first 15 min of wind tunnel flights. C Mean values of RQ as a function of standardized flight duration. Only at 95% and 100% flight duration ($RQ = 0.97$) is RQ significantly lower than 1. (B and C from Jungmann 1984). D Example of thorax surface temperature differences ΔT_{ts} and correlated maximum metabolic power and relative metabolic power for three different locomotory activities of the same 100 mg bee (same measuring situation, no change in between)

Metabolic parameters during flying

A typical example for gas exchange measurements during wind tunnel flight is plotted in Fig. 6A. Mean RQ was 1.01 ± 0.05 . Mean values of RQ during the first 15 min of flight are plotted in Fig. 6B, the changes of RQ during long-time flights are given in Fig. 6C, showing values significantly smaller than 1 only during the last 5% of those flights.

The other results of metabolic aspects of flight are discussed in Feller and Nachtigall (1989), Jungmann et al. (1989) and Nachtigall et al. (1989). Relatively small thorax temperature differences of 3 degrees on average were measured; relative power taken from exhaustion flights of tethered wind tunnel bees was near 0.3 W g^{-1} (Nachtigall et al. 1989).

Discussion

Respiratory quotients

Absolute values during resting, walking and flying. Manometric measurements of 'resting' bees at lower temperatures showed a RQ of 1.012 ± 0.200 , and for walking at higher temperatures ($12.5^\circ\text{C} \leq T_a \leq 35^\circ\text{C}$), as well as flying at $15^\circ\text{C} \leq T_a \leq 35^\circ\text{C}$, RQ was not significantly different from 1.0. Except for the first few minutes of flight (Fig. 6B) scatter was small. In no case, except at the very end of long-time flights (Fig. 6C), was it found that after reaching steady-state conditions the bees used fuels other than carbohydrates for energy metabolism. Like many flies (*Phormia regina*) bees prefer to use the carbohydrates in glycogen stores (Beutler 1937; John 1958). This coincides with their high rates of glycolysis (Bailey 1975) and a high activity of glyceraldehydephosphate dehydrogenase (Beenackers 1969), whereas the activities of 3-hydroxyacyl-CoA dehydrogenase and other enzymes characteristic of fat metabolising insects such as locusts (Weis-Fogh 1952) are low. It is not known whether bees normally use fat or fat and carbohydrates as do many Lepidoptera (Zebe 1954). On the other hand, hemolymph amino-acid concentration is so low that oxidation of amino acids (as in bloodsucking flies e.g. *Glossina*) could only account for 0.1% of the flight metabolic power (Barker and Lehner 1972).

Preliminary experiments have shown that, contrary to normally fed bees, hungry specimens did not show measurable quantities of hemolymph carbohydrates (Parhon (1909) and Jongbloed and Wiersma (1934), for example, often found RQ not equal to 1). This means that our experimental ani-

Table 1. Relative metabolic power ($P_{m\ rel}$) of seemingly resting bees compiled from the literature

Author	Method	Relative metabolic power $P_{m\ rel}$ (mW g ⁻¹)	
		20 °C–30 °C	10 °C
Parhon (1909)	Volumetric, swarm	88.04	123.52
Steidle-Zander (1921)	Juvenile bees, swarm	9.20	–
Tauchert (1930)	Individuals, $n=5$	316.67–428.36	–
Farrar (1931)	?	21.02	–
Kosmin et al. (1932)	Manometric, Barcroft-Verzar, individuals (fliers)	27.59–114.32	1.31
Woodworth (1932)	?	2.63	–
Jongbloed and Wiersma (1934)	Volumetric, individuals	11.83	–
Rosow (1946)	?	5.26	–
Hocking (1953)	Manometric, individuals	18.40	–
Cahill and Lustick (1976)	Paramagnetic, groups of 5–10 animals	116.95	252.28
Withers (1981)	Electrochemical, individuals (fliers)	6.57–239.15	–
This paper	Manometric, Warburg, individuals (fliers)	(19.71 ± 21.38) ^a (5.65 ± 2.44) ^b	(1.31 ± 0.53) ^a (1.31 ± 0.53) ^b

Values are usually means but sometimes ranges are given. To convert values into ml O₂ g⁻¹ h⁻¹ multiply by 1/5.866=0.171; to convert into mMol O₂ g⁻¹ h⁻¹ multiply by 1/22.4=0.045.

^a All bees measured; ^b reduced to truly resting bees (compare Fig. 2B)

mals were well fed and in good physiological condition.

Change during flying. Towards the end of all long-time wind tunnel flights RQ decreased slightly (Fig. 6C). A similar decrease was observed in thorax temperature difference (Rothe 1983; Heinrich 1975) and wingbeat frequency (Sotavalta 1954). This may indicate power reduction due to exhaustion of carbohydrate fuel stores and switching to a 'reserve metabolism' (fat?, proteins?) a few minutes before flights cease. Corresponding to this, bees do not move their wings or legs after a long wind tunnel flight in the interval between the cessation of wingbeat and the decreasing of flight parameters to a constant level. Body masses in this phase were at their lowest level. For roundabout flights Sotavalta (1954) writes: "The state of 'apparent exhaustion' cannot be prolonged but leads quickly to the state of complete exhaustion." Therefore, the moment when a bee runs out of fuel in wind tunnel exhaustion flights (Feller 1985; Nachtigall et al. 1989) can be determined with some precision.

Values $RQ \neq 1.0$. The scatter of manometric values between the extremes of 0.35 and 1.95 is certainly correlated to gas exchange mechanisms rather than to metabolic effects. Since resting bees may regulate carbon dioxide loss by periodically opening their stigmas (Miller 1966), discontinuous exchange may take place (Kestler 1978; see Lighton 1988 for a general discussion) (Fig. 1E). It is apparently

more pronounced at lower temperatures and smaller CO₂ output. These conditions are typical for our manometric measurements. Due to the Warburg technology of our experiments, O₂ and CO₂ rates are determined one after the other, and result in a coincidental, symmetrical Gauß-like distribution of smaller and higher RQ values around the mean value of 1.0 (Fig. 1D). This may explain the deviating values found in small spot checks by other workers using this method e.g. Parhon (1909) and Jongbloed and Wiersma (1934). During walking and flying \dot{V}_{CO_2} and \dot{V}_{O_2} were measured at the same time so that no significant deviations from $RQ=1$ occurred.

Resting metabolic power P_m

Comparison of literature data and the problem of defining true resting conditions. In Table 1 relative metabolic power measurements of seemingly resting bees are compiled from the literature and compared to the data presented here. They differ widely, ranging from 2.63 to 428.36 mW g⁻¹ (factor 163) at 20 °C < T_a < 30 °C, and from 1.31 to 252.28 mW g⁻¹ (factor 192) at $T_a=10$ °C. Assuming accurate measuring methods, it would seem that in many cases bees were not really resting but actively thermoregulating.

Tauchert (1930) found exceedingly high values which are difficult to understand and have been omitted. Not enough information is available in Farrar (1931), Woodworth (1932) and Rosow (1946) to judge their methods. The differing results from

the following authors may be explained as follows: Parhon (1909), Steidle-Zander (1921) and Cahill and Lustick (1976) analyzed swarms and groups. Parhon, Cahill and Lustick obtained values 3–4 times higher than the (smallest) values for individual bees found by other authors. This indicates a warming effect in swarms and groups so that body temperature, and therefore metabolic power, is higher than ambient temperature and dependent on the number of individuals (Free and Spencer-Booth 1958; Southwick 1982). Whilst in a single bee thorax temperature approaches ambient temperature, ΔT_{ts} generally being smaller than 2 °C, a bee in a small group tends to have an enhanced temperature (e.g. in a 50-bee group $T_{ts} = 22$ °C at $T_a = 15$ °C), and a bee in a large swarm tends to sustain 35 ± 0.5 °C (Heinrich 1981). Cahill and Lustick's small groups showed $T_{ts} = 30$ °C at $T_a = 10$ °C, not indicating true resting metabolism but additional energy consumption for thermoregulation. The fact that this was not the case in Steidle-Zander's swarm bees may be due to their using juvenile bees not yet adapted to social thermoregulation.

Similarly the higher values of Kosmin et al. (1932) and Withers (1981) are certainly from bees that were partially actively thermoregulating, although sitting apparently motionless at the bottom of the measuring vessel. It has been shown in this paper that locomotor and thermoregulatory activity had stopped (no leg movement; $\Delta T_{ts} = 0$) at ambient temperatures of 10 °C. Our values coincide with those of Kosmin et al. (1932), who were the only other authors to analyze individuals at 10 °C, whereas Parhon's (1909) measurements – if they are correct – show a metabolic power increase of approximately 100-fold due to swarm effects, indicating extremely strong thermoregulatory heat production i.e. comparatively higher at a lower ambient temperature. Recently, Planke and Lensing (1989), who determined daily periodicity of CO₂ output from small groups or single bees living in respiration chambers, found nightly minimum values of approximately 2 ± 0.5 mg CO₂ bee⁻¹ h⁻¹ between 21 h and 4 h. These values were 15 times smaller than the maximum values recorded during day time. Assuming 100 mg bees, a RQ of 1.0, and standard conditions, corresponding values (for 2 mg CO₂ bee⁻¹ h⁻¹) are 20 mg CO₂ g⁻¹ h⁻¹, 10.2 ml CO₂ (O₂) g⁻¹ h⁻¹, 60 mW g⁻¹. This seems extraordinarily high, but unfortunately the temperature, which must have been very high, was not given in this paper.

At higher temperatures (20 °C $\leq T_a \leq 30$ °C) our calculations of 'true' relative metabolic power of 5.65 ± 2.44 mW g⁻¹ coincide with the smallest values mentioned in the literature. This is understandable if one compares our procedures of reduction (see legend to Fig. 2) to the mean value calculation of other authors: establishing true resting conditions is a matter of definition.

Variability at different ambient temperatures T_a . As Fig. 2A shows, variation is less at very low (10–15 °C) and very high (35 °C) temperatures, than at medium temperatures. This is understandable since individual differences in behaviour disappear with hypothermia at very low temperatures, and more homogenous metabolic rates occur at very high temperatures. Flying bees, as well as juvenile and worker bees, behave more 'calmly'

and reduce food intake and energy production at 35 °C compared, for example to 25 °C (Schreiner 1952). However, with the exception of a slight variability at $T_a = 10$ °C, which is assumed to be due to individual physiological differences rather than individual behavioural differences, variability may be due to the fact that at any T_a both truly resting as well as more or less actively thermoregulating animals were observed. It seems safe to assume that truly resting animals are always found in the lower P_m classes (Fig. 2A), and to define resting metabolic rates of truly resting bees at a certain T_a so that these values show the same percentage deviation as the smallest value. This is the case at 10 °C. Thus, any increase at higher T_a should only be due to the temperature dependency of metabolic events (Q_{10} effects), without additional thermogenesis. It is a matter of definition whether one accepts this rather than relating working metabolic rates to resting metabolic rates at certain temperatures (e.g. 'room-temperature'), or whether one takes the values for 'truly motionless bees at $T_a = 10$ °C' as a basis for comparison (see below).

Correlation to ambient temperature T_a . A positive correlation between resting metabolic power $P_{m \text{ rest}}$ and ambient temperature T_a was obtained (Fig. 2B, closed circles). Compared to $T_a = 10$ °C, the $P_{m \text{ rest}}$ values were 3.7 times higher at 20 °C, and 12.2 times higher at 35 °C. Similar values were obtained by Kosmin et al. (1932) from inactive animals (sitting completely motionless); compared to $T_a = 11$ °C the rates were 2.5 times higher at 18 °C, and 12.5 times higher at 35 °C. A positive correlation was also found by Steidle-Zander (1921) between 22.5 and 35 °C. Steidle-Zander's regression curve was somewhat steeper than ours, but showed identical values near $T_a = 25$ °C.

Contrary to these measurements on individual bees, negative correlations were found by Parhon (1909) in swarm bees, by Kosmin et al. (1932) in more or less active bees ('moving across the bottom of the vessel, cleaning themselves, wings folded and motionless'), and by Cahill and Lustick (1976) in bee groups. These findings are easily explained by the (mostly social) thermoregulatory reaction described previously. They coincide with Parhon's (1909) results that P_m of swarm bees is 1.5 times higher at 10 °C than at 20–30 °C.

Correlation to thorax surface temperature difference $\Delta T_{ts} = T_t - T_a$. The regression plot of Fig. 3D was shown to give reliable P_m values if one uses T_{ts} values for resting bees or bees moving slowly, not showing pronounced cooling effects due to forced

circulation. Comparing the P_m values read from the graph to those calculated for direct measurements of oxygen consumption, the following data were obtained. A value of $\dot{V}_{O_2} = 1.6 \text{ ml h}^{-1}$ (corresponding to 9.68 mW) and of $\Delta T_{ts} = 4.5 \text{ }^\circ\text{C}$ were measured simultaneously by Warburg methods. For $\Delta T_{ts} = 4.5 \text{ }^\circ\text{C}$ the regression plot indicates $1.13 \text{ ml O}_2 \text{ h}^{-1}$, corresponding to 6.63 mW. The heat production prior to cooling is due to shivering thermogenesis of the flight muscles, and the degree of efficiency (η) of flight muscles during flight is ≤ 0.2 . Assuming that the same efficiency is valid for shivering movements, P_m should be 1.25 times higher (see results), but it is possible that η is smaller during shivering than during flight, so that P_m calculated from the equation $P_m = 1.343 \Delta T_{ts} + 0.581$ is close to reality. Accepting $\eta = 0.2$ for a model calculation, metabolic power increases from 6.63 to 8.29 mW for a single bee. The remaining difference of 1.39 mW coincides with the resting value found at the corresponding T_a . The same holds for comparisons of direct gas analytical measurements. In Cahill and Lustick's (1976) bee groups with a mean $P_{m \text{ rel}}$ of 250 mW g^{-1} , T_{ts} was $30 \text{ }^\circ\text{C}$ at $T_a = 10 \text{ }^\circ\text{C}$ ($\Delta T_{ts} = 20 \text{ }^\circ\text{C}$). Calculation from the regression plot gives $4.679 \text{ (ml O}_2 \text{ h}^{-1}) \cdot 5.688 \text{ (mW (ml O}_2 \text{ h}^{-1})^{-1}) \cdot (1000/100) \cdot \text{g}^{-1} = 275 \text{ mW g}^{-1}$ (for a caloric equivalent of $21.117 \text{ J ml O}_2^{-1}$ and for 100 mg bees). This is equivalent to 343 mW g^{-1} when $\eta = 0.2$. The somewhat higher value may be due to the fact that the regression plot is valid for single bees, whereas Cahill and Lustick (1976) used small groups of ten bees (of another race). Thus the relatively smaller heat loss observed might have sustained ΔT_{ts} with less heat production.

Metabolic power and rate of increase in walking and flying

Walking. The shift in \dot{V}_{O_2} , T_{ts} and \dot{V}_{CO_2} which increased after walking began (Fig. 5A), reflects the expected course of the metabolic processes. Equilibrium between heat production and heat loss in resting air occurred at about $12 \text{ }^\circ\text{C}$. This value was more or less constant over 80–90% of the walking period, even at slightly different ambient temperatures. At medium temperatures T_{ts} was negatively correlated to T_a . The thorax temperature difference decreased only towards the end of a walking period. This indicates that a bee is able to regulate heat production and body temperature during walking, which is not surprising if one takes into account its enormous ability to regulate social temperature (Lindauer 1954; Wohlgemuth 1957),

and to discriminate temperature differences as small as $0.25 \text{ }^\circ\text{C}$ (Heran and Wanke 1952).

Resting metabolic power values reported in this paper were 19.7 mW g^{-1} ($20 \text{ }^\circ\text{C} \leq T_a \leq 30 \text{ }^\circ\text{C}$; at rest, but with actively thermoregulating bees included), 5.7 mW g^{-1} ($20 \text{ }^\circ\text{C} \leq T_a \leq 30 \text{ }^\circ\text{C}$; \pm truly resting bees), and 1.3 mW g^{-1} ($T_a = 10 \text{ }^\circ\text{C}$; truly resting bees; Table 1). The metabolic power necessary to sustain $\Delta T_{ts} = 12 \text{ }^\circ\text{C}$ is 16.7 mW for a single bee, or 167 mW g^{-1} , calculated for 100 mg bees. Compared to the rates just mentioned, this means an 8.4, 30 and 127-fold increase during walking. Only the first factor corresponds to the values reported for running cockroaches (factor 5; Hereid et al. 1981) and running ants (*Formica* sp. workers; factor 7; Jensen et al. 1980). We therefore assume that the resting rates mentioned by these authors were not determined under true resting conditions, but under the conditions 'seemingly resting, but actively thermoregulating'.

Flying. Thorax temperature differences during flights in the wind tunnel and in the round-about were high immediately after starting, but decreased afterwards to an average of approximately $3 \text{ }^\circ\text{C}$ (extreme values $0.2 \text{ }^\circ\text{C}$ and $5.2 \text{ }^\circ\text{C}$); ΔT_{ts} was not correlated to T_a . The same was found in bees flying 'freely' at the end of a wire (Esch 1960), and again under natural environmental conditions. The comparatively low ΔT_{ts} values are easily explained by highly effective convective cooling due to the flow produced by the beating wings. Another reason for these relatively low values may be that suspended bees usually do not attain full lift and thrust (Esch 1976). Heinrich (1979) found much higher ΔT_{ts} values (up to $15 \text{ }^\circ\text{C}$ at $15 \text{ }^\circ\text{C} \leq T_a \leq 25 \text{ }^\circ\text{C}$) for freely flying bees. In our experiments ΔT_{ts} was constant after 2 min in round-about flights of 0.7 m s^{-1} . At $T_a = 30 \text{ }^\circ\text{C}$, ΔT_{ts} was usually constant over 80 to 90% of the flight time, but often decreased towards the end of a flight, corresponding to a reduction in wing beat frequency (Brandes 1984). Thus, temperature is also regulated during flight. In our round-about experiments, mean ΔT_{ts} was $3.01 \pm 0.77 \text{ }^\circ\text{C}$ ($n = 36$). The P_m value necessary to sustain this temperature difference is 46.2 mW g^{-1} ; to sustain $\Delta T_{ts} = 15 \text{ }^\circ\text{C}$ (Heinrich 1979) it is 207 mW g^{-1} . Our lowest value for flights analyzed by the exhaustion flight method (Feller and Nachtigall 1989) was 240 mW g^{-1} at $T_a = 20 \text{ }^\circ\text{C}$. This indicates that ΔT_{ts} values obtained under forced convective conditions in suspended animals cannot be used in the equation indicated in Fig. 3D. Compared to 240 mW g^{-1} , the factor of increase during 'resting' at

$20\text{ }^{\circ}\text{C} \leq T_a \leq 30\text{ }^{\circ}\text{C}$ was 12; after excluding thermo-regulatory bees it was 43; during 'true resting' at $T_a = 10\text{ }^{\circ}\text{C}$ it was 185. Ludwig (1962) mentioned factors of increase of as high as 100 in insects rotating on a bent needle in a Warburg chamber.

Bartholomew and Casey (1978) determined flight metabolic power and resting metabolic power (mW) as a function of body mass (g) in hawk moths (Lepidoptera) hovering at $22\text{ }^{\circ}\text{C} \leq T_a \leq 24\text{ }^{\circ}\text{C}$. They obtained $P_{m \text{ hovering flight}} = 344.7 \cdot M_b^{0.82}$ and $P_{m \text{ rest}} = 2.3 \cdot M_b^{0.82}$. Since the exponents were similar, the 150-fold increase is independent of body mass. Hovering flight, however, is the most energy consuming flight situation. Ellington (1984) calculated $P_{m \text{ hovering flight}} = 832\text{ mW g}^{-1}$ for honey bees. This value is 3.5 times higher than our exhaustion flight measurements for tethered flight in the laboratory. Using this factor, and applying it to the data of Bartholomew and Casey (1978), 100 mg hawk moths (same body mass as honey bees) showing a relative $P_{m \text{ rest}}$ of 2.7 mW g^{-1} , should have used $P_m = 151.7\text{ mW g}^{-1}$ during forward flight (factor of increase 56). Relative $P_{m \text{ rest}}$ is half as high as our values for bees over the same temperature range ($\geq 20\text{ }^{\circ}\text{C}$), and twice as high as our values at $10\text{ }^{\circ}\text{C}$. This again shows that the reference values depend greatly on the ambient conditions.

Conditions for measuring resting metabolic rate in humans are exactly defined with regard to time after food intake, body posture and favoured temperature, whereas in poikilothermic invertebrates they are not. Therefore, contrary to humans, the value of calculating the factors of increase and of comparing them to those of other invertebrates or vertebrates is limited.

References

- Bailey E (1975) Biochemistry of insect flight. Part 2: Fuel supply. In: Candy DJ, Kilby BA (eds) Insect biochemistry and function. Chapman and Hall, London
- Barker RJ, Lehner Y (1972) Free amino acids in thoraces of flown honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). *Comp Biochem Physiol* 43B:163–169
- Bartholomew GA, Casey TM (1978) Oxygen consumption of moths during rest, pre-flight warm up, and flight in relation to body size and wing morphology. *J Exp Biol* 76:11–25
- Beenackers AMT (1969) Carbohydrate and fat as a fuel for insect flight. A comparative study. *J Insect Physiol* 15:353–361
- Beutler R (1937) Über den Blutzucker der Bienen. *Z Vergl Physiol* 24:71–115
- Brandes C (1984) Tanztempo, Zuckerverbrauch, Lauf-, Flugeschwindigkeit und Flügelschlagfrequenz von *Apis mellifera* bei subletaler Parathionvergiftung. *Zool Jb Physiol* 88:345–359
- Cahill K, Lustick S (1976) Oxygen consumption and thermoregulation in *Apis mellifera* workers and drones. *Comp Biochem Physiol* 55A:355–357
- Ellington CP (1984) The aerodynamics of hovering insect flight. *Phil Trans Roy Soc London B* 306:1–181
- Esch H (1960) Über die Körpertemperaturen und den Wärmehaushalt von *Apis mellifera*. *Z Vergl Physiol* 43:305–335
- Esch H (1976) Body temperature and flight performance of honey bees in a servomechanically controlled wind tunnel. *J Comp Physiol* 109:265–277
- Farrar MD (1931) The metabolism of the adult honeybee. *J Ecol Entomol* 24:611
- Feller P (1985) Windkanalflug von Honigbienen (*Apis mellifera carnica*): Untersuchungen zu Treibstoffverbrauch, Flugphysiologie und Thermoregulation. Diplomarbeit, Universität des Saarlandes, unpublished
- Feller P, Nachtigall W (1989) Flight of the honey bee. II. Inner- and surface thorax temperatures and energetic criteria, correlated to flight parameters. *J Comp Physiol B* 158:719–727
- Free JB, Spencer-Booth Y (1958) Observations on the temperature regulation and food consumption of honeybees (*Apis mellifera*). *J Exp Biol* 35:930–937
- Heinrich B (1975) Thermoregulation in bumblebees. II. Energetics of warm-up and free flight. *J Comp Physiol* 96:155–166
- Heinrich B (1979) Keeping a cool head: honeybee thermoregulation. *Science* 205:1269–1271
- Heinrich B (1981) The mechanisms and energetics of honeybee swarm temperature regulation. *J Exp Biol* 91:25–55
- Heran H, Wanke L (1952) Beobachtungen über die Entfernungsmeldung der Sammelbienen. *Z Vergl Physiol* 34:383–393
- Hereid CF II, Prawel DA, Full RJ (1981) Energetics of running cockroaches. *Science* 212:331–333
- Hocking B (1953) The intrinsic range and speed of flight of insects. *Trans R Entomol Soc Lond* 104:223–345
- Jensen TF, Holm-Jensen I (1980) Energetic cost of running in workers of three ant species. *Formica fusca* L., *Formica rufa* and *Camponotus herculeanus* L. (Hymenoptera, Formicidae). *J Comp Physiol* 137:151–156
- John M (1958) Über den Gesamtkohlenhydrat- und Glykogenhaushalt der Bienen (*Apis mellifera*). *Z Vergl Physiol* 41:204–220
- Jongbloed J, Wiersma CAG (1934) Der Stoffwechsel der Honigbiene während des Fliegens. *Z Vergl Physiol* 21:519–533
- Jungmann R (1984) Stoffwechselphysiologische Untersuchungen an in einem Miniaturwindkanal fliegenden Honigbienen (*Apis mellifera*). Diplomarbeit, Universität des Saarlandes, unpublished
- Jungmann R, Rothe U, Nachtigall W (1989) Flight of the honey bee. I. Thorax surface temperature and thermoregulation during tethered flight. *J Comp Physiol B* 158:711–718
- Kestler P (1978) Atembewegungen und Gasaustausch bei der Ruheatmung adulter terrestrischer Insekten. *Verh Dtsch Zool Ges* 1978:268
- Kosmin NP, Alpatow WW, Resnitschenko MS (1932) Zur Kenntnis des Gaswechsels und des Energieverbrauchs bei der Biene in Beziehung zu deren Aktivität. *Z Vergl Physiol* 17:408–422
- Lighton JRB (1988) Discontinuous CO₂ emission in a small insect, the formicine ant *Camponotus vicinus*. *J Exp Biol* 134:363–376
- Lindauer M (1954) Temperaturregulation und Wasserhaushalt im Bienenstaat. *Z Vergl Physiol* 36:391–432
- Ludwig HW (1962) Der Sauerstoffkonsum und RQ fliegender Insekten. *Verh Deutsch Zoolog Ges Wien* 1962:355–359
- Miller PL (1966) The regulation of breathing in insects. *Adv Insect Physiol* 3:279–354

- Nachtigall W, Rothe U, Feller P, Jungmann R (1989) Flight of the honey bee III. Flight metabolic power, calculated from gas analysis, thermoregulation and fuel consumption. *J Comp Physiol B* 158:729–737
- Parhon M (1909) Les échanges nutritifs chez les abeilles pendant les quatre saisons. *Ann Soc Nat Zool* 9:1–50
- Planke M, Lensing W (in press) Metabolic measurement of honey bee. *J Apid Res*
- Rosow SA (1946) Food consumption by bees. *Beekeeping* 23:8
- Rothe U (1983) Stoffwechselphysiologische Untersuchungen an ruhenden, laufenden und fliegenden Honigbienen (*Apis mellifica carnica*). Dissertation Universität des Saarlandes, unpublished
- Rothe U, Nachtigall W (1980) Zur Haltung von *Apis mellifica* in einem Flugraum. *Apidologie* 11:17–24
- Rothe U, Nachtigall W (1982) Erfahrungen mit einem Bienenflugraum. Spontane Königinnenaufzucht und Lebensdaueranalysen. *Apidologie* 13:241–246
- Schreiner T (1952) Über den Nahrungstransport im Darm der Honigbiene. *Z Vergl Physiol* 34:278–298
- Sotavalta O (1954) On the fuel consumption of the honeybee (*Apis mellifica* L.) in flight experiments. *Ann Zool Soc "Vanamo"* 16:1–27
- Southwick EE (1982) Metabolic energy in intact honey bee colonies. *Comp Biochem Physiol* 71A:277–281
- Steidle-Zander E (1921) Das Leben der Bienen. Stuttgart
- Tauchert F (1930) Weitere Stoffwechseluntersuchungen an Insekten. *Z Biol* 89:541–546
- Weis-Fogh T (1952) Fat combustion and metabolic rate in flying locusts. *Phil Trans R Soc Lond* 237:1–36
- Weis-Fogh T (1972) Energetics of hovering flight in hummingbirds and in *Drosophila*. *J Exp Biol* 56:79–104
- Withers PC (1981) The effect of ambient air pressure on oxygen consumption of resting and hovering honeybees. *J Comp Physiol* 141:433–437
- Wohlgemuth R (1957) Die Temperaturregulation des Bienenvolkes unter regeltheoretischen Gesichtspunkten. *Z Vergl Physiol* 40:119–161
- Woodworth CE (1932) Some effects of reduced atmospheric pressure at honeybee respiration. *J Ecol Entomol* 25:1036
- Zebe E (1954) Über den Stoffwechsel der Lepidopteren. *Z Vergl Physiol* 36:290–317