

Resting metabolic rate can vary with age independently from body mass changes in the Colorado potato beetle, *Leptinotarsa decemlineata*

Saija Piironen*, Leena Lindström, Anne Lyytinen

Centre of Excellence in Evolutionary Research, Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FI-40014 Jyväskylä, Finland

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ABSTRACT

Temperature and mass dependency of insect metabolic rates are well known, while less attention has been given to other factors, such as age. Among insect species that experience seasonal variation in environmental conditions, such as in temperate latitudes, age may also have indirect effects on the metabolic rate. We examined the effect of age on the resting metabolic rate of *Leptinotarsa decemlineata* during 11 days after adult emergence by using flow-through respirometry. Age had a significant mass-independent effect on metabolic rate of beetles. A twofold increase in metabolic rate occurred during the first 2 days of adult life after which metabolic rate decreased with age relatively slowly. Ten day-old adult beetles had a metabolic rate similar to newly emerged beetles. The beetles have to be able to complete their development and prepare for overwintering during the relatively short favourable summer periods. Therefore, the observed pattern in metabolic rate may reflect physiological changes in the pre-diapause beetles adapted to temperate latitudes.

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1. Introduction

The metabolic rate, the energetic cost of living under specific environmental conditions, is closely related to life-history traits (Chown and Gaston, 1999) and has been shaped by the environmental conditions under which organisms have evolved to cope and reproduce. This has resulted in substantial among- and within-species variation in metabolic rate (Chown and Nicolson, 2004). Variation in the metabolic rate among individuals has been linked to fitness (Crnokrak and Roff, 2002; Krasnov et al., 2004; Boratynski and Koteja, 2009; Ketola and Kotiaho, 2009) and it has been considered to be adaptive (Hoffmann and Parsons, 1989; Harshman et al., 1999; Reinhold, 1999; Davis et al., 2000; Addo-Bediako et al., 2002). For example, a higher metabolic rate of insect species or populations from colder environments than those of warmer environments may be beneficial by enabling the individual to develop and grow faster in relatively short and cool growing seasons (Ayres and Scriber, 1994; Chown and Gaston, 1999; Addo-Bediako et al., 2002).

Metabolic rate may also vary for other reasons than mentioned above. In insects, the main interest in metabolic rate variation to date has concentrated on the allometric relationship between metabolic rate and mass (e.g. Lighton and Fielden, 1995; Van Voorhies et al., 2004; Niven and

Scharlemann, 2005) and the temperature dependence of metabolic rate (e.g. Berrigan and Partridge, 1997; Nespolo et al., 2003; Terblanche and Chown, 2007). Besides mass and temperature, other factors can also affect metabolic rate including sex (Rogowitz and Chappell, 2000), age (Terblanche et al., 2004), season (Tauber et al., 1986; McCaughan et al., 2009) and different forms of activity (Berrigan and Partridge, 1997; Rogowitz and Chappell, 2000; Gray and Bradley, 2003; Terblanche and Chown, 2007), but these factors have less often been taken into account in metabolic studies. For example, age can have a pronounced effect on metabolic rate (Hack, 1997; Terblanche et al., 2004; Hetz, 2007). The relationship between metabolic rate and age in larval and pupal life-stages is well established (Rajagopal and Bursell, 1965; Dingha et al., 2005; Hetz, 2007), whereas it is less clear in adults. In general, metabolic rate is assumed to decrease with adult age (Hack, 1997; Roberts and Rosenberg, 2006) resulting from the general deterioration of the physiological condition associated with ageing (Grotewiel et al., 2005). However, other studies have observed no change (Promislow and Haselkorn, 2002; Marais and Chown, 2003) or have reported an increase of metabolic rate with adult age (Gray and Bradley, 2003; Terblanche et al., 2004; Melvin et al., 2007). This increase is considered to be due to higher energetic demands (May, 1989; Gray and Bradley, 2003; Terblanche et al., 2004).

Furthermore, the relationship between age and metabolic rate may be complicated by indirect effects and affected by body mass which may also change with age (Gray and Bradley, 2003; Nespolo

* Corresponding author. Tel.: +358 14 260 4254; fax: +358 14 260 2321.
E-mail address: saija.p.piironen@jyu.fi (S. Piironen).

et al., 2005). For example, metabolic rate may depend on age in an indirect way by the season (Tauber et al., 1986). In higher latitudes, where environmental conditions change predictably (short favourable summers, long harsh winters), insects have to adapt physiologically to unfavourable conditions (Tauber et al., 1986). Many insect species survive the winter by entering diapause which is a dynamic physiological process (Kostal, 2006) including changes in the energy metabolism which thereby, is indirectly age-related. For example, insect metabolism is greatly lowered at diapause compared to that of a non-diapausing stage (Denlinger et al., 1972; Hahn and Denlinger, 2007; Kostal et al., 2008).

The life stage of the individual may have a considerable effect on metabolic rate. Among temperate beetles, only a few studies have explored the age dependence of metabolic rate in the adult stage (Yocum et al., 2009). Yet in seasonal environments, the physiological changes can take place under relatively short time periods. Evidently, more information is needed to understand metabolic rate variation and how it is associated to life-history evolution. We investigated the age dependence of resting metabolic rate in the Colorado potato beetle (*Leptinotarsa decemlineata*) (Say) for 11 days, from adult emergence until the age of 10 days. To survive winter periods, the overwintering generation must prepare for diapause in a relatively short time period. Therefore, we expected to see a bell shaped pattern in metabolic rate with adult age.

2. Materials and methods

2.1. Study animals and rearing

L. decemlineata adults used in the study originated from beetles collected in Poland (Bonin, 54°09'N, 16°15'E) in June 2003 and had been maintained in the laboratory (L:D 18:6, average temperature 17–23 °C, overwintering periods at 5 °C, dark) for three generations. The fourth generation beetles (offspring of 63 families) used in this study were reared from egg to adult at a constant temperature of 23 °C under a fluctuating light regime of 18:6 h light:dark, including a 2 h sunrise and sunset in a controlled environmental chamber (Type B1300, Weiss technic). Beetles were kept in petri dishes (diameter 9 cm) lined with a moisturized filter paper and fed with fresh potato leaves (variety Van Gogh) supplied daily. To allow pupation, last instar larvae (4th) were placed in a plastic container filled with soil. Adult emergence was checked daily. After emergence the adult beetles were weighed and then kept individually under the same conditions as described above.

2.2. Metabolic rate measurement

CO₂ production was used as a measure of metabolic rate. Beetles ($n = 108$) were measured on 11 days from age 0 (the day when an adult emerged) to age 10 (10 day-old beetles are ready to enter winter diapause). To avoid CO₂ emissions resulting from the assimilation of food, the beetles were starved for 4 h before measurements. To minimize the effects of repeated starvation, each beetle was measured at 4-day intervals. Beetles were divided into four groups so that the first group of beetles ($n = 30$) was measured at ages 0, 4, and 8, the second group ($n = 28$) at ages 1, 5, 9, the third group ($n = 25$) at ages 2, 6, 10 and the fourth group ($n = 25$) at ages 3 and 7 days. Beetles that died during the experiment and could not be measured three times were replaced by new ones and therefore the number of individuals among measurement groups differs. Individuals were divided evenly into the groups and there was no difference in emergence mass among metabolic measurement groups (ANOVA, females: $F_{3,51} = 1.156$, $p = 0.336$; males: $F_{3,49} = 0.359$, $p = 0.783$).

CO₂ production was measured using a CO₂ analyzer Li-6252 (LiCor, Lincoln, USA) connected to a flow-through respirometry system. The entire system was set in a temperature controlled room where the temperature was kept at 19 ± 1 °C. The CO₂ analyzer was calibrated using a calibration gas (450 ppm CO₂ in nitrogen) every week. Ambient air was filtered for CO₂ (soda lime) and water vapour (Drierite), and was pulled through a cylindrical respirometry chamber (volume circa 1.7 ml) and the CO₂ analyzer with a pump (Sable Systems, Henderson, USA). The flow rate was controlled to be 150 ml min^{-1} . The CO₂ analyzer's output signal was captured by the acquisition and analysis software Datacan V (Sable Systems). The recording of the CO₂ production started when the CO₂ level had stabilized and lasted a minimum of 5 min during which the moving activity of the beetle was visually recorded. A beetle was considered active if it walked or moved its legs (active or not active). Movements of antennae were not considered as active movement since it did not produce any detectable change in the recording of the CO₂ production. A 5-min recording time was considered to be adequate based on preliminary measurements which showed that a continuous mode of gas exchange of the beetles remained stable for at least an hour. The sampling interval was 0.2 s. Baselines (empty chamber without the beetle) were recorded before and after each measurement. Beetles were weighed (to 0.001 g) on a Mettler AM100 analytical balance. Respirometry data was baseline corrected using the Expedata (Sable Systems) software. The resting metabolic rate ($\text{ml CO}_2 \text{ h}^{-1}$) was the mean value of the lowest 1-min period of the whole 5-min recording. This was to ensure that the value represents the metabolic rate of a resting beetle.

2.3. Statistical analyses

Metabolic rate was analysed with analysis of covariance (ANCOVA). Adult age was entered as a fixed factor and body mass as a covariate. Separate analyses were performed for different age groups (0–3, 4–7 and 8–10 day-old beetles). Sexes were analysed separately because of the significant dependency of the covariate mass on sex. Otherwise, an ANCOVA model with sex included might not give reliable results (Quinn and Keough, 2002). In the first age group (0–3 day-old beetles), there was a significant correlation between age and the covariate body mass. Since this may violate the reliability of ANCOVA, we also analysed the data with an analysis of variance (ANOVA) using mass-specific metabolic rate or residual metabolic rate from a linear regression of metabolic rate on body mass as a dependent variable. Results obtained were consistent with ANCOVA. Activity (active/inactive during measurement) was entered as a fixed factor only for the first age group (0–3 day-old beetles) but not for the other two groups, as there were only a few individuals (3 in the second group and 1 in the third group) that were active during the measurement. Excluding these individuals did not change the results, thus, they were included in the analysis. When the main effect of age was significant, pair-wise comparisons between ages were conducted based on estimated marginal means and Bonferroni-adjusted for multiple comparisons. The assumption of the homogeneity of within-factor regression slopes was checked for each analysis. Body mass differences between age groups was analysed with ANOVA. Separate analyses were performed for age groups 0–3, 4–7 and 8–10 day-old beetles. Changes in body mass with age within measurement groups (first group 0, 4, 8, second 1, 5, 9 and third 2, 6, 10 day-old beetles) were analysed with repeated ANOVA. An intra-specific mass-scaling relationship between metabolic rate and body mass was calculated by a linear regression of metabolic rate ($\log_{10} \mu\text{W}$) on body mass ($\log_{10} \text{g}$), assuming a respiratory quotient of 0.84 (Terblanche et al., 2005). As activity affected metabolic rate (see Section 3), active individuals were excluded from the regression analyses.

Table 1
ANCOVAs on metabolic rate ($\text{ml CO}_2 \text{ h}^{-1}$) of *L. decemlineata* for three age groups.

Age group	Sex	Effect	df	MS	F	p
0–3 days	Female	Age	3	0.006	11.666	<0.01
		Activity	1	0.004	8.831	<0.01
		Mass (covariate)	1	0.007	12.868	<0.01
		Error	49	<0.001		
	Male	Age	3	0.004	11.375	<0.01
		Activity	1	0.001	3.506	0.067
		Mass (covariate)	1	0.005	12.399	<0.01
		Error	47	<0.001		
4–7 days	Female	Age	3	0.004	7.737	<0.01
		Mass (covariate)	1	0.005	9.815	<0.01
		Error	45	<0.001		
	Male	Age	3	<0.001	0.668	0.577
		Mass (covariate)	1	0.003	4.850	0.033
		Error	43	<0.001		
8–10 days	Female	Age	3	0.002	4.175	0.025
		Mass (covariate)	1	0.014	25.536	<0.01
		Error	31	<0.001		
	Male	Age	3	<0.001	1.665	0.205
		Mass (covariate)	1	0.001	4.504	0.041
		Error	33	<0.001		

3. Results

3.1. Metabolic rate

In the age group of 0–3 day-old beetles, the interaction between age and activity was not significant, and therefore, was omitted from the final analysis (Sokal and Rohlf, 2003). Metabolic rate varied with adult age in both sexes (Table 1). Metabolic rate increased substantially right after emergence. It peaked for 2 day-old beetles so that it was 1.8 times higher than on the emergence day (Fig. 1). For both sexes, metabolic rate was significantly lower at age 0 compared to ages 1, 2 or 3 days (pair-wise comparisons, females: 0 vs. 1, $p < 0.01$; 0 vs. 2, $p < 0.01$; 0 vs. 3, $p < 0.01$; males: 0 vs. 1, $p < 0.01$; 0 vs. 2, $p < 0.01$; 0 vs. 3 days, $p < 0.01$), whereas beetles at ages 1, 2 and 3 days had a similar metabolic rate (pair-wise comparisons, females: 1 vs. 2, $p = 0.41$; 1 vs. 3, $p = 0.64$; 2 vs. 3, $p = 1.00$; males: 1 vs. 2, $p = 0.25$; 1 vs. 3, $p = 1.00$; 2 vs. 3 days, $p = 0.56$). Activity significantly elevated the metabolic rate (Table 1). Excluding active individuals from the analysis did not change the main result of age (females: $F_{3,34} = 5.368$, $p < 0.01$; males: $F_{3,29} = 8.317$, $p < 0.01$).

In the age groups of 4–7 and 8–10 day-old beetles, metabolic rate decreased with adult age (Fig. 1). The decrease was

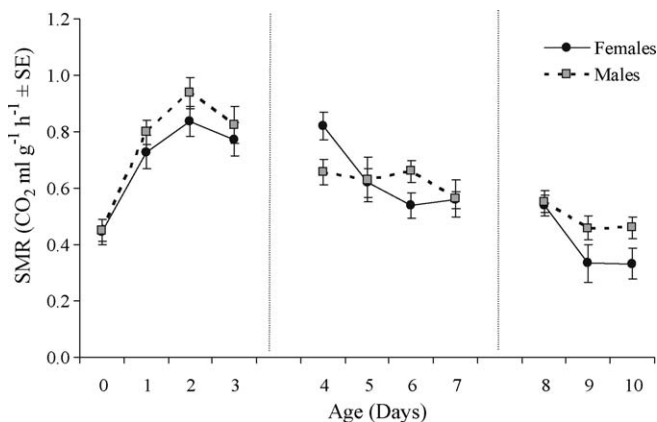


Fig. 1. Mass-specific metabolic rate ($\text{CO}_2 \text{ ml g}^{-1} \text{ h}^{-1} \pm \text{SE}$) of *L. decemlineata* for three age groups (0–3, 4–7, and 8–10 days old).

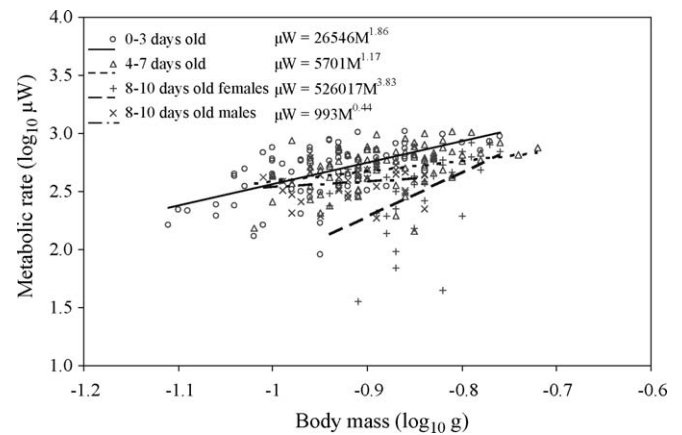


Fig. 2. The intra-specific scaling relationship between metabolic rate ($\log_{10} \mu\text{W}$) and body mass ($\log_{10} \text{g}$) for three age groups (0–3, 4–7, and 8–10 days old). M = body mass in g. The regression slopes for the third group (8–10 days old) differ significantly between sexes and therefore, the slopes are represented separately.

significant in females but not in males (Table 1). In females, the metabolic rate was significantly higher at age 4 days compared to older age classes (pair-wise comparisons, 4 vs. 5, $p = 0.03$; 4 vs. 6, $p < 0.01$; 4 vs. 7 days, $p < 0.01$), whereas between ages 5, 6 and 7 there were no differences in metabolic rate (pair-wise comparisons, 5 vs. 6, $p = 1.00$; 5 vs. 7, $p = 1.00$; 6 vs. 7 days, $p = 1.00$). In the group of 8–10 day-old beetles, females had a significantly lower metabolic rate at age 9 days compared to the age of 8 days (pair-wise comparisons, $p = 0.03$), whereas between ages 9 and 10 or 8 and 10 days there were no differences in metabolic rate (pair-wise comparisons, 9 vs. 10, $p = 1.00$; 8 vs. 10 days, $p = 1.00$). The covariate mass affected metabolic rate in all age groups for both sexes (Table 1).

The intra-specific mass-scaling slopes did not differ between sexes in the first two age groups (ANCOVA, 0–3 days old, $F_{1,69} = 0.066$, $p = 0.798$; 4–7 days old, $F_{1,91} = 0.086$, $p = 0.926$), and thus, sexes were pooled (Fig. 2). However, the regression slopes in the third group (8–10 days old) differed significantly between sexes (ANCOVA, $F_{1,67} = 8.691$, $p < 0.004$), therefore, the regression slopes are represented separately for both sexes (Fig. 2). The regression slopes between the first two age groups did not differ significantly from each other (ANCOVA, $F_{1,164} = 2.986$, $p = 0.089$) (Fig. 2).

3.2. Body mass

There was no significant interaction between body mass and sex in any of the age groups (0–3, 4–7 and 8–10 day-old beetles) but females were heavier than males in all three age groups (Table 2). Weight increased significantly with age in the age groups of 0–3 and 4–7 days but not in the age group of 8–10 day-old beetles (Fig. 3).

Table 2
ANOVAs on body mass (g) of *L. decemlineata* for three age groups.

Age group	Effect	df	MS	F	p
0–3 days	Age	3	0.003	14.679	<0.01
	Sex	1	0.009	43.742	<0.01
	Error	103	<0.001		
4–7 days	Age	3	0.001	3.881	0.012
	Sex	1	0.017	95.682	<0.01
	Error	94	<0.001		
	Error	94	<0.001		
8–10 days	Age	2	<0.001	0.172	0.842
	Sex	1	0.010	46.167	<0.01
	Error	68	<0.001		

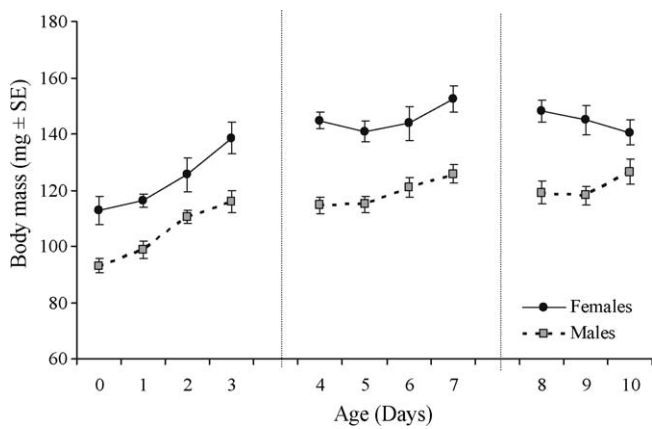


Fig. 3. Body mass (mg ± SE) of *L. decemlineata* for three age groups (0–3, 4–7, and 8–10 days old).

Repeated analysis of variance for the first three measurement groups (first group measured at ages 0, 4, 8, second 1, 5, 9 and third 2, 6, 10 days) confirmed that body mass increased significantly with age (females: first group, $F_{2,22} = 60.276$, $p < 0.01$; second, $F_{2,26} = 32.338$, $p < 0.01$; third, $F_{2,16} = 4.745$, $p = 0.02$; males: first group, $F_{2,20} = 50.841$, $p < 0.01$; second, $F_{2,22} = 14.311$, $p < 0.01$; third, $F_{2,22} = 13.372$, $p < 0.01$). Tests of within-subject repeated contrasts showed that body mass increased with adult age between the first and second measurements (females: first group, $F_{1,11} = 137.214$, $p < 0.01$; second, $F_{1,13} = 69.022$, $p < 0.01$; third, $F_{1,8} = 4.818$, $p = 0.06$; males: first group, $F_{1,10} = 72.586$, $p < 0.01$; second, $F_{1,11} = 14.971$, $p < 0.01$; third, $F_{1,11} = 15.980$, $p < 0.01$) but not between the second and third measurements (females: first group, $F_{1,11} = 0.351$, $p = 0.57$; second, $F_{1,13} = 1.070$, $p = 0.32$; third, $F_{1,8} = 0.156$, $p = 0.70$; males: first group, $F_{1,10} = 1.275$, $p = 0.29$; second, $F_{1,11} = 2.392$, $p = 0.15$; third, $F_{1,11} = 2.763$, $p = 0.13$). In other words, the weight of the beetles had reached a stable level for 4 day-old beetles (Fig. 3).

4. Discussion

Adult age had a significant effect on resting metabolic rate of *L. decemlineata* even after taking the effect of body mass into account (Table 1). Similar to previous work (Yocum et al., 2009), the metabolic rate formed a bell shaped pattern with age (Fig. 1). First, metabolic rate increased almost twofold from the emergence day to the first day after emergence and peaked in 2 day-old beetles. Then, it decreased gradually until 10 day-old beetles had metabolic rates that were at the same level as those of newly emerged beetles (Fig. 1) and close to metabolic rates of diapausing *L. decemlineata* beetles in the study by Vanatoa et al. (2006). The body mass of the beetles followed a different pattern with age. It increased steadily with age for the first 4–5 days of adult life after which the body mass stayed constant (Fig. 3). Hence, changes in metabolic rate could not be explained only by the body mass.

The bell shaped pattern of metabolic rate with age might reflect physiological changes that take place in the adult pre-diapause *L. decemlineata*. An initial, large increase in metabolic rate observed during the first few days of adult life (Fig. 1) may partly be due to flight muscle development which is energetically expensive (May, 1989; Marden, 2000; Terblanche et al., 2004). The time period closely corresponds to the time (5–10 days) that it takes for adult *L. decemlineata* to complete their flight muscle development (Weber and Ferro, 1996). An increase in metabolic rate also coincides with an observation that the beetles started to feed ferociously after the first day of emergence (personal observations), indicating high

energetic demands. The development of reproductive organs, especially ovaries in females, may also contribute to increases in the metabolic rate of insects (Terblanche et al., 2004). However, the impact of developing ovaries on metabolic rate is relatively small compared to that of development of flight muscles (May, 1989). Maximal rates of ovary development in *L. decemlineata* have been reported to occur for 5–6 day-old beetles (May, 1989). In our study, compared to males, females seemed to have a prolonged peak of metabolic rate that lasted until age 4 days (Fig. 1), potentially reflecting the energetic costs of developing ovaries.

As beetles got older their metabolic rate decreased gradually. This reflects the preparation for overwintering rather than general deterioration in physiological conditions associated with ageing as reported in crickets (Hack, 1997). During the preparation period, flight muscles undergo significant degeneration in *L. decemlineata* (Stegwee et al., 1963), which reduces energetic expenditures. Although, flight muscle development has been shown to occur also in older *L. decemlineata* beetles (de Kort, 1990), it did not contribute or elevate metabolic rate of the older beetles in this study. This is because the rate of muscle tissue growth is considerably decreased in older beetles compared to younger ones (May, 1989; de Kort, 1990) and its contribution to energy metabolism becomes relatively smaller.

Beetles in this study showed signs of being in pre-diapause and probably were ready to enter diapause at age 10 days. Several observations point to this. The body mass of 10 day-old beetles had reached a stable level indicating that the beetles had stored enough energy reserves for overwintering (Boman et al., unpublished). In addition, while body mass itself remained stable, there was a decrease in whole body metabolic rate, indicating that the proportion of non-metabolizing lipid tissues was increasing. Beetles also had ceased feeding by the age of 10 days. Furthermore, although we did not observe the diapause propensity of the beetles in this study, beetles originating from the same population are prone to enter diapause under these light conditions (approximately 50% enter diapause by age 20 days) (personal observations). It is also worth noting that after 10 days the metabolic rate of the beetles continues to decrease to a small extent, after which a stable, low level of metabolism is reached (May, 1989; Piironen et al., unpublished).

In addition to age, activity had an effect on metabolic rate. It was also age-dependent in that beetles showed active movement at younger ages. The pattern of sharp increase in metabolic rate during the first 2–3 days of adult life remained large and significant even when the actively moving beetles had been excluded from the analysis.

Our study, along with previous studies (May, 1989; Yocum et al., 2009), shows that the bell shaped pattern of metabolic rate with age is a characteristic of pre-diapause *L. decemlineata*. The pattern of age-related changes in the mass-specific oxygen consumption of *L. decemlineata* found by May (1989) closely resembles the pattern of mass-specific CO₂ production observed in this study. In both studies, a peak in metabolic rate occurred for 2–3 day-old beetles. In contrast, Yocum et al. (2009) found that a large increase in mass-specific CO₂ production did not occur until the beetles were 4–7 days old and reached a stable level by day 15. This discrepancy among studies may be due to the use of mass-specific metabolic rates (Packard and Boardman, 1999) or, secondly, differences in photoperiod. *L. decemlineata* uses critical day length as the main cue to initiate diapause (de Kort, 1990), and thus the rearing photoperiod regime may influence the age-related patterns in metabolic rate (May, 1989). Thirdly, populations may differ in their response to the same photoperiod and subsequently differ in metabolic rate. In the study by de Wilde and Stegwee (1958) *L. decemlineata* beetles that were reared under a photoperiod of 18:6 h light:dark were not induced to initiate

diapause and energy metabolism did not decrease with adult age. Also, de Wilde and Hsiao (1981) observed only a few diapausing beetles when they were reared under these light conditions. Interestingly, the beetles in this study showed signs of preparation for diapause as their metabolic rate decreased significantly until age 10 days (see Section 4) despite being reared under the same photoperiod as in above studies (de Wilde and Stegwee, 1958; de Wilde and Hsiao, 1981).

This third possible explanation of the differences in results among studies could stem from a different genetic background and/or physiological adaptation of the populations. *L. decemlineata* used in this study originate from higher temperate latitudes (54°09'N) compared to studies by May (1989) and Yocum et al. (2009), thus the beetles may have adapted to a longer critical day length for diapause induction. This is crucial since at higher latitudes, summer periods are shorter and insects have to enter winter diapause under relatively longer day lengths compared to lower latitudes (Tauber et al., 1986). In the study by de Wilde and Hsiao (1981), *L. decemlineata* originate from the same region (Poland) as beetles in this study and therefore the difference in diapause state is intriguing. However, the long time interval between the study populations (approximately over forty generations) may have allowed the populations to evolve physiologically. It is well known that adaptive changes in physiological and life-history traits can happen in very short time periods (e.g. Huey et al., 2000). Furthermore, diapause development is also affected by factors other than light conditions (temperature, soil parameters, food quality) and differences could arise as a response to the experimental conditions.

A comparison of the metabolic rates shows that the metabolic rate obtained in this study is within the same range as in previous studies of *L. decemlineata* (May, 1989; Yocum et al., 2009), and in other coleopteran species of the same size (Terblanche et al., 2005). However, the intra-specific mass-scaling components among different age groups in this study varied substantially; the group of 8–10 day-old females had especially exceptional values (Fig. 2). Other studies have also found high variability in intra-specific scaling exponents among and within species (see references in Van Voorhies et al., 2004; Chown et al., 2007). In our study, the substantial differences in the relationship between body mass and metabolic rate between different age groups are most likely due to the small size range of the beetles in each age group. Also, the relatively small sample sizes may have partly had an influence. Our more comprehensive data ($n = 1312$) measured from 6 day-old beetles gives a mass-scaling relationship of $MR (\mu W) = 2931 M^{0.768}$ (unpublished data). It is noted that this is close to the consensus relationship between metabolic rate and body mass for Coleopteran species ($MR (\mu W) = 1294 M^{0.740}$) (Terblanche et al., 2005). However, intra-specific scaling relationships are not directly comparable with inter-specific relationships as different factors affecting metabolic rate may differ between them (Chown et al., 2007).

In conclusion, this study demonstrates that adult age has a pronounced, mass-independent effect on resting metabolic rate in *L. decemlineata*. The age-related bell shaped pattern of metabolic rate reflects the physiological adaptation of this insect species to high temperate latitudes where the time window for the preparation of winter diapause is narrow. In a broader view, the possible variation in age-related patterns in resting metabolic rate among populations (e.g. comparing this study to May, 1989; Yocum et al., 2009) could reflect different physiological adaptations to specific environmental conditions. Evidently, age can have a significant effect on metabolic rate and this should be taken into account in studies of energy metabolism and its relationship to life-history evolution.

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