The Journal of Experimental Biology 204, 805–814 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB2927

MITE NOT MAKE IT HOME: TRACHEAL MITES REDUCE THE SAFETY MARGIN FOR OXYGEN DELIVERY OF FLYING HONEYBEES

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Accepted 14 November 2000; published on WWW 1 February 2001

Summary

Many physiological systems appear to have safety margins, with excess capacity relative to normal functional needs, but the significance of such excess capacity remains controversial. In this study, we investigate the effects of parasitic tracheal mites (*Acarapis woodi*) on the safety margin for oxygen delivery and flight performance of honeybees. Tracheal mites did not affect the flight metabolic rate of honeybees in normoxic (21 % oxygen) or hyperoxic (40 % oxygen) air, but did reduce their metabolic rate relative to uninfected bees when flying in hypoxic air (5 or 10 % oxygen), demonstrating that mites reduced the safety margin for tracheal oxygen delivery. The negative effects of mites on flight metabolic rate in hypoxic atmospheres were graded with the number of mites per trachea. For example, in 10 % oxygen atmospheres, flight metabolic rate was reduced by 20 % by moderate mite infection and by 40 % by severe mite infection. Thus, the safety margin for oxygen delivery in honeybees allows them to retain normal flight metabolic rate and behavior despite tracheal mite infection under most conditions. However, the reduction in tracheal gas-exchange capacity may constrain activities requiring the highest metabolic rates, such as flying in cool weather. In support of this hypothesis, bees that were unable to return to the hive during late-winter flights showed significantly higher levels of mite infection than bees that returned safely.

Key words: honeybee, *Apis mellifera*, tracheal mite, *Acarapis woodi*, oxygen delivery, safety margin, symmorphosis, metabolic rate, temperature.

Introduction

The extent to which natural selection has resulted in a matching between the capacities of physiological systems and their functional needs (symmorphosis) is a central and controversial topic in comparative physiology (Lindstedt and Jones, 1987; Weibel et al., 1991; Weibel et al., 1998). Comparative studies of variation in maximal system performance and tissue morphology and physiology have tested for evidence of such structure–function matching using a variety of systems including mammalian and invertebrate skeletal structures (Alexander, 1981; Alexander, 1998; Biewener, 1990), vertebrate oxygen delivery systems (Weibel et al., 1991; Maina, 1998) and mammalian fuel delivery systems (Weibel et al., 1996). In some cases, these studies have found changes in system structural components that are proportional (within an appropriate mathematical model) to changes in maximal organismal functional needs. These results support the symmorphosis hypothesis that the effects of natural selection tend to alter quantitive aspects of physiological structures so that functional needs are met without excessive biosynthetic, maintenance or spatial costs (Weibel et al., 1991; Weibel et al., 1996). However, such studies have been criticized for failing to control for phylogenetic effects and for failing to differentiate quantitative matching from constant excess capacity. Furthermore, evolutionary biologists tend to regard such optimality models of animal function as tools for understanding organismal function rather than likely results of natural selection (Garland and Huey, 1987; Dudley and Gans, 1991).

In some studies, the changes in the functional capacity of tissues or structures do not seem to match closely variation in either organismal maximal needs or the changes in the functional capacity of other elements of a physiological series (Gehr et al., 1981; Karas et al., 1987; Alexander, 1998). The apparent lack of concordance between structure and function found in such studies may represent evidence against optimal design (symmorphosis) and/or may reflect the need for physiological structures to perform multiple functions in the context of an unpredictable environment, with imperfectly predictable developmental systems, using components that may vary in their costs (Garland and Huey, 1987; Dudley and Gans, 1991; Alexander, 1998; Weibel et al., 1991; Weibel et al., 1998). In summary, the appealing concept of symmorphosis remains controversial and difficult to test.

An important component of the theoretical arguments surrounding the principles of animal design in general, and symmorphosis in particular, concerns the observation that many physiological systems that have been examined exhibit a safety margin, in which system capacities exceed apparent functional needs. Safety margins may be evidence against symmorphosis (Garland and Huey, 1987; Dudley and Gans, 1991), but alternative explanations such as historical selective pressures to survive and function under unpredictable stressful conditions such as accidents or disease have been suggested (Alexander, 1981; Biewener, 1990; Diamond, 1993; Diamond, 1998). Evolutionary biologists have found increasing evidence for disease as a selective agent (Williams and Neese, 1991; Schmid-Hempel, 1998). One important problem in the larger question of resolving the role of natural selection in animal design will be to examine the effects of naturally occurring diseases on safety margins for physiological function.

Although the safety margin for oxygen delivery is very high for resting insects, it can be nonexistent during insect flight (Harrison and Lighton, 1998). However, insects such as the honeybee *Apis mellifera* appear to have considerable safety margins for oxygen delivery even during flight because flight metabolic rates (MR, $W\,g^{-1}$) remain constant in atmospheres ranging between 40 % and 10 % oxygen (Joos et al., 1997). In the present study, we test the hypothesis that the safety margin for oxygen delivery in honeybees can function to ameliorate the pathogenic effects of the tracheal mite *Acarapis woodi* (Rennie). We examine the effect of this tracheal mite on honeybee flight metabolic rate, thoracic temperature (T_{th}, °C), and natural flight ability under stressful winter conditions. We interpret these results to determine (i) whether the safety margin for oxygen delivery is reduced by tracheal mite infection, (ii) whether the possession of a safety margin in tracheal oxygen delivery helps conserve the function of infected bees, and (iii) whether the reduction in tracheal oxygen delivery capacity is a primary mechanism of pathology for these mites.

Biology of the honeybee tracheal mite

The tracheal mite has been found in honeybee colonies throughout Europe for many years, but has only recently been introduced into North America, where it has had a major economic impact (Delfinado-Baker, 1988; Finley et al., 1996; Fore, 1996). The mechanisms by which tracheal mites cause mortality in honeybees are unknown. *Acarapis woodi* mites infect honeybees primarily during the first days of adult life, entering the trachea *via* the first thoracic spiracle (Bailey and Ball, 1991). The mites reproduce within the tracheae, generally increasing in number as bees age (Pettis and Wilson, 1996). By most reports, tracheal mites have little effect on honeybee mortality, except in winter (Bailey, 1958; Eischen, 1987; Maki et al., 1988; Gary and Page, 1989). The winter dependence of tracheal mite effects on honeybees may be due to increases in mite infection levels associated with greater bee age in winter or may be due to specific stressful winter conditions (Bailey, 1958; Bailey and Ball, 1991). Mites accumulate in the major tracheae of the thorax (Delfinado-Baker, 1988), suggesting that they may interfere with the gas-exchange needs of the flight muscles. Tracheal mites may deprive honeybees of nutrients as they feed on hemolymph through the tracheal walls. Tracheal mites may also cause morbidity indirectly because of viral transmission or because of stress effects associated with the immune response of the honeybees to the infection (Shimanuki et al., 1995; Hung et al., 1995).

Testing for a reduction in oxygen transport capacity by tracheal mites

Prior studies have documented that tracheal-mite-infected honeybees are flight-capable and carry pollen and nectar loads similar to uninfected bees (Gary and Page, 1989; Downey et al., 2000). We hypothesized that mite infection causes a partial blockage of the tracheae, reducing tracheal oxygen delivery capacity, and that the safety margin for oxygen delivery allows the conservation of flight ability despite infection. Honeybees ventilate during flight by abdominal pumping, at times inspiring and expiring through the thoracic spiracles (Bailey, 1954). It seems most likely that mites might interfere with gas exchange by increasing the resistance to convective flow through the thoracic trunks. We tested indirectly for such an effect by examining the consequences of tracheal mite infection on the safety margin for flight metabolic rate. Specifically, we examined the interactive influences of atmospheric oxygen level (5, 10, 21 and 40 % oxygen) and mite infestation level on flight metabolic rate and T_{th}. We predicted that, if mites are partially blocking the tracheae to the flight muscle, increasing mite infection should be correlated with decreasing flight metabolic rate and T_{th} under hypoxic conditions that challenge the oxygen-delivery capacity of the tracheal system. Conceivably, tracheal blockage by mites could be sufficient to affect flight metabolic rate and T_{th} in normoxic air. If so, we would predict that flight metabolic rate and T_{th} should be elevated by hyperoxia in tracheal-mite-infested honeybees. Alternatively, tracheal mites could have an insignificant effect on tracheal oxygen delivery capacity, and flight metabolic rate and T_{th} could be depressed in mite-infected bees regardless of atmospheric oxygen levels. Such a result would be evidence against the idea that the safety margin in tracheal oxygen delivery in honeybees has functional consequences during this disease state, and would refute tracheal blockage as an important pathology of these mites.

Since honeybee flight metabolic rate can vary inversely with air temperature (T_{air}; Harrison et al., 1996; Roberts and Harrison, 1999), and a minimal T_{th} is required for flight (Coehlo, 1991), reductions in maximal tracheal oxygen delivery capacity may be more likely to affect flight performance at cooler T_{air}. We tested for this possibility by comparing the effects of mites on flight metabolic rate and T_{th} under normoxic conditions at 16 °C and 24 °C, predicting that mite infestation should more strongly affect flight metabolic rate at cooler air temperatures. In addition, if a primary

mechanism of mite-mediated mortality relates to tracheal blockage, then we would predict that tracheal-mite-infected bees would be more likely to die during flights outside the hive in cool weather when high flight metabolic rates are required to thermoregulate. To test this prediction, we compared the mite infestation levels of honeybees that were successful or unsuccessful in returning to the hive on cool winter days.

Materials and methods

Effects of mite infestation on tracheal oxygen delivery capacity

We measured the flight metabolic rate of honeybees during mid-winter (January, 1998) at State College, Pennsylvania, USA. We used a colony containing both uninfected and infected adult bees, thereby allowing us to examine the effects of mite infection within a group of related individuals. The colony was placed on the roof of the laboratory building, within a few minutes walk of the room where respirometric measurements were made. At intervals, the colony was opened, and 1–5 bees were captured and transferred to the laboratory for respirometry. All bees were assayed for their metabolic rate during agitated flight (Harrison and Hall, 1993) within 30 min of capture; preliminary studies indicated no correlation between time since capture and flight metabolic rate over this time period.

The initial study of the interaction between atmospheric oxygen level (5 %, 10 %, 21 % or 40 % oxygen) and mite infestation level was conducted over 2 days. To control for possible circadian or sequential effects (for example, less healthy bees might have been easier to catch and therefore tended to be caught earlier in the study), we measured the flight metabolic rate of approximately 15 bees in a particular oxygen atmosphere, and then switched to a new test atmosphere. We measured the flight metabolic rate of bees in 21 % oxygen atmospheres in four series of 15–20 bees at intervals from the beginning of the first day to the end of the second day. For the 5, 10 and 40 % oxygen tests, bees were measured in 2–3 series of approximately 15 bees, at different times of the day. All flights were conducted at 24±1 °C.

Following each respirometric assay, we measured the T_{th} of the bee within 5 s of removal from the chamber using a Physitemp MT 29/1B microprobe thermocouple thermometer connected to a Physitemp Bat-12 thermometer (Roberts and Harrison, 1999). Bees were then weighed (±0.1 mg) and dissected to determine the level of mite infection. We recorded the number of eggs, larvae and adults within each of the tracheal trunks arising from the prothoracic spiracle.

Effects of mite infestation on flight metabolic rate at cool temperatures

Since prior studies have found that flight metabolic rate increased with air temperature down to air temperatures of approximately 20 °C (Harrison et al., 1996), and we suspected that winter bees might be capable of generating a high metabolic rate at even lower air temperatures because of acclimation effects, we examined the effect of mite infestation level on flight metabolic rate at 16±1 °C in 21 % oxygen atmospheres. These studies were conducted on a separate day, with the entire respirometry system moved into a temperature-controlled room. After respirometry, T_{th} and mite infestation levels were measured as described above.

Mite infection and flight ability under natural cold conditions

Honeybees occasionally fly on sunny days in winter (primarily brief flights to defecate), and in doing so they may encounter low temperatures and wind. At these times, it is not uncommon to find bees crawling on the ground, unable to return to the hive. These bees are subsequently killed by night-time freezing temperatures. To test the hypothesis that tracheal mite infection impairs flying during cool weather, we observed the entrance and snow-covered surrounding area of two colonies (one on 10 February 1998 and the other on 23 January 1999). The T_{air} was cool (7–12 °C) on both days, but it was sunny and many bees were leaving the colony for defecation flights. We collected two groups of bees from each colony: (i) bees that were flying or had just returned successfully to the hive entrance, and (ii) bees that flew from the hive, but failed to return and were found on the ground, alive but unable to fly. These bees were subsequently dissected and total mite infestation determined as described above.

Respirometric assays

Worker honeybees fly vigorously and sometimes aggressively in response to physical agitation, both within and outside respirometry chambers, providing the behavioral basis for our ‘agitated flight’ respirometric assay. The agitated flight protocols we used have been criticized on the basis that the flight durations may be too short to achieve steady-state conditions, that the small container size used to achieve high temporal sensitivity may affect flight behavior and that the flight behavior of individuals is quite variable (Heinrich and Esch, 1997; Stevenson and Woods, 1997). However, at least in summer-caught honeybees, flying animals achieve steady-state body segment temperatures within 30 s (Roberts and Harrison, 1999). Since we discarded data from the first 30 s of our respirometry assay, steady-state conditions should have applied in this study. Comparisons of flight metabolic rates for honeybees in relatively large containers with those for the 0.3 l chambers used in this study have found no consistent differences (Harrison et al., 1996; Joos et al., 1997; Roberts and Harrison, 1999). These findings suggest that the agitated flight protocol is useful for metabolic comparisons when large number of bees must be examined and consistency of behavioral performance is not required or desired. In this case, since we were specifically interested in the effects of tracheal mites on bees, and mite infestation potentially affects general bee motivation and tendency to fly, it was important to assay all bees regardless of the quality of their flight behavior in the respirometer. We categorized the flight behavior of each bee as excellent (continuous flight throughout the assay), good

Table 1. *Mite life stages and pattern of infestation for the various treatment groups*

Treatment	*N*	Side 1			Side 2		
		Eggs	Larvae	Adults	Eggs	Larvae	Adults
5 % oxygen	50	1.5±0.42	1.5±0.41	6.9±1.12	0.9±0.36	1.3±0.44	5.3±1.0
10 % oxygen	55	1.9±0.34	1.4±0.34	5.3±0.65	1.3±0.29	0.5±0.16	3.2±0.66
21 % oxygen	87	1.3±0.18	1.6±0.34	5.4±0.57	0.5±0.12	0.7±0.19	2.8±0.49
40 % oxygen	30	1.5±0.34	0.7±0.28	4.1±0.99	0.9±0.27	0.5±0.29	3.4±0.87

Values are means ± S.E.M.

If a bee was unilaterally infected, the infected side was designated side 1; if the bee was bilaterally infected, the side with more mites was designated side 1.

(flying the majority of the time) or poor (flying less than 50 % of the time).

Gas flows from nitrogen and oxygen cylinders were adjusted to obtain oxygen mixtures ranging from 5 to 40 % at a flow rate of approximately 2±0.01 l min^{-1} (monitored with an 820 mass flow-meter, Sierra Instruments; Gilbert, Arizona, USA), calibrated with a soapfilm bubble-meter. Incurrent gas mixtures passed through a 0.3 l Plexiglas respirometry chamber and then to a LiCor (Lincoln, Nebraska, USA) model 6252 carbon dioxide analyzer. Voltage output from the carbon dioxide analyzer was digitized (Sable Systems, Henderson, Nevada, USA) and recorded on a computer. Bees were placed individually into the chamber and continuously agitated to fly for 1.5 min; CO_2 emission rates were averaged over the final minute of this flight period. To convert CO_2 emission rates to metabolic rates, we assumed a respiratory quotient of 1.0 (Rothe and Nachtigall, 1989) and an energy conversion factor of 21.31 J ml^{-1} CO_2.

Statistical analyses

Statistical analyses were performed with SYSTAT (Wilkinson, 1989). Statistical significance was chosen at $P<0.05$. The ranked behavioral data were analyzed by Kruskal–Wallis or Mann–Whitney *U*-test (oxygen effects) or Spearman's correlation test (total mite effect). The effects of atmospheric oxygen level and mite infection level on metabolic rate and T_{th} were analyzed by multiple linear regression, followed by a test for the effect of total mite number on the dependent variable within each oxygen atmosphere using linear regression analysis. Values are presented as means ± S.E.M.

Results

Tracheal mite infection levels

The infected honeybees contained eggs, larvae and adult *A. woodii* (Table 1). The *A. woodi* eggs, larvae and adults are similarly sized (Fig. 1) and are therefore expected to cause similar tracheal blockage. Since 18 of 26 possible correlations between mite stage infection levels were significant, and total mite number was significantly correlated with the numbers of each mite life stage (Pearson correlation coefficients all greater than 0.59), for subsequent statistical analysis, we condensed these multiple variables to a single index of infection, the total number of mites per individual.

The total number of mites (all life stages pooled) found within honeybee tracheae varied considerably, from 0 to 70. At each atmospheric oxygen level, there were 8–10 bees without any mites, and there was no significant difference in

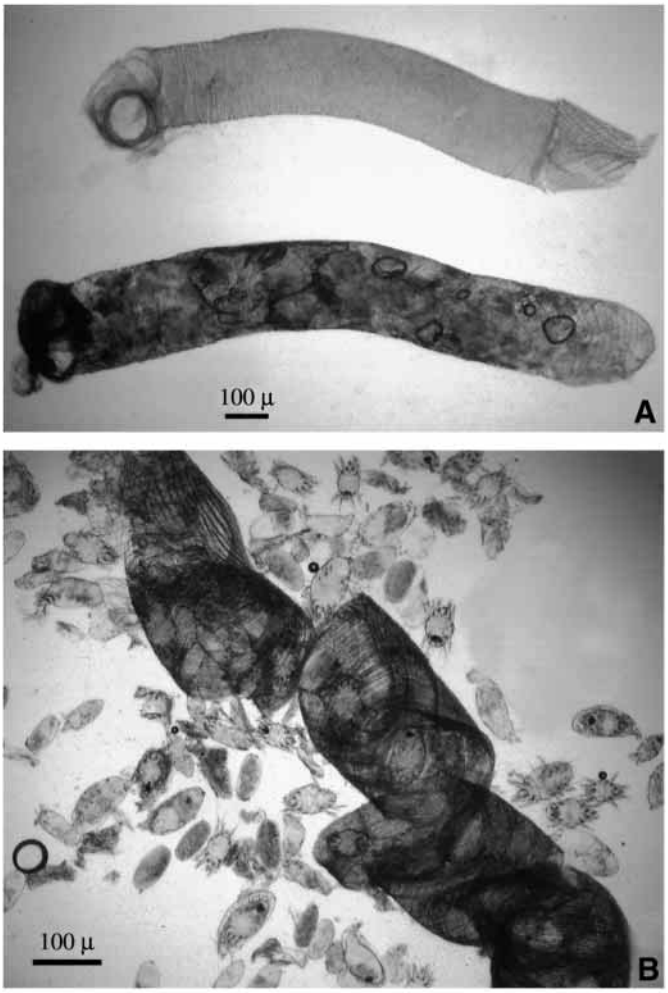

Fig. 1. Light micrographs of the prothoracic tracheal trunks of a honeybee showing *Acarapis woodi* mites. (A) Non-infested (top) and infested (bottom) tracheal trunks. (B) The infested trunk from A opened to show adults (eight legs), larvae (note feeding appendages) and eggs (simple ovals). Scale bars, 100 µm.

Table 2. *Flight behavior of bees in the respirometric assay under the various conditions*

	Flight behaviour			Correlation with total mite number (Spearman's)
	Excellent	Good	Poor	
24 °C, 40 % oxygen	4	22	4	0.258
24 °C, 21 % oxygen	16	22	15	–0.173
24 °C, 10 % oxygen	10	26	18	–0.336*
24 °C, 5 % oxygen	3	8	39	–0.232
16 °C, 21 % oxygen	3	8	39	–0.030

Numbers in each column indicate the number of bees that received each rating within that treatment group.

An asterisk indicates a significant correlation (P<0.05).

the infection level of bees tested at the different atmospheric oxygen levels (ANOVA, P=0.25). At high infection levels, mites can accumulate to considerable densities within the prothoracic tracheal trunks (Fig. 1). Of the mite-infected bees, approximately half were unilaterally infected with tracheal mites and half were bilaterally infected, with bilateral infection being correlated with higher total mite infestation (Pearson's r=0.65, P<0.001).

Effects of oxygen, temperature and tracheal mite infection on flight behavior

The quality of flight behavior, according to our three-tiered rank categorization, was significantly affected by oxygen level (Table 2; Kruskal–Wallis statistic 9.78, P=0.007). However, only the flight behaviors in the 5 % oxygen atmospheres differed significantly from those in the 21 % oxygen atmospheres at 24 °C (Mann–Whitney U-tests). Total mite number was negatively correlated with flight behavior in the 10 % oxygen atmospheres, but not in any of the other atmospheres (Table 2). Flight behavior in 21 % oxygen atmospheres was poorer at 16 °C than at 24 °C (Table 2; Mann–Whitney U-test).

Effects of tracheal mite infection on flight metabolic rate and gas-exchange capacity

There were no significant effects of being unilaterally compared with bilaterally infected in multiple regression analyses with flight metabolic rate as the dependent variable and total mite numbers or atmospheric oxygen level or both as independent variables (multiple linear regression, all P values for unilateral *versus* bilateral infection >0.2). Therefore, for further analyses, unilaterally and bilaterally infected bees were considered together.

Flight metabolic rate was significantly affected by atmospheric oxygen level and total mite infection number, and there was a significant interaction effect between oxygen level and mite infestation (multiple linear regression; Table 3). The significant interaction effect indicates that the effect of mite infection on flight metabolic rate depended on atmospheric oxygen level. To explore this interaction further, we examined the effect of mite infestation level within each atmospheric oxygen treatment group. In hyperoxic (40 % oxygen) and normoxic (21 %) atmospheres, total mite number did not affect metabolic rate (Fig. 2A). However, in hypoxic atmospheres (10 % and 5 % oxygen), metabolic rate decreased as total mite number increased (Fig. 2B,C). For uninfected bees, the critical oxygen level below which flight metabolic rate began declining was between 5 % and 10 % oxygen (Fig. 3), as in uninfected summer bees tested in a previous study (Joos et al., 1997). In 10 % oxygen atmospheres, flight metabolic rate was reduced by 18 % by moderate mite infection and by 36 % by severe mite infection (Fig. 3; in 10 % oxygen, 0 mites, metabolic rate 0.33±0.049 W g^{-1}; moderate infection, metabolic rate 0.27±0.022 W g^{-1}; severe infection, metabolic rate 0.21±0.021 W g^{-1}, N=8, 21, 21, respectively).

Effects of tracheal mite infection and atmospheric oxygen on thoracic temperatures

Higher atmospheric oxygen level increased T_{th}, and higher levels of total mite infestation decreased T_{th} during flight (Table 3). The oxygen × mite number interaction was not significant in the multiple regression analysis (Table 3). However, the relationships between total mite number and T_{th} within each atmospheric oxygen level were similar to those observed for flight metabolic rate. In 21 % or 40 % oxygen atmospheres, there were no significant effects of mite infestation on T_{th}, but in 10 % and 5 % atmospheres, T_{th} was decreased by increasing mite infestation (Fig. 2D–F). Thoracic temperature increased linearly with flight metabolic rate (MR),

Table 3. *Results of multiple linear regression with oxygen level and total number of mites as independent variables and either flight metabolic rate (W g^{-1}) or flight thoracic temperature (T_{th}, °C) as the dependent variable*

Variable	Flight metabolic rate coefficient	P	Flight T_{th} coefficient	P
y-intercept	0.19±0.025	<0.0001	32.0±0.72	<0.0001
Oxygen level	0.0046±0.00121	<0.0001	0.13±0.035	<0.0001
Total number of mites	–0.0040±0.00118	0.001	–0.11±0.034	0.001
Oxygen × total number of mites	0.00015±0.000065	0.031	0.003±0.002	0.114

Coefficients are presented as means ± S.E.M.

Both multiple linear regression models were highly significant and explained approximately 30 % of the variation in the dependent variables.

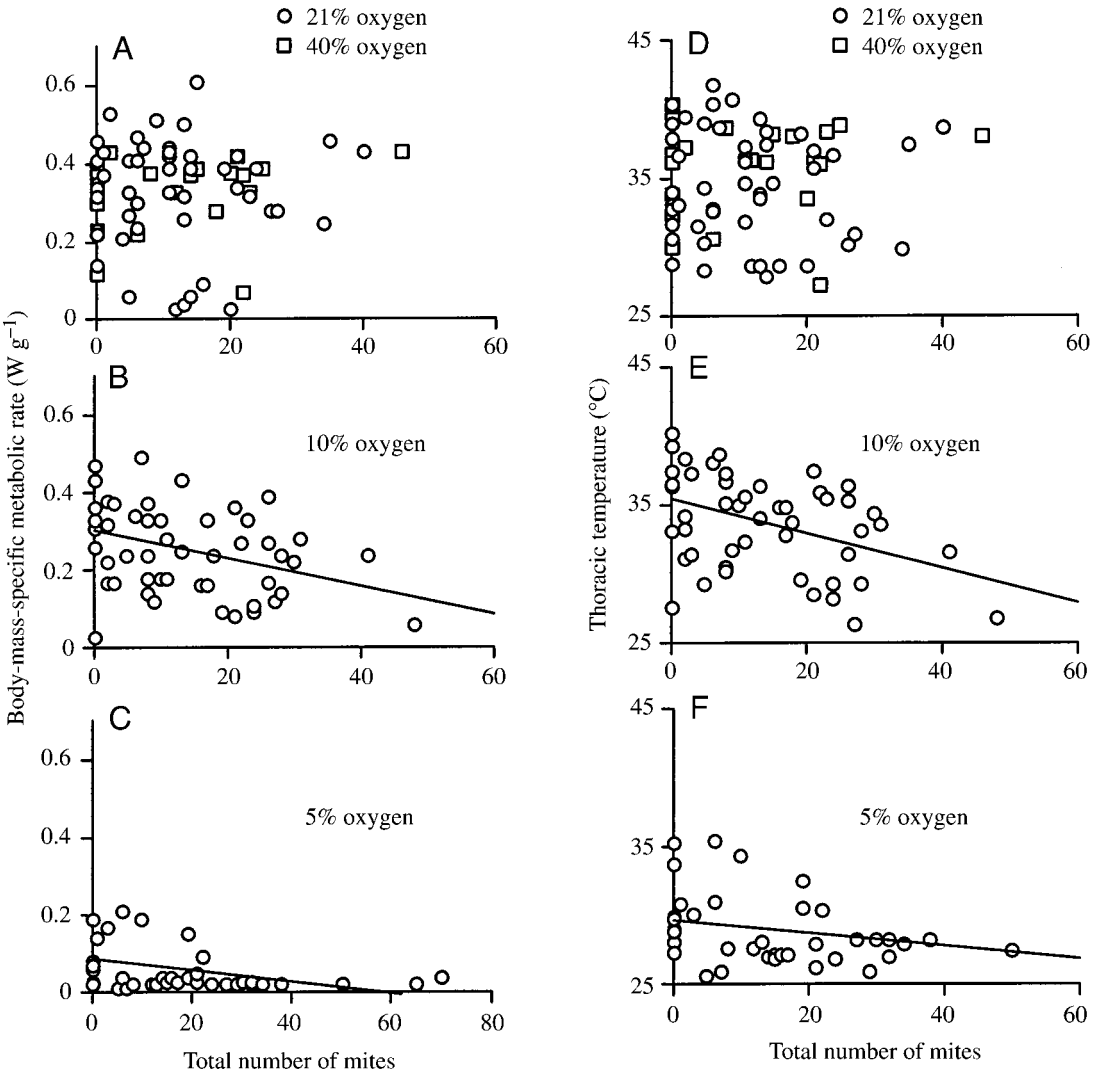

Fig. 2. The effect of total mite number on body-mass-specific metabolic rate (MR, left-hand column) and thoracic temperature (T_{th}, right-hand column) during agitated flight. (A) 40 % oxygen (squares) and 21 % oxygen (circles). MR was not affected by increasing mite number in 40 % oxygen (r^2=0.03, P=0.42) or in 21 % oxygen (r^2=0.00, P=0.90), so these regression lines are not drawn. (B) 10 % oxygen. MR=0.304–0.004N_m (where N_m is total mite number), r^2=0.14, S.E.M. of slope=0.001, F=7.70, P=0.008. (C) 5 % oxygen. MR=0.086–0.001N_m, r^2=0.16, S.E.M of slope=0.0005, F=6.94, P=0.012. (D) 40 % oxygen (squares) and 21 % oxygen (circles). T_{th} was not affected by increasing mite number in 40 % oxygen (r^2=0.00, P=0.99) or in 21 % oxygen (r^2=0.004, P=0.87), so these regression lines are not drawn. (E) 10 % oxygen. T_{th}=(35.5±0.07)–(0.126±0.039)N_m, r^2=0.18, F=10.5, P=0.002. (F) 5 % oxygen. T_{th}=(29.7±0.56)–(0.048±0.023)N_m, r^2=0.11, F=4.4, P=0.04.

pooling all atmospheric oxygen treatments together for the bees flown at 24 °C [Fig. 4; T_{th}=(27.1±0.25)+(24.8±0.88)MR; r^2=0.81, F=795, d.f.=1,182, P<0.001].

Effects of tracheal mite infection on flight metabolic rate and thoracic temperature at 16 °C and 21 % oxygen

Under the normoxic conditions tested, these winter-acclimated bees showed a decreased flight metabolic rate at 16 °C compared with 24 °C (24 °C, 0.33±0.019 W g^{-1}, N=53; 16 °C, 0.22±0.0219 W g^{-1}; N=50, t-test: t=3.2, P=0.002), as found in preliminary studies for summer bees (Harrison et al., 1996). As at 24 °C in normoxic atmospheres, there was no effect of mite infestation level on flight metabolic rate in 16 °C air (linear regression, F=0.16, P=0.69). Similarly, there was no effect of mite infestation level on T_{th} (linear regression, r^2=0.01, P=0). Thorax temperature and flight metabolic rate were strongly correlated for the bees flown at 16 °C [Fig. 4; T_{th}=(17.3±0.54)+(29.7±1.98)MR; F=225, d.f.=1,30, P<0.001, r^2=0.88].

Effects of tracheal mite infection on flight ability under natural cold conditions

Grounded bees had greater mite levels than bees able to return successfully to the hive in both hives (Fig. 5). These differences were significant (Mann–Whitney U-test, P=0.005 and P=0.02 for hives 1 and 2, respectively.)

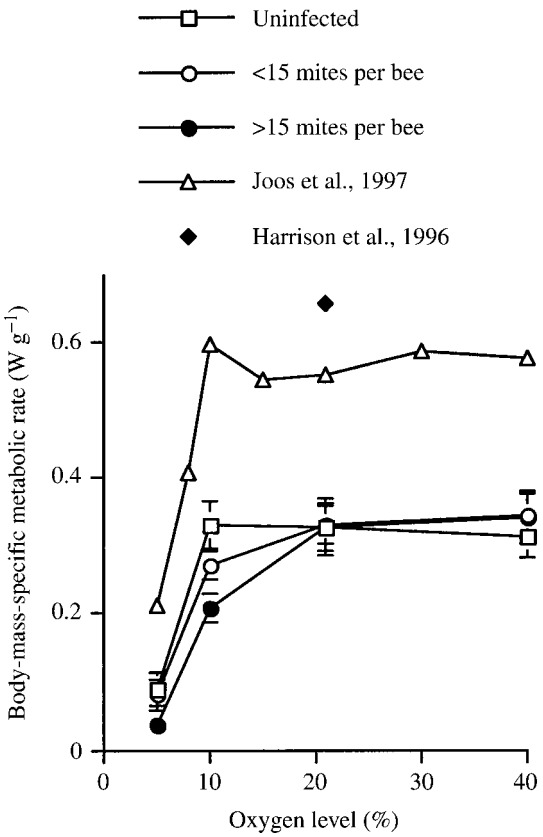

Fig. 3. The effects of atmospheric oxygen level on the body-mass-specific metabolic rate (MR; mean ± S.E.M.) of uninfected honeybees (squares, zero mites per bee), mildly infected honeybees (open circles, less than 15 mites per bee) and highly infected honeybees (filled circles, more than 15 mites per bee). For uninfected bees, MR was unaffected by varying atmospheric oxygen between 10 % and 21 %, but decreased in 5 % oxygen. For both mildly and highly infected honeybees, MRs in 5 % and 10 % oxygen were lower than in 21 % or 40 % oxygen atmospheres (Duncan's multiple-range tests). Data for uninfected summer bees (Harrison et al., 1996, filled diamond; Joos et al., 1997, open triangles) are also shown.

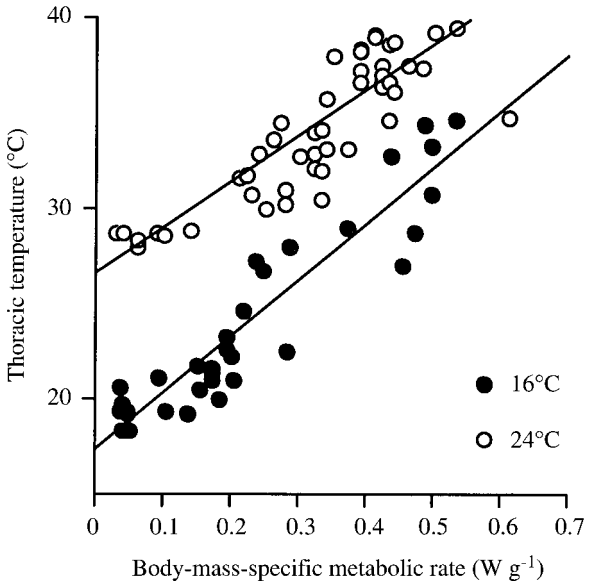

Fig. 4. The effects of body-mass-specific metabolic rate (MR) on thoracic temperature (T_{th}) at air temperatures of approximately 24 °C (open circles, range 23.5–24.5 °C) and 16 °C (filled circles, range 14.3–17.1 °C). The results for bees from all atmospheric oxygen levels were pooled, since there was no significant effect of oxygen level on this relationship when tested in a general linear model (SYSTAT, P>0.05).

Discussion

Effects of mites on the safety margin for oxygen delivery

The finding that flight metabolic rate is unaffected by mite infection in normal or hyperoxic atmospheres, but is reduced by increasing mite levels when the oxygen delivery system is stressed by hypoxia, indicates that tracheal mites reduce the safety margin and maximal capacity (conductance) for oxygen delivery by the honeybee tracheal system. The lack of an effect of mite infection on flight metabolic rate in normal atmospheres, together with the absence of a correlation between mite number and bee mass (r=–0.067, P=0.35) argues against mites causing a marked reduction in worker bee vigor and general condition. The safety margin for oxygen delivery allows bees to be able to fly and carry loads under summer conditions (Gary and Page, 1989; Downey et al., 2000), despite the reduction in oxygen delivery capacity due to the tracheal mites.

An estimate of the effect of mite infection on maximal oxygen delivery capacity (maximal tracheal conductance) can be made from the flight metabolic data in 10 % oxygen atmospheres, approximately half normal oxygen levels and the minimum atmospheric oxygen content that allows normal flight metabolic rate in uninfected bees. The tracheal conductance equals the oxygen flux divided by the partial pressure gradient for oxygen from air to mitochondria (Piiper et al., 1971). If the P_{O_2} in the region of the mitochondria is kept constant during these hypoxic exposures, then halving the

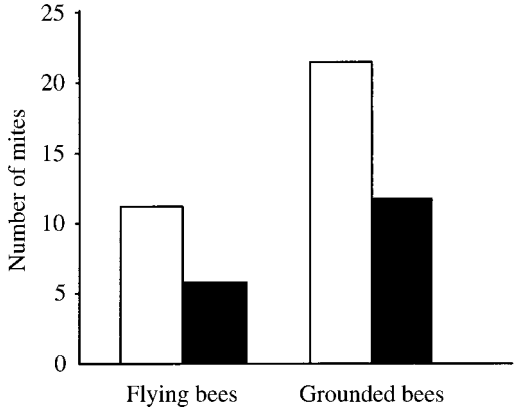

Fig. 5. Mean tracheal mite infestation levels for live, but grounded, bees collected on the snow compared with that of bees able to return to the hive. Hive 1, open columns; hive 2, filled columns.

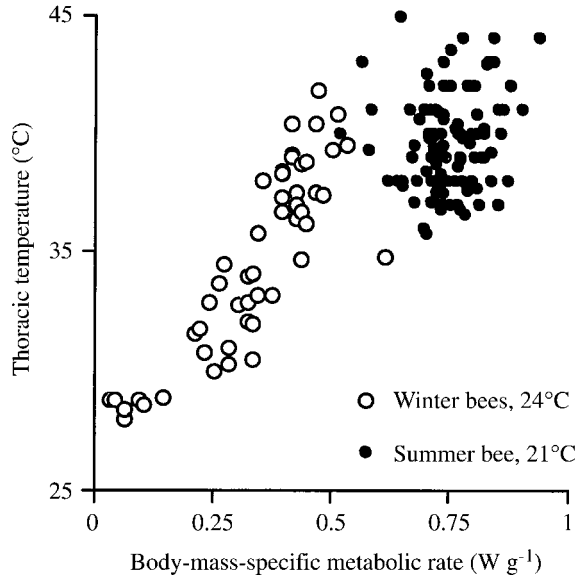

Fig. 6. The relationship between body-mass-specific metabolic rate (MR in $W g^{-1}$) and thoracic temperature (T_{th} in °C) for winter-collected honeybees flown at 24 °C [open circles, T_{th}=(26.6±0.73)+(24.1±2.03)MR, r^2=0.74, F=141, d.f.=1,50, P<0.001] and for summer-collected bees flown at 21 °C (filled circles; reanalyzed from Harrison et al., 1996; F=0.014, d.f.=1,104, r^2=0.00, P=0.91).

atmospheric oxygen level (and the air-to-mitochondria P_{O_2} gradient) while maintaining metabolic rate (as for uninfected bees) requires a doubling of tracheal conductance. The most likely mechanism for increasing tracheal conductance in flying honeybees is by increasing convective air flow through the tracheae. Evaporative water loss increases by 40 % when honeybees fly in 10 % relative to 21 % oxygen atmospheres, suggesting that ventilation is strongly increased during flight under hypoxic conditions (Joos et al., 1997). The severely mite-infected bees are at best able to increase tracheal conductance by approximately 1.6-fold when transferred from 21 % to 10 % oxygen atmospheres, since their flight metabolic rates are reduced by approximately 40 % in 10 % oxygen atmospheres (Fig. 3). These data suggest that roughly half the safety margin and scope for oxygen delivery is lost by severe tracheal mite infestation; measurements of flight muscle P_{O_2} are required to make this estimate more accurate. We hypothesize that tracheal mites impede convective flow through the tracheae, reducing maximal tracheal conductance, the safety margin for oxygen delivery, maximal metabolic rate and the ability to perform the most energetic flight behaviors.

Our findings support the hypothesis that the safety margin for oxygen delivery in honeybees has functional significance. Tracheal mites, a common natural pathogen, clearly reduce the gas-exchange capacity of honeybees, but the safety margin for oxygen delivery allows normal flight under most circumstances despite tracheal mite infection. Whether the apparent excess capacity in oxygen delivery actually evolved in response to such pathogens is unclear and will require further study. It would be particularly interesting to examine the safety margin for oxygen delivery in populations of bees that differ in their historical exposure to tracheal mites. Future studies of the effects of common pathogens on physiological function, and the evolutionary response of physiological systems to such infections, seem likely to be helpful in understanding the evolution of animal design.

Comparisons of winter and summer honeybees

Winter bees appear to have a dramatically lower flight metabolic rate than summer bees under similar conditions (Harrison et al., 1996; Joos et al., 1997; Fig. 3). The finding of lower flight metabolic rate in winter compared with summer bees is consistent with prior findings that winter bees have longer lifespans and lower hemolymph vitellogenin and juvenile hormone levels and pharyngeal gland sizes (Fluri et al., 1982; Sasagawa, 1989). In summer honeybees, elevations in juvenile hormone level are believed to cause the onset of foraging behavior (Huang et al., 1994), and flight metabolic rate increases at this time (Harrison, 1986). Conceivably, low juvenile hormone levels could produce biochemical differences in the flight musculature that lead to the lower flight metabolic rates. Alternatively, such biochemical changes could occur as a result of thermal (reverse acclimation) or nutritional effects.

The flight metabolic rate we measured for these winter bees at 24 °C was similar to those we have measured for summer bees at high (40 °C) air temperatures (Harrison et al., 1996; Roberts and Harrison, 1999). It would be interesting to examine the wing kinematics of winter bees to determine whether the decreasing flight metabolic rate is correlated with decreasing mechanical power output or increasing efficiency of conversion of metabolic to mechanical power. Although much of the focus of flight physiologists has been on understanding maximal metabolic rate, an equally interesting question is what determines the minimum metabolic rate required to sustain flight. Assessing this question, however, would require new protocols to attain verifiable minimum flight metabolic rates.

The lower flight metabolic rate attained by these winter bees leads to different thermal relationships from those for summer bees. For winter bees flying at either 16 °C or 24 °C, T_{th} increased strongly with increasing metabolic rate (Fig. 4). In comparison, the vast majority of summer bees tested using similar protocols at a T_{air} of 21 °C attained a metabolic rate and a T_{th} in the range of the highest values measured for these winter bees (Fig. 6; data reanalyzed from Harrison et al., 1996). For summer bees tested at 21 °C, there was no correlation between flight metabolic rate and T_{th}, perhaps because of the low variance or perhaps due to variation in the magnitude of heat loss among bees.

However, as noted above, these apparent metabolic differences between winter and summer bees should be considered to be preliminary findings since these results are only for bees from one hive. Also, in this study, bees were collected from inside the hive (since no foraging was occurring

in mid-winter), while outgoing foragers were collected in the prior studies. It is conceivable that bees disturbed during their long within-hive period during the winter take a considerable time to activate behaviorally for flight. It will be important to measure the flight metabolic rate of winter bees caught during voluntary flights outside the hive to exclude the possibility that the differences in flight metabolic rate we have measured are due to behavioral (rather than metabolic capacity) differences between winter and summer bees.

Is the reduction in tracheal capacity a primary mechanism of pathology for tracheal mites?

It is possible that a reduction in oxygen delivery capacity by the tracheal mites is the primary mechanism of pathology for these mites. At first glance, the finding that tracheal mites do not reduce the flight metabolic rate of honeybees in normoxic atmospheres suggests that impediment of oxygen delivery cannot be a primary pathology of tracheal mites. However, if winter bees have similar relationships between flight muscle temperature and force production to summer honeybees, they will be unable to generate sufficient lift to counteract their body weight at a T_{th} below 28 °C (Coelho, 1991). As noted above, heavily mite-infected honeybees should, at most, be able to increase their flight metabolic rate by 60 % (from 0.33 W g^{-1}, the mean value in 21 % oxygen, to 0.53 W g^{-1}). Since the slopes of the lines relating T_{th} and metabolic rate are similar at 24 °C and 16 °C (Fig. 4), it seems likely that thermal conductance is constant across this temperature range, so we can relate honeybee T_{th} to metabolic rate and T_{air} in a single model calculated from the data shown in Fig. 4:

$$T_{th} = 0.63 + 25.6\mathrm{MR} + 1.1T_{air}\,.$$

This equation predicts that the lowest T_{air} at which a honeybee with a maximal metabolic rate of 0.53 W g^{-1} could maintain a minimal flight-capable T_{th} (28 °C) is 12.5 °C. Heavily mite-infested bees should be unable to sustain flight at lower T_{air}.

This interpretation leads to the prediction that tracheal-mite-infected honeybees should frequently die outside the hive during flights in cold weather as a result of thermoregulatory failure. Indeed, our field study confirms that mite-infected bees are more likely to die during flights on days with temperatures below 12 °C (Fig. 5). In support of the hypothesis that tracheal blockage leads to flight failure at low T_{air} and subsequent death outside the hive, a typical symptom of a colony heavily infected with tracheal mites is to find that the colony has dwindled to a small number of bees, but that very few dead bees are found within the hive (S. Camazine, personal observations).

Although tracheal mites unequivocally reduce the safety margin for oxygen delivery in honeybees, our hypothesis that this mechanism represents a primary pathology of tracheal mites in honeybees will require further testing. It remains possible that other, more general, effects of mites (mediated by nutritive losses, viral infection or immune responses) reduce the functional capacity of honeybees. There is evidence that tracheal mites affect flight muscle structure (Komeili and Ambrose, 1991), suggesting that the effects of the mites are not limited to the tracheal system. Further studies of the physiological effects of tracheal mites and experimental studies of the effects of mites on the flight capacity of honeybees under various conditions (for example, in a large room at 10 °C) are necessary to test further the hypothesis that tracheal-mite-induced limitations on oxygen delivery and maximal metabolic rate represent the primary pathology of tracheal mites. However, given the economic losses associated with mite infection, it may not be premature to test the hive management implications of this hypothesis. If this hypothesis is correct, blocking hive exits until springtime air temperatures are consistently high or providing honey and pollen in spring to reduce foraging flights in cool weather are two management strategies that might reduce individual and colony mortality due to tracheal mites.

This work was supported in part by grants from the Pennsylvania Department of Agriculture (ME 447105), the USDA-CSREES (97-36200-2245), and the Mid-Atlantic Apiculture Research and Extension Consortium (MAAREC) to S.C., by NSF grants IBN 9728444 and IBN 9317784 and USDA grant 97-35302-4395 to J.F.H. and by NSF grants IBN-9600840 and IBN-9722196 to J.H.M.

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