1131

ENERGY METABOLISM OF EUCALYPTUS-BORING BEETLES AT REST AND DURING LOCOMOTION: GENDER MAKES A DIFFERENCE

GORDON L. ROGOWITZ[1],* AND MARK A. CHAPPELL[2]

[1]*Department of Biology, PO Box 23360, University of Puerto Rico, San Juan, Puerto Rico 00931-3360* and
[2]*Department of Biology, University of California, Riverside, CA 92521, USA*

*e-mail: rogowitz@rrpac.upr.clu.edu

Summary

We studied metabolic rates during rest, maximal running exercise and tethered flight in the long-horned eucalyptus-boring beetles *Phoracantha recurva* and *P. semipunctata*. Simultaneous measurement of rates of O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) indicated that $\dot{V}_{CO_2}$ closely approximated $\dot{V}_{O_2}$ and hence was a good index of aerobic metabolic rate. The resting metabolic rate (RMR), peak $\dot{V}_{CO_2}$ during running-wheel locomotion (MR$_{run}$) and factorial scope during running (MR$_{run}$/RMR) are similar to published values for several other insect taxa. MR$_{run}$ was repeatable for most test groups over intervals of 48–96 h. Studies of *P. semipunctata* show that MR$_{run}$ is relatively insensitive to changes in ambient temperature (T_a) between 20 and 30 °C, whereas resting metabolic rate increases with T_a with a normal Q_{10} (2.4). Consequently, factorial scope declines at the higher T_a: mean factorial scopes for male and female *P. semipunctata* are 17.7 and 13.6 at 20 °C *versus* 8.9 and 5.5 at 30 °C, respectively. Flight activity requires a considerably greater metabolic rate than terrestrial activity: at T_a values of 20–30 °C, the mean factorial scope for flight activity of male *P. semipunctata* is 72 (range 36–110). Nevertheless, our measurements of flight metabolic rate in *Phoracantha* spp. are considerably lower than predicted from allometric equations for other insects. Our most interesting finding was that males of both species had a substantially and significantly higher MR$_{run}$ and aerobic scope than females. The gender differences in MR$_{run}$ are consistent with differences in activity levels of males and females during mate-seeking behavior.

Introduction

In insects, the metabolic foundation of most locomotor activity is aerobic respiration. Accordingly, the upper limit to power production during locomotion, and hence the upper limits to sustainable speed and endurance, is set by the maximal rate of oxidative metabolism. Insects have extremely high metabolic rates during flight (Casey, 1989), but in many species the flight motor seems to have a relatively fixed power output across a wide range of flight speed (Ellington et al., 1990; Casey, 1992). In contrast, power output during terrestrial locomotion is an approximately linear function of running speed (Herreid and Full, 1984; Full et al., 1990). Although there is considerable interest in the energetics of terrestrial locomotion and costs of transport in insects (e.g. Lighton et al., 1993), the upper limits to power production during terrestrial exercise are relatively little studied. Even less is known of the possible effects of gender on these variables, although many insect species show strong sexual dimorphism in size, morphology or behavior.

The aim of this study was to examine the energy metabolism of males and females of two cerambycid beetles (the long-horned eucalyptus borers *Phoracantha recurva* and *P. semipunctata*; Coleoptera: Cerambycidae) during rest and terrestrial activity. These two species have similar morphology, behavior and ecology (T. D. Paine, personal communication). Gender comparisons are of interest for eucalyptus borers because males and females have quite different mating strategies and associated locomotor activity levels. Adults of both species fly at night to eucalyptus trees, where mating occurs and eggs are laid (Hanks et al., 1996a,b). After arriving at a tree, females are relatively sedentary. In contrast, males spend long periods running rapidly along the bark surface searching for mates. Males also engage in fights with one another to secure access to potential mates; these fights can be vigorous enough to result in the loss of appendages. Accordingly, we predicted that males would have a greater ability to support terrestrial locomotion and activity than females, expressed as a higher maximum aerobic power output and a greater metabolic expandability (scope) during exercise.

Although our primary goal was to compare the maximal

terrestrial exercise metabolic rate of males and females, we also examined the repeatability of individual differences in exercise metabolic rate. Repeatability is a measure of the fraction of individual variability that is consistent over time and, hence, is potentially heritable and subject to selection. We also studied the thermal dependence of metabolic rate, since *Phoracantha* spp. are active over a range of ambient temperatures. Finally, we evaluated metabolic rates during tethered flight activity as a comparison with power output during terrestrial exercise.

Materials and methods

Animals

Long-horned eucalyptus borers (*Phoracantha* spp.) are native to Australia. They arrived in California with their host plants, which are various species of eucalyptus trees. We obtained *P. recurva* and *P. semipunctata* from a laboratory colony maintained by Dr Timothy Paine at the University of California, Riverside, CA, USA. The laboratory stock were derived from free-living beetles obtained by cutting eucalyptus trees and collecting animals that came to the damaged wood (T. Paine, personal communication). Beetles were held in small screen-wire cages containing a paper shelter where they could hide. The cages were maintained at room temperature (22–24 °C). Food (20 % sucrose solution and eucalyptus pollen) was available *ad libitum* (except during experiments that required fasting); water was always available.

Metabolic measurements

An open-flow respirometry system was used to measure rates of CO_2 production ($\dot{V}_{CO_2}$; ml h^{-1} or ml g^{-1} h^{-1}) during rest, running exercise and tethered flight. For all measurements, we used a LiCor 6251 CO_2 analyzer capable of resolving differences of 0.2 parts per million (p.p.m.) of CO_2 in air. The analyzer was calibrated weekly against a precision gas mixture; there was almost no drift between calibrations. Flow rates of dry, CO_2-free air were maintained at ±1 % by a Tylan mass flow controller. Outputs from both instruments (as well as ambient temperature measured with thermocouples) were recorded on Macintosh computers equipped with National Instruments A/D converters and custom-designed software for data acquisition and analysis ('WartHog Systems', written by M. A. Chappell, University of California, Riverside, CA, USA).

Validation of $\dot{V}_{CO_2}$ as a metabolic index

We present data for $\dot{V}_{CO_2}$ rather than the rate of O_2 consumption ($\dot{V}_{O_2}$) because the sensitivity and stability of the LiCor 6251 are approximately 100-fold better than that of the best available O_2 analyzer. This is particularly important when the rate of CO_2 production is small. Conversion of $\dot{V}_{CO_2}$ into energy equivalents requires careful consideration because the energy equivalent of $\dot{V}_{CO_2}$ is strongly dependent upon the substrate used to fuel respiration (lipid, protein or

carbohydrate) and because released CO_2 may come from buffered storage in body fluids as well as directly from respiration. Therefore, we made preliminary measurements of both $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ during rest and forced exercise in a small-volume open system, which generated concentration changes in both gases that were large enough for accurate measurements. Reference air measurements were taken frequently (approximately every 5–8 min) to correct for drift of the oxygen analyzer. We used modified 30 ml syringes as metabolic chambers in these tests. Flow rates of dry, CO_2-free air were 75–90 ml min^{-1}. Excurrent gas was dried (using magnesium perchlorate), passed through the LiCor 6251, then scrubbed of CO_2 with Ascarite, redried and passed through the sensor of an Applied Electrochemistry S-3A oxygen analyzer. Changes in gas content were recorded as described previously. We calculated $\dot{V}_{O_2}$ as:

$$\dot{V}_{O_2} = \dot{V}(F_{IO_2} - F_{EO_2})/(1 - F_{IO_2}), \qquad (1)$$

where $\dot{V}$ is flow rate corrected to standard temperature and pressure (STP; 0 °C and 760 mmHg; 1 mmHg=0.1333 kPa), F_{IO_2} is the initial fractional concentration of O_2 (0.2095), and F_{EO_2} is the final fractional concentration of O_2. We calculated $\dot{V}_{CO_2}$ as:

$$\dot{V}_{CO_2} = \dot{V}(F_{ECO_2} - F_{ICO_2})/\{1 - F_{ECO_2}[1 - (1/RQ)]\}, \qquad (2)$$

where F_{ICO_2} and F_{ECO_2} are the initial and final fractional concentrations of CO_2, respectively, and RQ is the respiratory quotient. Because incurrent air was scrubbed of CO_2 (using soda lime and Ascarite), F_{ECO_2} was zero. Equation 2 was calculated using an assumed RQ of 0.85 (small deviations in RQ have a negligible effect on $\dot{V}_{CO_2}$).

Validation tests were performed on 10 beetles at rest and during rapid movement (stimulated by rolling and shaking the syringes). The RQ was identical during rest and exercise (0.88; $t=0.007$, $P=0.99$), and factorial scopes (determined by dividing peak values by resting values) were equivalent for both $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (4.67±0.56 and 4.65±0.42, respectively; means ± S.E.M., $N=10$; $t=0.06$, $P=0.96$). Hence, measurements of $\dot{V}_{CO_2}$ appear to be accurate indices of energy expenditure and aerobic metabolic power output both at rest and during exercise, at least for these species of eucalyptus borers. To provide additional information appropriate for conversion of $\dot{V}_{CO_2}$ to aerobic metabolic rate, we determined the mean RQ for nine beetles fasted for 48 h (0.75±0.02, $N=9$).

Resting metabolic rate

Resting metabolic rate (RMR; estimated as $\dot{V}_{CO_2}$ calculated from equation 2) was measured at flow rates of 100–200 ml min^{-1} in chambers constructed from 10 and 20 ml syringes. The syringes were maintained at constant ambient temperature (T_a; ±0.5 °C) in an environmental cabinet. RMRs were determined as the mean minimal steady-state $\dot{V}_{CO_2}$ during periods of at least 45 min. Many beetles breathed cyclically during these tests; in such cases, we averaged over an integral number of ventilation cycles (i.e. starting and ending on peaks; Lighton, 1994, 1996).

Metabolic rate during terrestrial locomotion

We determined the peak metabolic rate for terrestrial locomotion (MR_{run}) as the highest 1 min mean value of $\dot{V}_{CO_2}$ during forced exercise in a running wheel. The wheel (diameter 5.8 cm, internal volume approximately 100 ml) was constructed of clear Lucite and equipped with airtight bearings, a variable-speed motor and a tachometer output to the computer. Flow rates were maintained at 200–500 ml min^{-1} (depending on the size of the beetle). We applied the 'instantaneous' (z) correction (Bartholomew et al., 1981) to the results of equation 2 to compensate for mixing characteristics and to resolve short-term events accurately. At the start of each exercise test, we allowed the beetle 5–30 min to adjust to the stationary wheel (most animals were quiescent during this period, and we used some of the resulting $\dot{V}_{CO_2}$ data for RMR estimates). After the initial rest period, we started the wheel at low speed (4–5 cm s^{-1}) and then gradually accelerated it in small steps to the highest speed the beetle would sustain without initiating flight movements (extending the wings from the elytra). During initial tests in which running speed was monitored, peak $\dot{V}_{CO_2}$ occurred at speeds of 12–18 cm s^{-1} (approximately 4–6 body lengths s^{-1}).

We measured MR_{run} for each individual three times at 48 h intervals to determine whether the responses were consistent (repeatable). Food was available in the holding cages for the first two measurements (day 1 to day 3) but was withdrawn 48 h before the final test on day 5 (water remained available at all times). On the last day, RMRs were determined as described above. Most MR_{run} and RMR data were obtained at 30 °C (*N*=10–12 of each sex tested per species). Additional measurements were taken for *P. semipunctata* at 20 °C (*N*=8–10 of each sex).

Metabolic rate during tethered flight

The metabolic rate during tethered flight activity (MR_F; measured as $\dot{V}_{CO_2}$) was determined only in male *P. semipuncta* (*N*=14 individuals tested at 17–28 °C). We used a clear Lucite box (volume 1000 ml) as a metabolic chamber. Flow rates were 500–800 ml min^{-1}; as for the running wheel tests, we used equation 2 with the 'instantaneous' correction of Bartholomew et al. (1981) to provide accurate resolution of short-term changes. The MR_F data we report are the highest values of $\dot{V}_{CO_2}$ averaged over 1 min continuous periods of wing movement. Beetles were suspended by a 27 gauge hypodermic needle containing a thermocouple inserted 3–5 mm into the dorsal surface of the thorax and held in place using beeswax. Some individuals exhibited vigorous wing movement immediately after insertion of the probe and others did not commence activity until more than 1 h later. Our values are likely to be conservative estimates of flight costs because suspension from a rigid support may decrease energy expenditures for maneuvering and opposing the force of gravity. However, we were unable to obtain measurements for unrestrained animals. In preliminary tests, untethered beetles immediately terminated flight whenever an appendage touched a side of the metabolic chamber. Similarly, beetles suspended by a thin string failed to sustain flight (they climbed the string).

Statistical analyses

We report data as means ± S.E.M. To compensate for the effects of body mass, we employed analysis of covariance (ANCOVA) with mass as covariate. To compensate for mass effects during repeatability studies, we compared mass residuals for subsequent tests. Probabilities of <0.05 were considered significant. Tests were performed using Statistica/Mac (StatSoft), a statistics package for the Macintosh.

Results

Resting metabolic rate

In tests at 30 °C, RMR increased with body mass in both *P. recurva* (*r*^{2}=0.30, *P*=0.02) and *P. semipunctata* (*r*^{2}=0.79, *P*<0.001). To provide a basis for further comparison with published data, the effect of body mass is also given using allometric scaling coefficients for log/log relationships (Table 1). Two-way ANCOVA, with mass as covariate, indicated that RMR at 30 °C was slightly higher for *P. recurva* than for *P. semipunctata* (*P*=0.015; Table 2). For each species, RMR was also marginally higher for males than for females (*P*=0.05). However, in additional tests of *P. semipunctata* at 20 and 30 °C, RMR did not differ between sexes (*P*=0.42) and varied only with T_a (*P*<0.001, Table 3). Pooling data for both sexes of *P. semipunctata*, values of RMR were 0.0040±0.0002 and 0.0096±0.0005 ml CO$_2$ g^{-1} min^{-1} at 20 and 30 °C, respectively, yielding a thermal coefficient (Q_{10}) of 2.4.

Metabolic rate during terrestrial locomotion

We first determined the peak respiratory output for running wheel locomotion (MR_{run}) at 30 °C. We compared MR_{run} between two days without food restriction using a repeated-measures ANCOVA in which major effects of species and gender were included. MR_{run} did not differ significantly between species, but the effect of gender was highly significant (*P*<0.0001; Table 4). The MR_{run} of males averaged 40–50 % higher than that of females (Table 2), and 'absolute' aerobic scope (MR_{run}−RMR; the portion of aerobic power output available for activity) was 50–60 % higher in males. Fig. 1 illustrates the differences in MR_{run} relative to body masses of males and females. Although there was some overlap among individuals measured on different days, the contrast in MR_{run} between sexes is substantial. A small but significant (*P*=0.003) decline in MR_{run} occurred between day 1 and day 3, seen primarily in *P. semipunctata* (species × test day interaction, *P*=0.04) (Table 4). A smaller decline was evident for male *P. recurva*, and no decline was apparent for female *P. recurva* (Table 2).

To determine whether food restriction affected MR_{run}, we compared values for non-fasted beetles (day 1, day 3) with those for the same individuals on day 5, obtained after 2 days of fasting. This test (repeated-measures ANCOVA) revealed

Table 1. *Scaling equations describing the influence of $\log_{10}$(body mass) (g) on $\log_{10}$(peak $\dot{V}_{CO_2}$ during running-wheel exercise) (MR_{run}, ml CO_2 min^{-1}) and $\log_{10}$(resting metabolic rate) (RMR, ml CO_2 min^{-1})*

	a	b	r^2	P
Phoracantha recurva (30 °C)				
MR_{run}, males, fed	−1.39 (0.088)	0.56 (0.136)	0.44	0.0004
MR_{run}, males, fasted	−1.39 (0.171)	0.61 (0.235)	0.53	0.04
MR_{run}, females, fed	−1.33 (0.102)	0.87 (0.170)	0.59	<0.0001
MR_{run}, females, fasted	−1.46 (0.202)	0.70 (0.311)	0.42	0.06
RMR, both sexes	−2.34 (0.103)	0.41 (0.149)	0.34	0.014
P. semipunctata (30 °C)				
MR_{run}, males, fed	−1.20 (0.050)	0.77 (0.090)	0.79	<0.0001
MR_{run}, males, fasted	−1.22 (0.063)	0.75 (0.102)	0.88	0.0002
MR_{run}, females, fed	−1.32 (0.133)	0.89 (0.232)	0.45	0.001
MR_{run}, females, fasted	−1.90 (0.249)	0.08 (0.367)	0.01	0.83
RMR, both sexes	−2.16 (0.062)	0.75 (0.096)	0.81	<0.0001
P. semipunctata (20 °C)				
MR_{run}, males, fasted	−1.00 (0.142)	1.40 (0.327)	0.69	0.003
MR_{run}, females, fasted	−1.31 (0.142)	0.90 (0.310)	0.52	0.02
RMR, both sexes	−2.47 (0.080)	0.84 (0.180)	0.55	0.0002

The equations take the form $\log_{10}y=a+b\log_{10}x$.

Standard errors are given in parentheses. Results for days 1 and 2 were combined to increase the statistical power for tests of non-fasted ('fed') beetles.

Table 2. *Mean values of body mass, peak metabolic rate during forced locomotion in a running wheel (MR_{run}), resting metabolic rate (RMR) and factorial scope (MR_{run}/RMR) at 30 °C*

	N	Initial mass (g)	MR_{run} (ml CO_2 g^{-1} min^{-1})			RMR (ml CO_2 g^{-1} min^{-1})	Factorial scope
			Day 1	Day 3	Day 5		
Phoracantha recurva							
Males	12	0.26±0.035	0.084±0.006	0.075±0.008	0.079±0.008	0.014±0.001	6.13±0.588
Females	10	0.26±0.02	0.055±0.003	0.058±0.003	0.053±0.004	0.011±0.001	5.49±0.508
P. semipunctata							
Males	11	0.32±0.032	0.091±0.004	0.078±0.004	0.085±0.005	0.010±0.004	8.88±0.410
Females	10	0.28±0.030	0.064±0.005	0.051±0.005	0.054±0.006	0.009±0.001	5.50±0.576

Values are means ± S.E.M.

MR_{run} was measured three times at 48 h intervals with food available during day 1 and day 3 only.

RMR was measured on day 5.

Initial mass, taken prior to the first measurement of MR_{run}, did not vary significantly by species (P=0.22) or gender (P=0.64; two-way ANOVA).

no obvious effect of food restriction (day 3 *versus* day 5, P=0.74, Table 2). However, metabolic rate was lower on day 5 than on day 1 (P=0.02).

The effect of ambient temperature on MR_{run} was evaluated only for *P. semipunctata* (Tables 2, 3). Two-way ANCOVA yielded the unexpected result that the MR_{run} did not vary significantly with T_a between 20 and 30 °C (P=0.60). As shown in other tests, MR_{run} did vary by gender (P=0.0002).

Factorial scope of terrestrial locomotion

The factorial scope for $\dot{V}_{CO_2}$, which reflects the ability of an animal to change aerobic metabolic rate, was calculated as MR_{run}/RMR. In tests at 30 °C, males of each species had substantially and significantly higher factorial scopes than females (Table 2). Two-way ANCOVA revealed that factorial scope varied significantly by gender (P=0.001) and that differences between species occurred only for males (species by gender interaction, P=0.003). On average, *P. semipunctata* males had the largest factorial scopes, since MR_{run} was slightly higher and RMR slightly lower than those of *P. recurva* males. Two-way ANCOVA for *P. semipunctata* tested at 20 and 30 °C showed that factorial scope varied with T_a (P<0.001) in addition to gender (P=0.004). Factorial scopes for males and females were 17.7 and 13.6 at 20 °C, and 8.9 and 5.5 at 30 °C, respectively. Factorial scopes were higher at 20 °C than at 30 °C because RMR declined

Table 3. *Mean resting metabolic rate (RMR), peak metabolic rate during forced locomotion in a running wheel (MR$_{run}$) and factorial scope (MR$_{run}$/RMR) for* Phoracantha semipunctata *at 20°C*

	N	Body mass (g)	MR$_{run}$ (ml CO$_2$ g^{-1} min^{-1})	RMR (ml CO$_2$ g^{-1} min^{-1})	Factorial scope
Males	10	0.38±0.027	0.069±0.004	0.004±0.0002	17.7±1.51
Females	10	0.36±0.025	0.054±0.003	0.004±0.0002	13.6±0.92

Values are means ± S.E.M.
Metabolic rate was measured after a 2-day period of fasting.

Table 4. *Repeated-measures ANCOVA on peak* $\dot{V}_{CO_2}$ *during forced locomotion in a running wheel (MR$_{run}$)*

Effect	d.f.	Mean square effect	Mean square error	F	P
A, species	1,38	0.0007	0.0003	2.0	0.16
B, sex	1,38	0.0149	0.0003	43.6	<0.00001
C, test day	1,39	0.0012	0.0001	9.8	0.003
A×B	1,38	0.0002	0.0003	0.5	0.49
A×C	1,39	0.0006	0.0001	4.6	0.04
B×C	1,39	0.0001	0.0001	1.2	0.28
A×B×C	1,39	0.0002	0.0001	1.2	0.28

In this analysis, species and gender were included as major effects, and MR$_{run}$ was compared between day 1 and day 3.

Major effects (rows 1–3) and interactions (rows 4–7) are shown.

Body mass was a significant covariate ($F_{1,38}$=12.6, P=0.001).

considerably at the lower T_a, whereas MR$_{run}$ was more thermally stable.

Variance and repeatability of MR$_{run}$

Exercise metabolic rate showed considerable variability, with coefficients of variation of 12–30 % (after correction for body mass). The repeatability (or consistency) of MR$_{run}$ was evaluated by Pearson product-moment correlation, in which residuals obtained by linear regression of MR$_{run}$ on body mass were used to correct for individual variation in body size. We expected that individuals with a relatively high MR$_{run}$ on one test day would have a relatively high MR$_{run}$ on another day, and *vice versa*. Correlations between initial and final residuals were always positive and were frequently significant, particularly in *P. recurva* (Table 5). Fewer comparisons were significant for *P. semipunctata*, although the results for males and the combined data for both sexes were highly repeatable over the longest interval tested (96 h).

Relationship between MR$_{run}$ and RMR

We examined whether RMR was predictive of MR$_{run}$, using residuals obtained by linear regression on body mass to correct for any size-dependence. In tests of individuals of each species and gender, we examined Pearson product-moment correlations between residuals of RMR and residuals of MR$_{run}$. Correlation coefficients were calculated for males, females and both sexes combined, as follows: −0.23, −0.38 and 0.15 for *P.*
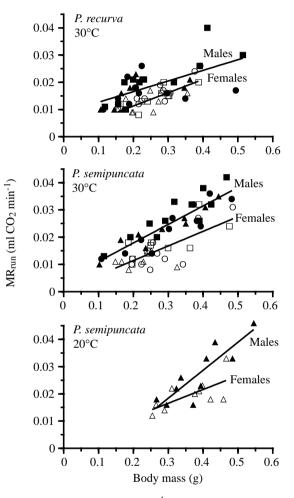

Fig. 1. The mass-dependence of peak $\dot{V}_{CO_2}$ during terrestrial exercise (MR$_{run}$) for *Phoracantha recurva* at 30 °C and for *P. semipunctata* at 30 °C and 20 °C. Lines of best fit (obtained by least-squares regression) illustrate general differences among males and females (all data are combined, irrespective of whether the animals were fed or fasted). Equations for specific effects are given in Table 1. Symbols indicate results for males (filled) and females (open) on day 1 (squares), day 3 (circles) and after fasting (triangles).

recurva at 30 °C, 0.24, 0.26 and 0.20 for *P. semipunctata* at 30 °C, and 0.34, 0.40 and 0.41 for *P. semipunctata* at 20 °C. In all cases, the correlations were non-significant (P>0.3), suggesting that the two variables are functionally independent. This conclusion is consistent with our findings that RMR has a normal Q_{10} of 2.4 while the Q_{10} of MR$_{run}$ is much lower.

Table 5. *Repeatability of maximal $\dot{V}_{CO_2}$ during forced terrestrial exercise (MR_{run})*

	Repeatability		
	Day 1 *versus* 3	Day 1 *versus* 5	Day 3 *versus* 5
Phoracantha recurva (30 °C)			
Males	0.75*	0.72*	0.80*
Females	0.44	0.67*	0.68*
Combined	0.64**	0.64**	0.69**
P. semipunctata (30 °C)			
Males	0.29	0.75*	0.22
Females	0.22	0.37	0.28
Combined	0.067	0.63**	0.19

Values are Pearson product-moment correlation coefficients indicating the consistency of peak $\dot{V}_{CO_2}$ between days (whether individuals with a given value of MR_{run} on one day have a similar value of MR_{run} on another day).

All correlations are for mass-corrected residuals. Significance is indicated by an asterisk (*$P<0.05$; **$P<0.01$).

$N=10$–12 for each sex and species (see Table 2); $N=16$–22 for both sexes combined.

The rate of CO2 production during flight activity

As expected, peak $\dot{V}_{CO_2}$ (ml min^{-1}) for male *P. semipunctata* during flight activity increased with body mass ($r^2=0.76$, $P<0.001$; Fig. 2). However, there was no significant effect of T_a on the MR_F of beetles over the range 17–28 °C ($r^2=0.003$, $P=0.86$). The mean value for MR_F over this T_a range was 0.30 ± 0.02 ml CO$_2$ g^{-1} min^{-1} ($N=14$) and the range was 0.17–0.58 ml g^{-1} min^{-1}.

Factorial scopes for flight activity were determined as MR_F/RMR, where RMR was estimated from the results given above using a Q_{10} correction to actual thoracic temperature (T_{th}). The mean, minimum and maximum values of factorial scope were 78 ± 7.4 (mean ± S.E.M., $N=14$), 36 and 138, respectively. Factorial scope was independent of body mass ($r^2=0.004$, $P=0.83$), but did vary with T_a ($r^2=0.57$, $P=0.002$). As for the factorial scope for running, this effect occurred because RMR increased with increasing T_a but MR_F did not. Excluding values for two beetles tested below 20 °C, the mean, minimum and maximum values of factorial scope were 72 ± 6.6 ($N=12$), 36 and 110, respectively.

Flight activity had little effect on T_{th}; in no case did T_{th} increase by more than 0.5 °C during flight, even over flight durations of several minutes. Similarly, we found no evidence of pre-flight endothermic warm-up.

Discussion

The main focus of this study was to determine whether the different mate-seeking behavior patterns of male and female *Phoracantha* spp. correspond to gender differences in exercise capacity. We found substantial and significant differences in maximal aerobic exercise metabolic rate between the sexes.
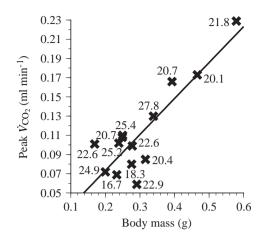

Fig. 2. Peak $\dot{V}_{CO_2}$ of male *Phoracantha semipunctata* during continuous tethered flight. Body temperatures (°C) are indicated beside the data points for individual beetles. The line of best fit was determined by least-squares regression (S.E.M. given in parentheses): $y=-0.001(\pm0.0198)+0.37(\pm0.061)x$.

Before discussing the possible effects of these differences, it is useful to compare the energy metabolism of eucalyptus borers with that of other species.

Resting metabolic rate and MR_{run}

In most respects, the resting and terrestrial exercise metabolic rates in *P. recurva* and *P. semipunctata* are similar to those reported for other insect taxa. As expected, resting metabolic rates were affected by both mass and temperature, with Q_{10} values within the normal physiological range of 2–2.5. After correcting for these factors and converting $\dot{V}_{CO_2}$ data to microwatts, the RMRs of the two *Phoracantha* species at 30 °C (approximately 760 µW, assuming RQ=0.85 and 20.5 J ml^{-1} O$_2$) were considerably higher than predicted values for flightless insects of similar mass (approximately 460 µW for a 0.26 g insect at 30 °C), but within the expected range for flight-capable species (Lighton and Fielden, 1995, 1996). In a more narrowly focused comparison, *Phoracantha* spp. RMR was approximately 40 % higher than predicted by an allometric equation for resting beetles (Bartholomew and Casey, 1977).

For comparative purposes, maximal aerobic capacities for terrestrial exercise can be judged either from absolute rates of aerobic metabolism or from the factorial increase above resting metabolic rate (metabolic scope). The energy cost of running locomotion has been extensively studied in cockroaches (e.g. Herreid et al., 1981a,b; Herreid and Full, 1984; Full and Tullis, 1990) and ants (e.g. Lighton et al., 1993). There have also been a few studies of beetles (Bartholomew and Casey, 1977; Morgan, 1987; Full et al., 1990; Kram, 1996). Although some of the previous studies were not designed expressly to measure maximal aerobic exercise capacity, our results for *Phoracantha* spp. are within the range of MR_{run} reported for other insect taxa. After correction for mass and temperature, the MR_{run} for male *Phoracantha* spp. was 70–75 % of the metabolic rate of tropical beetles during terrestrial exercise (Bartholomew and Casey, 1977). Values for female *Phoracantha* spp. were

somewhat lower (Table 1). The aerobic scope for *Phoracantha* spp. (5.5–17.7, depending on sex and temperature) was similar to that reported for cockroaches (4–12; Herreid et al., 1981a,b; Herreid and Full, 1984) and rhinoceros beetles (*Xylorctes thestalus*) walking carrying weights (approximately 16; Kram, 1996). Some scarabs (*Pleocoma* spp.) have considerably higher aerobic scopes during terrestrial activity (Morgan, 1987). However, these species, unlike *Phoracantha* spp., apparently employ the flight musculature for endothermic heat production when walking at low ambient temperatures, and the high metabolic rates are not directly related to the power of locomotor movements.

Surprisingly, we found no significant effect of temperature on MR_{run}. If the low thermal sensitivity of exercise $\dot{V}_{CO_2}$ is an accurate reflection of the power output of the musculature driving leg movement, then maximal sustainable running speed should show a similarly low thermal sensitivity. This would enable *Phoracantha* spp. to maintain a similar locomotor capability over a range of ecologically relevant temperatures. Hanks et al. (1996a) showed that the walking speeds of *P. semipunctata* were significantly higher at 28 °C than at 17 °C (19.5 *versus* 12.1 cm s^{-1} respectively), which contrasts with our findings for MR_{run}. However, it is worth noting that Hanks et al. (1996a) measured voluntary walking, while our work focused on maximal exercise capacity, and that Q_{10} during voluntary walking was only 1.47 (considerably less than the physiological norm of 2–2.5).

Repeatability of MR_run

To our knowledge, the repeatability of MR_{run} or other aspects of energy metabolism (i.e. the consistency over time of an individual's performance ranking within a population) has not been previously reported for any insect. Although we did not find significant repeatability of MR_{run} in all tests (Table 5), the repeatability coefficient (r) was always positive, which indicates some consistency. Moreover, for both species, repeatability for both sexes combined was highly significant over the longest time interval examined (96 h). Despite a low sample size compared with that typical of repeatability studies, the repeatability of *Phoracantha* spp. MR_{run} is in many cases as high or higher than most repeatabilities for locomotor or aerobic performance in vertebrates (sprint speed, travel distance and endurance, Tolley et al., 1983; Garland, 1985; Huey and Dunham, 1987; Djawdan and Garland, 1988; van Berkum et al., 1989; Austin and Shaffer, 1992; Rogowitz et al., 1999; maximal oxygen consumption, Hayes, 1989a,b; Garland and Bennett, 1990; Hayes and Chappell, 1990; Chappell et al., 1995, 1996).

Flight metabolic rate

Although factorial scopes for running wheel activity of *Phoracantha* spp. were relatively large, particularly at 20 °C, the metabolic power output (and hence factorial scope) during flight activity was considerably higher (mean scope 72, maximum scope 110 at a T_a of 20–30 °C). This suggests that MR_{run} was probably not limited by constraints on tracheal gas

transport to the leg muscles. However, the flight metabolic rate of tethered *P. semipunctata* is only 25–42 % of the flight metabolic rate of similar-sized non-tethered moths (Bartholomew and Casey, 1978; Casey, 1989), and the factorial scope during tethered flight of the eucalyptus borers is correspondingly lower than that of these moths (which can exceed 150). Similarly, the MR_F of *P. semipunctata* is approximately 40 % of the MR_F of the scarab *Cotinus texana* after correction for mass and body temperature differences (Chappell, 1984). The low MR_F in *Phoracantha* spp. may be a real phenomenon resulting from low wing loading, or it may be a consequence of the trauma of thermocouple insertion or of suspending the test animals from a rigid support, which may lower the power output of the flight muscles (for a review, see Casey, 1989). If low MR_F is not an artifact of the measurement technique, it would allow *Phoracantha* spp. to sustain flight for relatively long periods per unit of stored metabolic fuel. However, this would not necessarily produce a low cost of transport (the energy used to move a unit of mass over a unit of distance), since low power output during flight is probably indicative of low flight speed.

The relatively low MR_F in *Phoracantha* spp., coupled with a lack of insulation on the thorax, is presumably why these beetles do not elevate their thoracic temperature significantly when flying. Even if metabolic rates during natural untethered flight were similar to those of moths of similar mass (i.e. 2–4 times higher than the MR_F we measured), thoracic temperature should not increase by more than 2 °C above air temperature. In contrast, many large beetles such as scarabs show impressive endothermy before and during flights (Bartholomew and Heinrich, 1978; Morgan and Bartholomew, 1982; Chappell, 1984; Morgan, 1987).

Gender differences in MR_run

Perhaps the most interesting finding of this study is the substantial difference between the MR_{run} of males and females, even though the body masses and overall morphology of the two sexes are similar (except for larger antenna size in males; Hanks et al., 1996a,b). We assume that the higher peak $\dot{V}_{CO_2}$ of males reflects a greater capacity for aerobic power production during running and, hence, a higher maximal sustainable running speed. The relationship between speed and metabolic rate is approximately linear in running insects (Herreid et al., 1981a,b; Herreid and Full, 1984). Therefore, the 50–60 % greater absolute scope in males suggests that they have a maximal sustainable running speed approximately 1.5–1.6 times higher than that of females.

High running speed is very important for male mating success in *Phoracantha* spp. Mating occurs on the trunks of eucalyptus trees, which both sexes visit during nocturnal flights (damaged or injured trees are particularly attractive to these beetles). After arriving at a tree, females move slowly along the trunk while inspecting potential oviposition sites under loose bark; they do not actively seek males. In contrast, males spend long periods searching for females by running rapidly along the trunk with their long antennae extended laterally. If

a male's antennae touch a female, he stops and initiates courtship behavior. The number of such encounters is a function of the rapidity with which a male can 'sweep' a large area of trunk. Therefore, a male's fitness (mating success) is probably strongly influenced by his sustainable running speed, and hence by MR_{run}. This is particularly important for *Phoracantha* spp. males because most movement during mate-seeking is on vertical surfaces, and the metabolic cost of running at a given speed increases considerably with slope (Full and Tullis, 1990).

If a male encounters a female that is already accompanied by another male, the two males fight vigorously, with the larger individual usually displacing the smaller one (Hanks et al., 1995, 1996a,b). The role of MR_{run} in determining success in these aggressive confrontations is uncertain. Although body size is a major determinant of success in male–male combat in *Phoracantha* spp. (Hanks et al., 1996a,b), we speculate that fighting ability is also positively correlated with MR_{run}, since strength (metabolic power output) is presumably important in winning fights. However, aerobic performance during combat is probably less important than anaerobic (burst) exercise capacity unless fights are prolonged.

In conclusion, we hypothesize that selection for high mobility during mate seeking, and possibly for a capacity for sustained combat, is associated with the evolution of high MR_{run} in male *Phoracantha*. We have shown that one of the necessary prerequisites for natural selection, substantial and repeatable variance in MR_{run}, exists in *Phoracantha* spp. However, our data do not reveal whether the variance in MR_{run} is heritable. Only if it is, and if there are no constraining functional trade-offs or genetic or phenotypic correlations, can MR_{run} continue to evolve under selection.

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