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RESEARCH ARTICLE

A long life in the fast lane: positive association between peak metabolic rate and lifespan in a butterfly

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SUMMARY

High peak metabolic rates may provide a performance advantage, but it may also entail a physiological cost. A long-held assumption is that high mass-specific energy expenditure is associated with short lifespan. To examine the relationship between energy expenditure and lifespan we asked two questions. First, do individuals have a consistent rate of metabolism throughout their life? Second, is metabolic rate correlated with lifespan? We analysed the repeatability of measurements of resting (RMR) and peak flight metabolic rate (MR_{peak}) throughout the life of the Glanville fritillary butterfly (*Melitaea cinxia*). Measurements of MR_{peak} showed significant repeatability. Senescence occurred only shortly before death. RMR showed a U-shaped relationship with age and very low repeatability. Intraspecific association between metabolic rates and lifespan was tested under three conditions: in the laboratory, under field conditions and in a laboratory experiment with repeated flight treatments. There was a significant correlation between MR_{peak} and lifespan in all three experiments, but the correlation was positive, not negative. RMR was not correlated with lifespan. Both MR_{peak} and lifespan may reflect physiological condition and therefore be positively correlated. Individuals with a large resource pool may be able to invest in mechanisms that slow down ageing. Individuals with high metabolic capacity may also possess adaptations against ageing. Molecular polymorphism in the gene phosphoglucose isomerase (*Pgi*) was significantly associated with both MR_{peak} and lifespan, and may have coevolved with defence mechanisms against senescence. Generalisations such as 'live fast, die young' may be too simple to explain the complex processes affecting ageing and lifespan.

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INTRODUCTION

Maximum metabolic capacity and maximum endurance are likely to have direct positive effects on individual fitness in many species. High sustained metabolic rate enhances shivering thermogenesis of small birds wintering in harsh conditions (Swanson and Garland, 2009), survival of rodents at high altitudes (Hayes and O'Connor, 1999) and continuous flight by birds migrating across vast hostile areas (Hedenström, 2008; Gill et al., 2009). High maximal metabolic capacity may also be sexually selected, as in the case of the remarkable display flights of hummingbird males (Clark, 2009; Clark and Dudley, 2009).

High energy expenditure is costly *per se* but it may also result in a cost in the form of physiological trade-offs. According to the 'rate of living' theory, high mass-specific metabolic rate is associated with short lifespan (Rubner, 1908; Pearl, 1928). The classic theory posits that an equal amount of tissue will consume the same total amount of oxygen during the lifetime of an individual, be it a mouse or an elephant (Schmidt-Nielsen, 1984). A fast rate of living thus leads to a short maximum lifespan. The old theory has subsequently evolved into the 'free radical theory of ageing' and even further to the 'oxidative stress theory', as the proximate mechanism of ageing is thought to be oxidative damage; free radicals, typically reactive oxygen species (ROS) and other harmful by-products of energy metabolism, cause somatic damage, leading to senescence and death (Harman, 1956; Barja, 2004; Dowling and Simmons, 2009; Buttemer et al., 2010). Defence mechanisms such as antioxidants are known to hinder oxidative damage, but there is clear indication that net oxidative stress increases significantly due to exercise such as extended flight in pigeons (Costantini et al., 2008) and high reproductive effort in zebra finches (Alonso-Alvarez et al., 2004). The rate of oxidative damage to mitochondrial DNA has been shown to be inversely related to the maximum lifespan of several mammal species (Barja and Herrero, 2000; Barja, 2002).

The rate of living theory is an appealing generalisation, but recent research demonstrates that the relationship between metabolic rate, oxidative damage and lifespan is a highly complex one. For example, there are systematic differences in the lipid composition of cell membranes among different taxa, which seem to be related to the susceptibility to oxidative damage (Hulbert et al., 2007). Cell membrane fatty acid composition may be an important factor in explaining the long-recognised phenomenon of birds having higher metabolic rates than similar-sized mammals, yet birds live on average twice as long as similar-sized mammals (Hulbert et al., 2007). Membrane composition is also size-dependent: large bird and mammal species have membranes that are less vulnerable to oxidative damage (Brand et al., 2003; Buttemer et al., 2010). Furthermore, the rate of ROS production does not seem to be directly related to metabolic rate as the rate of living theory would suggest.

ROS production is higher during resting metabolism than during activity when the mitochondrial membrane potential is low (Barros et al., 2004; Nicholls, 2004; Kowaltowski et al., 2009). Low mitochondrial membrane potential is also achieved through the means of uncoupling proteins, which carry protons through the membrane and thus reduce the ATP production rate, but at the same time lower the membrane potential (Speakman et al., 2004). Uncoupling proteins may serve as a protective mechanism against the damaging effects of energy consumption and thereby contribute to longer lifespan (Speakman, 2005).

In the empirical literature, metabolic rate, and more specifically resting metabolic rate (RMR) and the more strictly defined basal metabolic rate, has been related to lifespan across a number of animal species (Schmidt-Nielsen, 1984; Furness and Speakman, 2008). However, a difficulty with this relationship is that both metabolic rate and lifespan correlate strongly with body mass and it is therefore not clear whether metabolic rate has any causal role in the relationship. The traditional way of dividing metabolic rate with body mass in order to obtain mass-specific metabolic rate does not remove the size effect because small animals have higher metabolic intensity than large animals and therefore higher mass-specific metabolic rates. Instead, mass-independent metabolic rates should be calculated as residuals from a linear regression between metabolic rate and body mass. Studies using such residual metabolic rates have found no correlation between metabolic rate and lifespan across vertebrate species (Speakman, 2005; de Magalhães et al., 2007; Furness and Speakman, 2008).

Many studies have examined differences in lifespan across species but less is known about the relationship between metabolic rate and lifespan within single species. In well-studied species of *Drosophila*, no clear patterns have emerged (Promislow and Haselkorn, 2002; Marden et al., 2003; Van Voorhies et al., 2003; Hulbert et al., 2004; Melvin et al., 2007). In some cases the intraspecific relationship between energy expenditure and lifespan has been negative, such as in crickets (Okada et al., 2011); in other cases the relationship has been positive, for instance in dogs (Speakman et al., 2003), hamsters (Oklejewicz and Daan, 2002) and mice (Speakman et al., 2004). Experimental manipulation of energy expenditure has resulted in mixed effects on lifespan. For instance, decreased activity has resulted in increased lifespan when low temperatures or small cages have been used to restrict activity in house flies and fruit flies (Ragland and Sohal, 1975; Sohal and Buchan, 1981; Yan and Sohal, 2000; Magwere et al., 2006), or when the foraging time of worker honeybees has been limited (Rueppell et al., 2007). In contrast, experimentally increased energy consumption had no effect on lifespan in mice (Selman et al., 2008), rats (Holloszy, 1993) or voles (Vaanholt et al., 2009). In contrast, exercise has been reported to have increased lifespan in mice (Chigurupati et al., 2008). In a butterfly, experimental flight treatments shortened lifespan in some populations but the effect was absent in populations that were adapted to frequent flight due to landscape structure (Gibbs and Van Dyck, 2010). In another butterfly, experiment flight treatments alone did not affect lifespan, unless coupled with starvation at the larval stage. Butterflies that had experienced starvation as larvae lived longer than control individuals when forced to fly (Saastamoinen et al., 2010).

Here, we examine the relationship between metabolic rate and lifespan in the Glanville fritillary butterfly (Fig. 1). The long-term persistence of a well-studied classic metapopulation of the Glanville fritillary in Finland entirely depends on frequent recolonisation of small meadows, which can only support small local populations with a high risk of extinction (Hanski, 1999; Nieminen et al., 2004).

Fig. 1. The Glanville fritillary butterfly (*Melitaea cinxia*) inhabits dry meadows. All important life-history traits depend on flight, which is energetically costly. Photo courtesy of Lea Heikkinen.

Natural selection favours high dispersal rate in this metapopulation (Zheng et al., 2009), and dispersal rate is known to be associated with high flight metabolic rate (Niitepõld et al., 2009). Apart from dispersal, virtually all other life-history traits in butterflies depend on flight, which is energetically very expensive (Bartholomew and Casey, 1978) and may lead to physiological trade-offs potentially affecting fitness. Flight metabolic rate is known to be highly variable among local populations in the Glanville fritillary metapopulation and it is associated with molecular variation in the gene phosphoglucose isomerase (*Pgi*), which encodes for a glycolytic enzyme (Haag et al., 2005; Niitepõld, 2010). Furthermore, molecular variation in *Pgi* is associated with variation in several other life-history traits (Saastamoinen, 2007; Saastamoinen and Hanski, 2008; Niitepõld et al., 2009), including lifespan (Saastamoinen et al., 2009). The questions we ask here are: are measurements of resting and peak flight metabolic rate repeatable, and are there therefore consistent differences among individuals in the rate of energy expenditure throughout their life; and are high metabolic rates associated with short lifespan?

MATERIALS AND METHODS

Rearing of butterflies

We conducted four experiments in which Glanville fritillary butterflies [*Melitaea cinxia* (Linnaeus 1758)] were reared in common garden conditions. The larvae originated from the Åland Islands in SW Finland, except for the repeatability experiment on males only, in which the larvae were from southern France. In all experiments larvae were reared under controlled conditions in sibling groups and

fed *ad libitum* with fresh leaves of the host plant *Plantago lanceolata*. Pupae were weighed and moved to individual containers. Newly eclosed butterflies were weighed and marked with a felt tip pen on the hind wing. Prior to experiments marked individuals were kept in large cylindrical flight cages (50×40 cm) at room temperature with a natural light cycle.

Measurement of metabolic rates

RMR and peak flight metabolic rate (MR_{peak}; the highest rate of CO_2 production during sustained flight) were measured in the same trial. All butterflies were post-absorptive (fed the day before) or they had never been fed due to their young age (see below). Each butterfly was placed in a transparent cylindrical 1 litre respirometry chamber that was covered with a black cloth. Dry, CO_2-free air was flowed through the chamber at a rate of 1 l min^{-1} using a SS3 subsampler (Sable Systems, Las Vegas, NV, USA). The air temperature inside the chamber was measured with an NTC thermistor probe (Sable Systems). The respirometry chamber was kept covered for ca. 15 min, during which time all ambient CO_2 was flushed out and the metabolic rate settled to a stable, slightly cyclical baseline. The use of a large respirometry chamber reduces temporal precision in the CO_2 signal, but a positive consequence of this setup is a conveniently smoothed RMR reading. Butterflies remained practically immobile inside the darkened respirometry chamber, but if an individual became active during the measurement, an increase in the CO_2 level was immediately observed. On these rare occasions, more time was allowed for the butterfly to settle down to complete rest. As a measure of RMR we used the average over 40 s of stable CO_2 production.

Following the measurement of RMR the cloth was removed and the butterfly was agitated to fly under a light source that emitted visible and UV light. The measurement of flight metabolic rate lasted for 10 min, during which the butterfly was forced to fly as continuously as possible by shaking the chamber whenever the butterfly landed on the wall of the chamber. Butterflies typically reached the highest metabolic rate during the first minutes of the experiment, after which the metabolic rate often declined. After 10 min the chamber was again covered with the black cloth and the CO_2 level returned to baseline. The differential CO_2 analyser (Li-Cor 6251; Li-Cor Biosciences, Lincoln, NE, USA) was calibrated against a zero CO_2 gas several times a day. More details about the respirometry setup are presented elsewhere (Niitepõld et al., 2009).

Repeatability of metabolic rate measurements

The RMR and MR_{peak} of 12 unmated males were measured repeatedly during their entire life. The first measurement took place on the first day after eclosion and the measurement was subsequently repeated every second day. Individuals were measured in a random order and the identity of the butterfly was recorded only after the measurement had been completed and the butterfly was placed on a sponge to feed on 20% honey-water solution. On the days between measurements butterflies were kept in cages under broad-spectrum artificial lighting and provided water in a sponge for 1 h.

In the second repeatability experiment we measured the RMR and MR_{peak} of male (N=9) and female (N=24) butterflies. Individuals were allowed to mate under controlled conditions. Of these individuals 11 females and two males mated. The measurements took place at the ages of 3, 9 and 15 days. Between the measurements, individuals were subjected to a treatment of 10 min of flight in a cage every third day. Individuals were fed with 20% honey-water every second day *ad libitum*. On the days with no feeding, water was provided.

Longevity and metabolic rate

Longevity in the laboratory

A total of 146 females were reared in the laboratory and a subset of 71 females was used for the measurement of metabolic rate. Metabolic rates were measured on the second day following eclosion. After the measurement butterflies were kept in mesh flight cages in a greenhouse with controlled temperature and light conditions. The temperature was 15°C during the 12 h night and reached a maximum of 25°C during the day. Individuals were fed every second day with 20% honey-water solution and given water on alternative days. Dead individuals were collected on a daily basis.

Longevity in the field

Newly eclosed laboratory-reared butterflies were marked individually and released in a network of meadows on a small (1.6 km^2) island in SW Finland. Individuals were followed using mark–recapture protocols. A subset of butterflies was collected from the field and fed with 20% honey-water solution on the day of capture. On the following day, their metabolic rates were measured, after which each butterfly was returned to the meadow where it had been captured. Butterflies were 3 to 13 days old during the measurement. When individuals that were 10 days old or older were recaptured in the field, they were transferred to cylindrical flight cages that were kept outdoors under natural conditions. Butterflies were fed as above until the end of their life. Because we caught an insufficient number of females, only males (N=21) were used in the analysis.

Experiment with flight treatment

We recorded the lifespans of females in the repeatability experiment (above). In addition to these 24 females we included 11 females that were not included in the repeatability analysis because they died before the second measurement at the age of 9 days. Butterflies were forced to fly every third day and spent their lives in cages in the laboratory.

Genotyping

Individuals in the first longevity experiment and the field experiment were genotyped for a non-synonymous single nucleotide polymorphism in the gene *Pgi* using methods described by Orsini et al. (Orsini et al., 2009). DNA was extracted from a small piece of wing. The focal single nucleotide polymorphism *Pgi-111* has been found to be significantly associated with phenotypic variation in the Glanville fritillary (Saastamoinen and Hanski, 2008; Niitepõld et al., 2009; Saastamoinen et al., 2009). Most individuals represent the genotypes AA or AC, while the CC homozygotes are very rare in the Åland Islands (Orsini et al., 2009).

Statistical analyses

We obtained values for mass-independent metabolic rates by regressing whole-animal metabolic rate against wet body mass on the day after emergence (Fig. 2). In the field experiment (see below) we used the pupal mass as a measure of body mass as a high-precision scale was not available for measuring adult body mass (Fig. 2B,D). If measurement temperature had an effect on metabolic rate, it was included in the regression model and thus its effect was removed from the residual that was used in all subsequent analyses. Repeatability (r) was calculated as described by Lessells and Boag (Lessells and Boag, 1987). Analyses with repeated measurements on the same individuals were carried out using a linear mixed model with individual as a random factor (Proc Glimmix in SAS 9.2; SAS Institute, Cary, NC, USA). We used ar(1) as the covariance structure

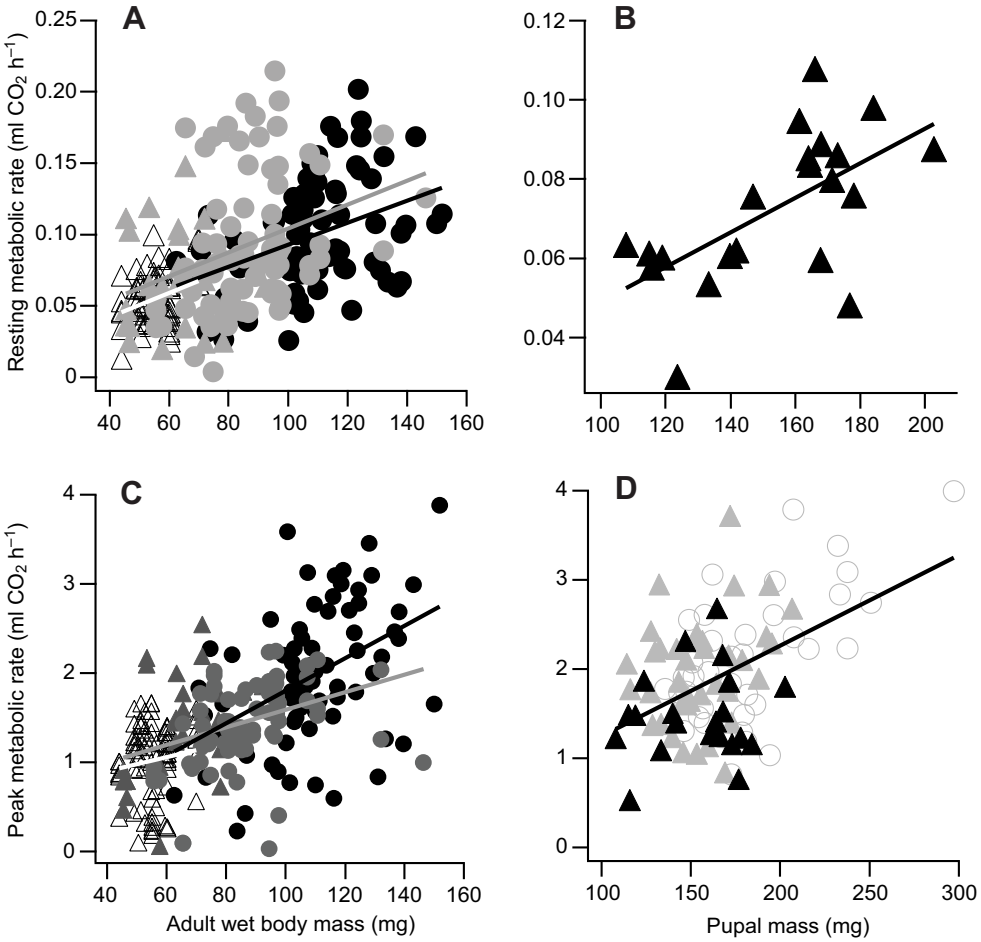

Fig. 2. Metabolic rate was affected by body mass in all experiments in the Glanville fritillary. Residuals from these regressions were used in subsequent analyses instead of uncorrected whole-animal metabolic rate. (A) Repeated measurements of resting metabolic rate in 12 males (white triangles; y=0.0008449x+0.008374), the males (grey triangles) and females (grey circles) of the second repeatability experiment (y=0.0008372x+0.02056), and the females from the laboratory longevity experiment (black circles; y=0.0007718x+0.01552). (B) Resting metabolic rate of the males used in the field longevity experiment plotted against pupal wet mass (black triangles; y=0.0004377x+0.005227). (C) Peak flight metabolic rate of males from the first repeatability experiment (white triangles; y=0.007865x+0.5875), the males and females from the second repeatability experiment (grey triangles and circles, respectively; y=0.009844x+0.6045), and the females from the laboratory longevity experiment (black circles; y=0.01823x–0.0205). (D) Peak flight metabolic rate plotted against pupal mass in individuals from the field longevity experiment (y=0.01014x+0.2361). Longevity data were available for a subset only (black triangles).

to account for temporal autocorrelation. Linear regression and ANCOVA were used to analyse associations between metabolic rate, *Pgi* genotype and lifespan. When metabolic rate was used as an explanatory variable, only mass-independent residuals were used.

RESULTS

Repeatability of metabolic rates

In the experiment on 12 frequently measured males, both whole-animal RMR (Table 1) and mass-independent RMR (Table 2) were significantly repeatable, though the level of repeatability was relatively low (r=0.24 and 0.17 for whole-animal and mass-independent RMR, respectively; Fig. 3A, supplementary material Fig. S1A). Fitting a repeated-measures mixed model to the data revealed a U-shaped relationship with age: whole-animal RMR was highest at young age, declined as the butterfly grew older and began to increase towards the end of the life (age, $F_{1,73}$=2.75, P=0.10; age squared, $F_{1,73}$=36.52, P<0.0001). Correcting for the effect of body mass did not change the pattern; the relationship between mass-independent RMR and age was also nonlinear (age, $F_{1,73}$=2.84, P<0.10; age squared, $F_{1,73}$=36.88, P<0.0001). In contrast, MR_{peak} remained relatively stable until the oldest ages, when there was a dramatic drop and the butterfly died 0–2 days later (Fig. 4A, supplementary material Fig. S2A). The mean (±s.e.m.) age was 15.3±0.58 days. We visually estimated a cut-off point at the age of 13 days and omitted measurements for older butterflies while calculating repeatability. One individual died at the age of 10 days and we removed its last measurement, which showed strong senescence. The data set therefore consisted of 11 males with six measurements and one male with five measurements. The repeatability value was 0.76 for both whole-animal and mass-independent MR_{peak}, and both were highly significant (Tables 1, 2).

In the second experiment involving nine males and 24 females, the repeatability of whole-animal RMR was not significant in the pooled sample ($F_{32,52}$=0.73, P=0.82) and repeatability was very low, –0.11 (supplementary material Fig. S1B). Similarly, mass-independent RMR showed very low repeatability (r=–0.24, $F_{32,52}$=0.51, P=0.98; Fig. 3B). RMR was highest during the first measurement and significantly lower during the following two measurements (whole-animal RMR, $F_{2,50}$=28.83, P<0.0001; mass-independent RMR, $F_{2,50}$=27.11, P<0.0001). We repeated the analyses for the last two measurements only. Now age had no significant effect on whole-animal RMR ($F_{1,18}$=1.33, P=0.26) or mass-independent RMR ($F_{1,18}$=0.78, P=0.39), but repeatability remained low and nonsignificant (whole-animal RMR, $F_{18,19}$=1.60, P=0.16, r=0.23; mass-independent RMR, $F_{18,19}$=0.75, P=0.73, r=–0.1). We then analysed repeatability for unmated males, mated females and unmated females separately. The sample included only two mated males so they were omitted from the analysis. RMR was non-repeatable in all subgroups (Tables 1, 2).

As the measurement interval was 6 days and the maximum age at the time of the last measurement was 15 days, only a few individuals showed signs of senescence in MR_{peak}. One male and one female died on the day of their last measurement and one female died the day after. We therefore omitted the last data points for these individuals from the analysis of repeatability of MR_{peak}. Repeatability was significant for the pooled sample: r=0.61 for whole-animal MR_{peak} ($F_{31,49}$=4.95, P=0.0000) and r=0.54 for mass-independent MR_{peak} ($F_{31,49}$=3.95, P=0.0000). Calculating

Table 1. Repeatability of the measurements of uncorrected (no correction for body mass) resting metabolic rate (RMR) and peak metabolic rate (MR_{peak})

	Repeatability	Statistics
RMR		
Unmated males[a]	0.24*	$F_{11,75}$=3.26; P=0.001
Unmated males	–0.07	$F_{6,10}$=0.85; P=0.56
Mated females	–0.20	$F_{10,20}$=0.53; P=0.85
Unmated females	–0.13	$F_{12,19}$=0.72; P=0.72
MR_{peak}		
Unmated males[a]	0.76***	$F_{11,58}$=19.97; P<0.0001
Unmated males	0.91***	$F_{5,9}$=24.91; P<0.0001
Mated females	0.50*	$F_{10,19}$=3.70; P=0.0068
Unmated females	0.46*	$F_{12,18}$=3.00; P=0.018

[a]Measured every second day. Others were measured every sixth day.
*P<0.05; ***P<0.001.

repeatability separately for mated and unmated males and females revealed differences among the groups. Unmated males had exceptionally high repeatability: r=0.91 for whole-animal MR_{peak} (Table 1, supplementary material Fig. S2B) and r=0.90 for mass-independent MR_{peak} (Table 2, Fig. 4B). Unmated females showed lower repeatability. Whole-animal MR_{peak} repeatability was 0.46, which was statistically significant (Table 1B, supplementary material Fig. S2C), but mass-independent MR_{peak} was not significantly repeatable (r=0.12, P=0.28; Table 2, Fig. 4C). Repeatability was significant in mated females: r=0.50 and 0.46 for whole-animal and mass-independent MR_{peak}, respectively (Tables 1, 2, Fig. 4C, supplementary material Fig. S2C).

Association between metabolic rates, lifespan and *Pgi* genotype

In the laboratory experiment, 146 females were maintained under controlled conditions in a greenhouse. Heterozygous individuals in *Pgi_111* (AC) lived for a mean (±s.e.m.) of 13.1±0.65 days, which was highly significantly longer than the average lifespan of the AA homozygotes, 9.7±0.78 days ($F_{1,144}$=11.2, P=0.001). A randomly selected subset of 71 females was used for the measurement of metabolic rates. The effect of *Pgi* on lifespan was also significant ($F_{1,69}$=4.39, P=0.04) in this sub-set of butterflies (13.4±1.1 *versus* 10.5±0.89 days in the AC and AA individuals, respectively).

We added mass-independent RMR to the model as a covariate, but it had no significant effect on lifespan, although there was a weak positive trend ($F_{1,68}$=1.84, P=0.18). In contrast, mass-independent MR_{peak} had a highly significant effect on lifespan whereas now the effect of *Pgi* genotype became nonsignificant. The final model thus consisted of MR_{peak} as the only explanatory variable, which had a highly significant effect (t_{70}=4.41, P<0.0001) and explained 22% of variation in lifespan (Fig. 5A). The effects of *Pgi_111* and MR_{peak} were exclusive as *Pgi* had a strong effect on MR_{peak} ($F_{1,69}$=27.21, P<0.0001). In contrast, there was no relationship between *Pgi_111* and RMR ($F_{1,69}$=0.09, P<0.77).

Table 2. Repeatability of the measurements of mass-independent (residual) RMR and MR_{peak}

	Repeatability	Statistics
RMR (residual)		
Unmated males[a]	0.17*	$F_{11,75}$=2.50; P=0.0099
Unmated males	–0.12	$F_{6,10}$=0.73; P=0.63
Mated females	–0.18	$F_{10,20}$=0.57; P=0.82
Unmated females	–0.33	$F_{12,19}$=0.40; P=0.95
MR_{peak} (residual)		
Unmated males[a]	0.76***	$F_{11,58}$=19.32; P<0.0001
Unmated males	0.90***	$F_{5,9}$=23.78; P<0.0001
Mated females	0.46*	$F_{10,19}$=3.31; P=0.01
Unmated females	0.12	$F_{12,18}$=1.34; P=0.28

[a]Measured every second day. Others were measured every sixth day.
*P<0.05; ***P<0.001.

In the field experiment, the lifespan of male butterflies older than 10 days was measured (see Materials and methods). Lifespan ranged from 10 to 18 days with a mean of 12.7 days. RMR had no effect on lifespan (t_{20}=–1.16, P=0.26), but the effect of MR_{peak} was positive and highly significant (t_{20}=3.07, P=0.006), explaining 33% of variation in lifespan (Fig. 5B). *Pgi_111* had no effect on lifespan ($F_{1,19}$=0.43, P=0.66), nor was there any relationship between *Pgi_111* and MR_{peak} or RMR ($F_{1,19}$=1.06, P=0.37 and $F_{1,19}$=2.16, P=0.15, respectively).

In the third experiment, butterflies were subjected to repeated flight treatments in the laboratory. The mean (±s.e.m.) lifespan was 14.0±0.86 days. We modelled lifespan using the first measurement of mass-independent MR_{peak} (age 3 days) as the explanatory variable. There was a significant positive correlation between lifespan and mass-independent MR_{peak} (t_{34}=2.31, P=0.027), explaining 14% of variation in lifespan (Fig. 5C). This data set allowed us to test the effect of MR_{peak} on lifespan at the ages of 9 and 15 days. The correlation was positive, although not significant, at the age of 9 days (t_{23}=1.58, P=0.12, supplementary material Fig. S3). At the age of 15 days the correlation was significant (t_{14}=3.28, P=0.006). Mass-independent RMR showed no significant correlation with lifespan at the age of 3 days (t_{34}=1.14, P=0.26), 9 days (t_{23}=0.68, P=0.51) or 15 days (t_{14}=1.01, P=0.33).

DISCUSSION

Repeated measurements of resting and flight metabolic rates

Repeatability is calculated as the intraclass correlation coefficient (Lessells and Boag, 1987), and it thus represents the proportion of variance that is explained by variation among individuals. High repeatability implies that measurement error is small and the measurements hence yield reliable estimates of the trait. Significant repeatability also means that the trait value remains consistent over time. The latter inference is relevant here, as it indicates that an individual butterfly with a high metabolic rate in a particular measurement tends to have high energy expenditure throughout its life.

MR_{peak} was found to be significantly repeatable in two experiments with somewhat different methodology. In the first experiment males were measured every second day throughout their life, while in the second experiment mated and unmated individuals of both sexes were measured every sixth day for a maximum of three times. We calculated the repeatability of both raw whole-animal MR_{peak} and mass-independent (residual) MR_{peak}. Both traits showed generally very similar repeatability, although the repeatability of whole-animal metabolic rate tended to be higher than that of mass-independent metabolic rate. Repeatability was higher in the first experiment (r=0.76; identical for both whole-animal and mass-independent MR_{peak}) than in the pooled sample of the second experiment (r=0.61 and 0.54 for whole-animal and mass independent MR_{peak}, respectively). However, analysing the different groups of the second experiment separately revealed differences related to mating status and sex. Unmated males had extraordinarily high repeatability (r=0.91 and 0.90). Curiously, mated females had higher repeatability than unmated females (r=0.50 *versus* 0.46 for whole-animal MR_{peak} and 0.46 *versus* 0.12 for mass-independent MR_{peak}). The difference between mated and unmated females may be due to differences in

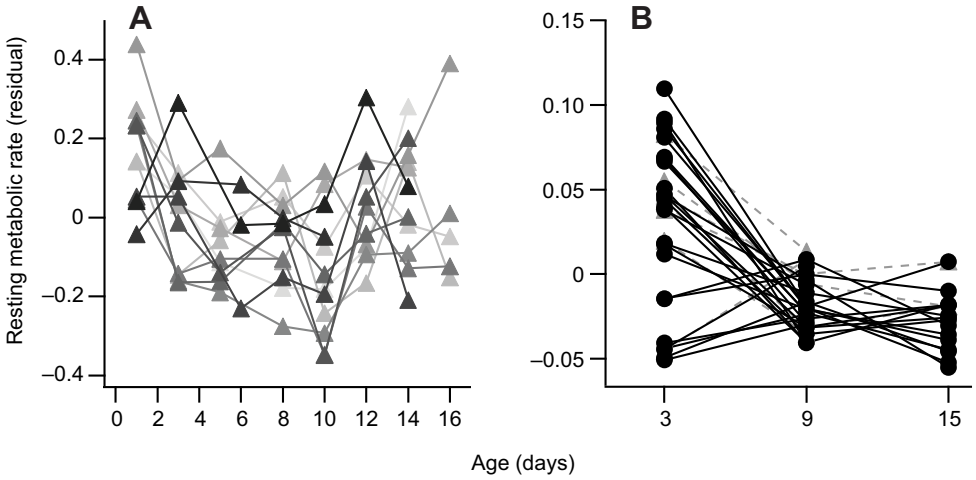

Fig. 3. Mass-independent (residual) resting metabolic rate (RMR) measured throughout the adult life of Glanville fritillaries. (A) RMR of 12 male butterflies, measured every second day. RMR showed a nonlinear relationship with age (see Results). The repeatability was significant but low (r=0.17). (B) Mass-independent RMR of nine male (grey triangles) and 24 female (black circles) Glanville fritillaries. RMR declined significantly after the first measurement. Repeatability was nonsignificant and very low (r=–0.24). Repeatability remained low even when analysing only data for the two last measurements (r=–0.1).

condition or health, as females were allowed to mate freely, which may have led to low-quality females not mating. Nevertheless, taken together, both experiments show that MR_{peak} is in most cases repeatable during the life of the butterfly. These results are consistent with an emerging pattern in the literature: studies on metabolic rates in species ranging from insects and reptiles to mammals and birds have shown metabolic rate to be a repeatable trait (Nespolo and Franco, 2007).

The repeatability of RMR was significant but low (r=0.24 and 0.17 for whole animal and mass-independent RMR, respectively) in the first experiment but clearly nonsignificant in the second experiment. In both experiments RMR was significantly elevated at the youngest ages and then declined as the individual grew older. RMR appears thus to be more prone to temporal variation than MR_{peak}. In another nymphalid butterfly, *Vanessa cardui*, RMR has been shown to be a repeatable trait in starved individuals but not in fed individuals (Woods et al., 2010). Factors such as absorptive and nutritional status may have a great effect on RMR, especially in species that are short-lived and reproductively active throughout most of their adult life. At the individual level, RMR is known to be affected by a number of genetic and environmental factors and their interactions (Arnqvist et al., 2010; Burton et al., 2011), and it also appears that there is a high level of within-individual plasticity in RMR. Measurement error is likely to play some part in the low repeatability of RMR, especially as the measured CO_2 concentrations are low. However, measurements of RMR are reliable enough to allow detection of the effects of body mass, temperature and time of day (Niitepõld, 2010). It is noteworthy that *Pgi_111* has an effect on MR_{peak} but not on RMR in the Glanville fritillary. RMR also seems to be a poor predictor of metabolic capacity as it is not correlated with MR_{peak} (Niitepõld, 2010). Although undoubtedly there are genetic factors affecting RMR, these may be masked by processes related to the current physiological state of the individual, hence the low repeatability of RMR.

Senescence, a progressive and deleterious process leading to reduced organismal performance (Hulbert et al., 2007), is well known to occur in humans but has been rarely reported in wild animals until recently. Presently, a growing body of literature has reported senescence in a wide variety of species, including unicellular organisms (Ackermann et al., 2003; Stewart et al., 2005) and a large number of vertebrates in the wild (Jones et al., 2008). In *Drosophila melanogaster*, functional senescence occurs in traits such as wing beat frequency during tethered flight (Petrosyan et al., 2007), flight performance (flying towards a light source) (Miller et al., 2008), and negative geotaxis and phototaxis (crawling upwards after disturbance, and crawling towards a light source, respectively) (Leffelaar and Grigliatti, 1983). Metabolic senescence has been described in humans (Roberts and Rosenberg, 2006) and laboratory animals such as rats (Even et al., 2001), but its occurrence is not necessarily widespread, especially in the wild. Longitudinal studies

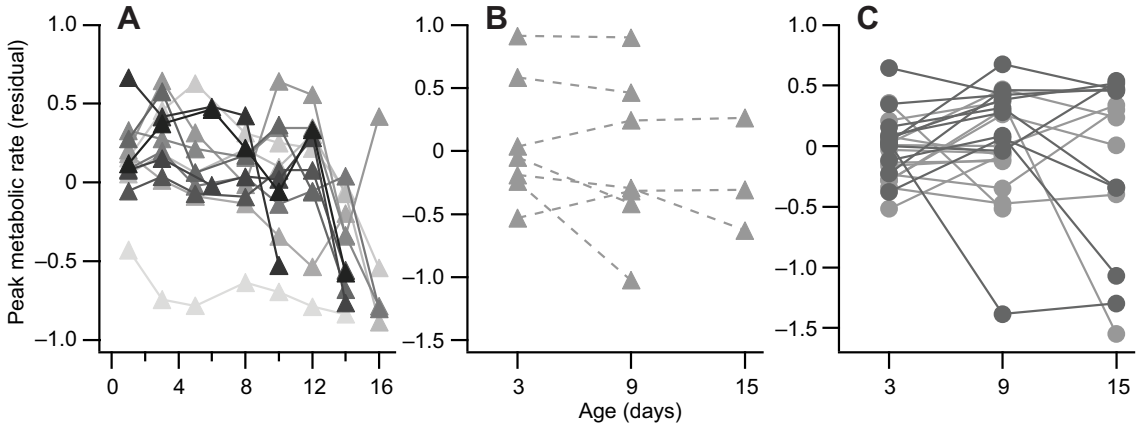

Fig. 4. Repeated measurements of mass-independent (residual) peak flight metabolic rate in (A) male Glanville fritillaries measured every second day, (B) males measured with an interval of 6 days and (C) females measured every 6 days. The repeatability of the measurements was 0.76 in the first experiment and 0.90 in the males of the second experiment. The repeatability was 0.46 for mated females (dark grey circles), but only 0.12 for unmated females (light grey circles).

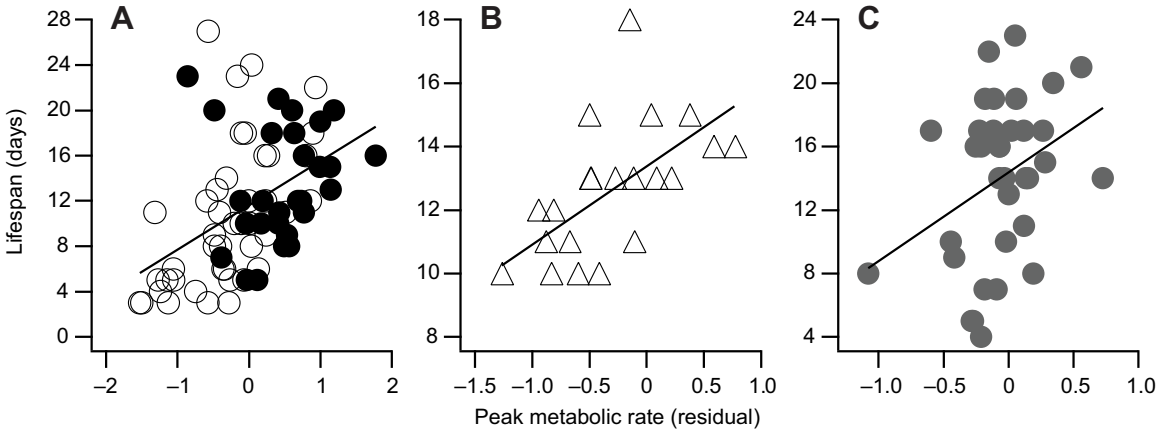

Fig. 5. Lifespan *versus* mass-independent peak flight metabolic rate in the Glanville fritillary. (A) Females maintained in cages in a greenhouse under controlled conditions. The grey squares represent AA homozygotes and black circles AC heterozygotes in *Pgi_111*. (B) Male butterflies reared in the laboratory and released into the field. Individuals older than 10 days were recaptured, brought to the laboratory and kept in cages outdoors to record their lifespan. (C) Females subjected to repeated flight treatments in the laboratory every third day. Peak flight metabolic rates are residuals from regressions of peak flight metabolic rate against adult body mass (A,C) or pupal mass (B).

on relatively long-lived vertebrates are rare and although some studies have detected metabolic senescence (Broggi et al., 2010), others have found no signs of it (Moe et al., 2007). The few studies that have looked into insect metabolic senescence have produced mixed results. In five species of *Drosophila*, none showed metabolic senescence (Promislow and Haselkorn, 2002). The house cricket, in contrast, appears to experience a reduction in mass-specific RMR as it ages (Hack, 1997). In the present repeatability experiment in which males were measured with an interval of 2 days, MR_{peak} showed a sharp decline 0 to 2 days before death (Fig. 3A). Senescence in flight metabolism thus appears late in the life of the butterfly and seems to have limited influence on performance during most of the life. At the same time, this result highlights the significance of maintaining flight capacity for as long as possible regardless of the energetic cost.

The patterns seen in RMR did not appear to reflect senescence even though RMR typically declined from the first measurements (Fig. 3). An initially high RMR in newly eclosed individuals may result from a number of processes that take place in a maturing individual, such as egg development in females (Clifford and Woodring, 1986) and preparation of spermatophore production in males. Similarly, the flight muscles of young insects undergo maturation as exemplified by changes in muscle troponin T composition (Marden et al., 2008; Schippers et al., 2010). As the individual grows older, investment in these energy-consuming processes decreases. Elevated metabolic rate in young individuals may be a general phenomenon, as the pattern reported here bears a striking resemblance to the relationship between metabolic rate and age in *D. melanogaster* by Khazaeli et al. (Khazaeli et al., 2005). The butterfly *Vanessa cardui* exhibits similarly elevated RMR at the very youngest age (Woods et al., 2010). In the Colorado potato beetle, RMR increases rapidly during the first days of the life of an adult individual and then begins to decrease (Piiroinen et al., 2010). This too suggests high physiological activity in maturing individuals. Our first experiment where males were measured until the end of their lives revealed a subsequent elevation in RMR towards the end of life (Fig. 3A). This pattern may represent increased investment in somatic maintenance as the individual ages, as reported for *Drosophila similans* (Melvin et al., 2007). The increasing RMR could therefore reflect the energetic cost of maintaining high metabolic capacity until the very end of life.

Relationship between metabolic rate and lifespan

We found no relationship between RMR and lifespan in any of our experiments. A similar result has been found in several previous studies that have compared energy consumption and individual lifespan within a particular species, for example in different species of *Drosophila* (Hulbert et al., 2004; Melvin et al., 2007). RMR represents the minimum metabolic rate in the absence of locomotion, but it is subject to substantial variation due to physiological processes. It is also questionable how well RMR correlates with lifetime energy consumption (Speakman, 2005), and therefore the supposed link between RMR and lifespan is not clear even from a theoretical point of view. The very low repeatability of RMR in the current experiments suggests that there are no consistent differences in RMR between individual butterflies. The contribution of RMR to between-individual differences in whole-life energy consumption would therefore be small. In contrast, there were systematic differences in MR_{peak} between individuals. Significant differences in lifetime energy consumption could thus arise from differences in flight metabolic rate and the frequency of flight. Previous work shows that flight metabolic rate and activity in the field are positively correlated in the Glanville fritillary (Niitepõld et al., 2009). Behaviour can therefore greatly amplify differences in lifetime energy consumption.

Peak flight metabolic rate was very clearly and positively correlated with lifespan (Fig. 5, supplementary material Fig. S3). The same positive correlation was found even among individuals that spent most of their lives in the field and exhibited the usual behaviours related to foraging, mate location and dispersal. Likewise, a positive correlation was present among females that were kept in the laboratory but forced to fly every third day for a period of time that roughly corresponds to one day's combined flight in the field (Ovaskainen et al., 2008). The connection between peak metabolic rate or $\dot{V}_{O_2,max}$ and lifespan has rarely been addressed so far, especially at the intraspecific level. This is probably due to methodological difficulties in measuring maximum metabolic rate in many animals. Insects such as butterflies flying freely inside a respirometry can be measured without complicated experimental setups and training of animals. A clear peak and subsequent fatigue is routinely seen during measurements of butterfly flight metabolic rate, suggesting that a true metabolic maximum is reached. Also,

adding lead weights to the thorax did not increase peak flight metabolic rate in a moth measured using the same technique as in our experiments (Marden et al., 2008). Because peak metabolic rate may be more directly linked with fitness than RMR, we hope to see more work focused on metabolic capacity and lifespan.

Why would individuals with the highest metabolic capacity and possibly high lifetime energy consumption live the longest? Elevated energy consumption due to activity such as flight is known to increase oxidative stress (Yan and Sohal, 2000; Costantini et al., 2008) and even reduce lifespan in some species (Sohal and Buchan, 1981; Magwere et al., 2006). However, the links between energy consumption, oxidative stress and lifespan are not straightforward (Van Voorhies, 2001; Speakman, 2005). Contrary to early assumptions, ROS production has been shown to vary between the different stages of mitochondrial respiratory activity and it is not linearly correlated with oxygen consumption (Barros et al., 2004; Kowaltowski et al., 2009). Mitochondria are the main source of ROS (Barja, 2002), but the rate of ROS production appears to be low at high rates of oxygen consumption when mitochondrial membrane potential is low (Nicholls, 2004; Kowaltowski et al., 2009). Membrane potential is also lowered by proton leak due to mild uncoupling performed by uncoupling proteins, which further reduces ROS production (Korshunov et al., 1997; St-Pierre et al., 2002). Furthermore, the fatty acid composition of cell membranes is not the same across species and such differences may lead to dissimilar levels of susceptibility to oxidative damage (Hulbert et al., 2007; Montgomery et al., 2011). Studies on long-lived mutant strains have shown that low metabolic rate is not a requirement for long lifespan (Marden et al., 2003; Van Voorhies et al., 2003). In addition, some very long-lived species do not appear to possess exceptional defence mechanisms against oxidative damage (Andziak et al., 2006; Speakman and Selman, 2011). Therefore, it appears that the argument of high energy expenditure leading to increased ROS production, elevated oxidative damage and shortened lifespan may not be universally true. Recent studies suggest that while ROS are undoubtedly associated with ageing, they are not the only factors that contribute to ageing (Kirkwood and Kowald, 2012).

Evolutionary theories of ageing recognise that the rate of ageing and longevity are adaptive traits that evolve under selection pressure set by the environment (Kirkwood and Austad, 2000). For example, it is well known that flying vertebrates with high mass-specific energy expenditure also have long lifespans, which implies that these groups have effective means of regulating ROS production or preventing or tolerating oxidative damage (Brunet-Rossinni, 2004; Hulbert et al., 2007; Munshi-South and Wilkinson, 2010). Butterflies with high metabolic capacity may therefore possess adaptations providing stronger defence mechanisms and higher resistance to oxidative stress. While houseflies and fruit flies can show a simple relationship between flight and longevity (Sohal and Buchan, 1981; Magwere et al., 2006), experimental work has shown that the effect of forced flight on butterfly longevity is far from uniform. Depending on the population of origin (Gibbs and Van Dyck, 2010) or the conditions experienced during development (Saastamoinen et al., 2010), butterflies may be seemingly immune to negative effects of flight on lifespan. It is also worth pointing out that we examined in our experiments existing individual variation in metabolic rate. We do not yet know how individuals with different metabolic rate and therefore dissimilar life-history strategies would react to experimentally increased energy consumption. Even though our experiment on free-flying males allowed males to express normal levels of flight activity, other environmental conditions could have yielded different results.

Because it is unlikely that a long lifespan would be a direct consequence of having a high metabolic rate, the positive intraspecific association between MR_{peak} and lifespan may arise from both traits being correlated with the general physiological condition of an individual. Condition in turn reflects the total amount of resources that can be allocated to various fitness-related somatic and reproductive processes (Tomkins et al., 2004; Ketola and Kotiaho, 2009), including protection against ageing. Individuals with high condition can afford to allocate energy to defence and repair mechanisms and still invest in flight and reproduction. For example, experimental work on free-living alpine swifts (*Apus melba*) has demonstrated that resistance to oxidative stress correlates positively with survival and fecundity (Bize et al., 2008). Differences in lifespan among individuals may be best explained by the size of the resource pool, including critical nutrients, and resource allocation strategies. When resources are not limited, a positive correlation may exist between traits that in other situations may compete for the same resources (Zera and Harshman, 2001). The curious transgenic PEPCK-C^{mus} mice are an example of the plasticity of organismal performance. The mice showing overexpression of a phosphoenolpyruvate carboxykinase isoform are hyperactive and apparently live and remain reproductively active for much longer than wild-type mice (Hakimi et al., 2007; Hanson and Hakimi, 2008). The most significant cost, apart from aggressive behaviour, is increased food intake. The high energy demand may explain why natural selection has not favoured high expression of the enzyme in wild mice. Lifespan can also show much plasticity. In the honeybee, lifespan largely depends on social function, not chronological age, and senescence can even be reversed if the worker's function changes (Tolfsen et al., 2011). The study of ageing may benefit from more attention on how resources are allocated among different processes (Boggs, 2009). This is particularly relevant while trying to understand how lifespan is affected by stressful conditions when resources are scarce (Gibbs and Van Dyck, 2010; Saastamoinen et al., 2010).

Pgi genotype, metabolic rate and lifespan

The *Pgi* genotype was significantly correlated with lifespan in the laboratory experiment on females but not in the field experiment on males. This is consistent with the results of Klemme and Hanski (Klemme and Hanski, 2009), who found the same association of *Pgi_111* with longevity in females but not in males. Saastamoinen et al. (Saastamoinen et al., 2009) have reported a significant effect in both sexes, but the effect was stronger in females.

The highly polymorphic *Pgi* gene encodes the glycolytic enzyme phosphoglucose isomerase (Watt, 1977) and has been found to have various effects on organismal performance and fitness in many butterflies (Watt et al., 1983; Karl et al., 2008; Saastamoinen and Hanski, 2008) and in the willow beetle *Chrysomela aeneicollis* (Dahlhoff and Rank, 2000). The *Pgi* genotype has a strong effect on flight metabolic rate in the Glanville fritillary (Haag et al., 2005; Niitepõld et al., 2009; Niitepõld, 2010). The effect interacts with ambient temperature, probably because of a molecular level trade-off between enzyme kinetics and thermal stability (Watt et al., 1983).

The effects of *Pgi* genotype on MR_{peak} and lifespan in the Glanville fritillary have been observed in several studies (see above) and are hence well established, but the explanation of the genotypic effect on lifespan remains unknown. Individuals with generally high energy expenditure can possess mechanisms preventing oxidative damage. Mechanisms that protect against various kinds of stress have been suggested to play a role in determining lifespan (Vermeulen and Loeschcke, 2007). One potential protective

mechanism linked to *Pgi* is high expression of heat shock proteins that protect proteins from denaturation in high temperatures but also under other types of stressful conditions (Feder and Hofmann, 1999). Thermally sensitive PGI alleles have been associated with high heat shock protein expression in the willow beetle (Dahlhoff and Rank, 2000). Although being costly to produce (Hoffmann and Hewa-Kapuge, 2000), heat shock proteins have been linked to defence against ageing and to increased lifespan in *C. elegans* and *Drosophila* (Morrow et al., 2004; Kenyon, 2005; Tower, 2009). Thus heat shock proteins or other protective mechanisms could contribute to the longer lifespans of active genotypes and phenotypes. We suggest that this could explain the higher lifespan of the AC heterozygotes in *Pgi_111*, which have higher flight metabolic rate than the AA homozygotes. Concerning the positive relationship between MR_{peak} and lifespan, our results indicate a similar relationship in both genotypes (Fig. 5A). As a next step, it would be informative to examine the relationships between *Pgi* genotype, MR_{peak} and lifespan under dissimilar environmental and nutritional conditions and under dissimilar levels of physical activity.

LIST OF ABBREVIATIONS

MR_{peak}	peak flight metabolic rate
RMR	resting metabolic rate
ROS	reactive oxygen species

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AUTHOR CONTRIBUTIONS

K.N. and I.H. designed and executed the study, interpreted the results and wrote the paper.

COMPETING INTERESTS

No competing interests declared.

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