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# Genotype by temperature interactions in the metabolic rate of the Glanville fritillary butterfly

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## SUMMARY

**Metabolic rate is a highly plastic trait. Here I examine factors that influence the metabolic rate of the Glanville fritillary butterfly (*Melitaea cinxia*) in pupae and resting and flying adults. Body mass and temperature had consistent positive effects on metabolic rate in pupae and resting adults but not in flying adults. There was also a consistent nonlinear effect of the time of the day, which was strongest in pupae and weakest in flying adults. Flight metabolic rate was strongly affected by an interaction between the phosphoglucose isomerase (*Pgi*) genotype and temperature. Over a broad range of measurement temperatures, heterozygous individuals at a single nucleotide polymorphism (SNP) in *Pgi* had higher peak metabolic rate in flight, but at high temperatures homozygous individuals performed better. The two genotypes did not differ in resting metabolic rate, suggesting that the heterozygotes do not pay an additional energetic cost for their higher flight capacity. Mass-independent resting and flight metabolic rates were at best weakly correlated at the individual level, and therefore, unlike in many vertebrates, resting metabolic rate does not serve as a useful surrogate of the metabolic capacity of this butterfly.**

Key words: allometry, flight, genotype by environment interaction (G×E), insect, metabolism, phosphoglucose isomerase.

## INTRODUCTION

Metabolic rate varies among individuals and within single individuals depending on their behaviour and physiological state. Genetic variation is known to affect the metabolic rate of insects (Laurie-Ahlberg et al., 1985; Nespolo et al., 2007), and quantitative trait locus (QTL) mapping has revealed associations between particular chromosomal regions and metabolic traits in *Drosophila melanogaster* (Montooth et al., 2003). However, not many specific loci linked with variation in metabolic rate have been identified so far, although this situation is likely to change with the rapid development of genomic tools even for non-model species (Ellegren and Sheldon, 2008).

One example of a candidate gene associated with flight metabolic rate is the polymorphic malate dehydrogenase (allozyme) locus in honeybees (Coelho and Mitton, 1988; Harrison et al., 1996). Different electromorphs of the enzyme have been shown to be associated with different levels of metabolic rate. Another promising candidate gene is phosphoglucose isomerase (*Pgi*), which codes for the glycolytic enzyme PGI, well known for functional variation in *Colias* butterflies (Watt, 1977; Watt et al., 2003) and in willow beetles (Dahlhoff et al., 2008; Dahlhoff and Rank, 2000). PGI is known to be sensitive to temperature. Different forms of the enzyme vary in their kinetics and thermal stability: homozygous isoforms with high activity ($V_{max}/K_m$) tend to be thermally unstable whereas those isoforms that tolerate high temperature have low enzymatic activity (Watt, 1983). Heterozygous forms of the enzyme may combine superior kinetics with good thermal stability, affecting organismal performance and fitness (Watt and Dean, 2000). In the Glanville fritillary butterfly (*Melitaea cinxia*), flight metabolic rate varies between the PGI allozyme electromorphs (Haag et al., 2005). Differences between PGI genotypes have recently been traced to single nucleotide polymorphisms (SNPs) in the *Pgi* sequence (Orsini et al., 2009), and a single SNP in *Pgi* is sufficient to explain a significant amount of variation in flight metabolic rate and dispersal in the Glanville fritillary (Niitepõld et al., 2009).

Flight is essential for butterflies and many other insects. Flight capacity may have direct fitness consequences because of processes such as escape from predators, foraging and searching for oviposition sites. The long-term persistence of the Glanville fritillary in fragmented landscapes is dependent on frequent dispersal between local populations and colonisation of new habitat patches (Hanski and Ovaskainen, 2000). Flight capacity may also have indirect consequences in the form of trade-offs with other energy-demanding processes such as reproduction (Nespolo et al., 2008; Saglam et al., 2008). The need for a high flight capacity may be reflected in the cost of maintenance, namely in the resting metabolic rate, but while a positive relationship between maximum and minimum metabolic rates is well established for vertebrates (Bennett and Ruben, 1979; Dutenhoffer and Swanson, 1996; Hinds et al., 1993; Walton, 1993; White and Seymour, 2004), it is less clear what this relationship is in invertebrates and in insects in particular (Niven and Scharlemann, 2005; Reinhold, 1999).

The goal of this study was to identify factors that affect resting and flight metabolic rates in pupae and adults of the Glanville fritillary butterfly. Apart from the effects of the time of the day and body mass on metabolic rates, I also examined the effect of the *Pgi* genotype and its interaction with temperature. The aim was to investigate how molecular variation in the *Pgi* locus translates to physiological performance under variable environmental conditions. The individual-level correlations between mass-independent pupal ($MR_{pupa}$), resting (RMR) and peak flight ($MR_{peak}$) metabolic rates were examined to assess whether the flight metabolic rate could be predicted with measurements of the minimum metabolic rate.

## MATERIALS AND METHODS

### Study species and design

The Glanville fritillary butterfly (*Melitaea cinxia* L.) is a north-temperate butterfly with a geographical distribution ranging from west Europe to south Siberia (Tolman and Levington, 1997). The species and its metapopulation dynamics have been intensively studied in the Åland Islands in SW Finland since 1991 (Ehrlich and Hanski, 2004; Hanski, 1999). These studies have addressed questions such as the effect of habitat fragmentation on population dynamics (Hanski and Ovaskainen, 2000) and inbreeding depression in natural populations (Saccheri et al., 1998). Recent advances in molecular techniques have made it possible to start building up a genome-level understanding of ecological processes (Vera et al., 2008). A major focus of the past work has been on dispersal, which is known to be functionally linked with flight metabolic rate (Niitepõld et al., 2009).

The present study consisted of two experiments. In the first experiment, the resting (RMR) and peak flight metabolic rates ($MR_{peak}$) of adult females were measured under suboptimal (26°C) and optimal (32°C) temperatures. In the second experiment, females were first measured as pupae and subsequently as adults at rest and in flight. The latter measurements were taken over a range of temperatures (29–35°C).

### Experiment on resting and flight metabolic rates in suboptimal and optimal temperatures

The experimental individuals originated from the Åland Islands and were the offspring of a generation kept in a large outdoor cage (Saastamoinen, 2007). The parents had been collected in the field as newly eclosed adults. The larvae were reared in controlled laboratory conditions (12 h:12 h L:D, and 25°C:20°C). Larvae had constant access to leaves of the host plant *Plantago lanceolata*. After eclosion butterflies were marked and weighed and a small piece from the edge of the hind wings (max. 2 mm×2 mm) was cut for molecular analyses.

The metabolic rates of 71 females were measured as $CO_2$ emission rate using flow-through respirometry on the second full day after eclosion. Each individual was measured once, either in suboptimal (26°C) or optimal temperature (32°C). RMR and $MR_{peak}$ were measured during the same trial, first RMR, when the respirometry jar was covered with a black cloth, then the measurement of $MR_{peak}$, when the cover was removed and the individual was exposed to UV light and agitated to fly. Individuals were kept in a 1-l cylindrical transparent polymethylpentene jar (Nalgene, Thermo Fisher Scientific, NY, USA) with dried and $CO_2$-free air flowing through at the rate of 1 l $min^{-1}$. The temperature inside the jar was measured using an NTC thermistor (Sable Systems, Las Vegas, NV, USA). The measurements were performed in two separate temperature-controlled rooms. Magnesium perchlorate was used to dry the air before it entered the Li-Cor 6251 $CO_2$ analyser (Li-Cor Biosciences, Lincoln, NE, USA). The analyser was calibrated against two gas mixtures before the experiment: one with no $CO_2$ and the other with a concentration corresponding to the highest levels produced by the butterflies. During the experiment the analyser was calibrated against the zero gas two to three times a day. The program ExpeData (Sable Systems) was used to record the data.

The experimental setup did not allow detection of detailed patterns in gas exchange during the resting stage, rather it gave a smoothed average. Measurements of resting metabolic rate with better temporal resolution have however shown no signs of discontinuous gas exchange in the Glanville fritillary (K.N., unpublished data). RMR appeared stable over long periods and rare events of activity inside the darkened chamber could be detected as rise in the $CO_2$ curve. If an individual became active before the curve had stabilised to a steady baseline, additional time was allowed for the butterfly to settle to complete rest. RMR was calculated as the mean $CO_2$ emission rate during a period of 40 s of stable $CO_2$ production. Half of the RMR measurements were performed at 26°C (s.d.=0.82, min.=23.3°C, max.=26.8°C), and the other half at 32°C (s.d.=0.73, min.=30.2°C, max.=33.0°C).

Following the measurement of RMR, the black cloth was removed and the butterfly was stimulated to fly as continuously as possible by shaking and tapping the jar for 10 min. The butterfly was thereby repeatedly forced to fly as soon as it attempted to land on the walls of the respirometry jar. Individuals typically flew more or less continuously for the first 3 min, after which the length of flight bouts became more variable. Some individuals showed clear signs of fatigue towards the end of the experiment, whereas others flew for the full 10 min period. The measurement temperature remained relatively stable during the measurement. The average temperature at the moment of the highest $CO_2$ production was 26.7°C (s.d.=0.53, min.=25.0°C, max.=27.4°C) in the lower temperature treatment, and 32.3°C (s.d.=0.55, min.=31.0°C, max.=33.0°C) in the higher temperature treatment.

### Experiment on pupal and adult resting metabolic rates and flight metabolic rate in a range of temperatures

In the second experiment butterflies originating from the Åland Islands, Estonia and China were used. All larvae were reared in common garden conditions starting from winter diapause. Individuals from Åland were the offspring of butterflies collected in the field as larvae, reared in the laboratory and mated in an outdoor cage. Estonian and Chinese individuals were collected as larvae in the respective field sites in the previous autumn. All larvae were reared in growth chambers (16 h:8 h L:D). The ambient temperature followed a stepwise programme with a maximum of 28°C at mid-day and 12°C in the night. Larvae were fed *ad libitum* with leaves of *Plantago lanceolata*. There were no differences in the metabolic rates of butterflies originating from the different source populations, and results for the pooled material are therefore reported in this study.

The metabolic rate of each individual was measured twice in this experiment: first as a pupa (on average 7.5 days before eclosion) and then as an adult on the second full day after eclosion. A pupa was gently placed in a 7 ml respirometry chamber with dried and $CO_2$-free air flowing through at the rate of 480 ml $min^{-1}$. The respirometer was kept in darkness during the measurement. The individuals recovered quickly from the handling and showed stable metabolic rates after the first 2 min. The gas exchange was continuous and fluctuated around a stable mean value. Individuals did not generally show signs of closing their spiracles fully for any extended periods. Pupal metabolic rate ($MR_{pupa}$) was calculated as the average $CO_2$ production of the last 5 min of the 10 min measurement period. The average measurement temperature was 25.3°C (s.d.=0.53, min.=23.9°C, max.=26.8°C). The pupae were stored in individual plastic cups with a moist piece of paper prior to and after the measurements.

After eclosion, butterflies were marked and weighed. Marked butterflies were moved to a mesh cage and left in shade. On the following day butterflies had flight practice in a cage exposed to sunlight for approximately 1 h. The measurements on adult butterflies were done on the second day after eclosion. The measurements of RMR and $MR_{peak}$ were performed as in the first experiment (above). The respirometry equipment was operated in

Table 1. Factors affecting resting metabolic rate and peak flight metabolic rate

| | RMR | | | $MR_{peak}$ | | |
|---|---|---|---|---|---|---|
| Variable | d.f. | *F* | *P* | d.f. | *F* | *P* |
| Body mass | 1,67 | 32.83 | **<0.0001** | 1,66 | 23.70 | **<0.0001** |
| Temperature | 1,67 | 135.91 | **<0.0001** | 1,66 | 3.55 | 0.0639 |
| *Pgi* | 1,67 | 0.32 | 0.5724 | 1,66 | 26.50 | **<0.0001** |
| Mass × *Pgi* | – | – | – | 1,66 | 3.75 | 0.0571 |

RMR, resting metabolic rate; $MR_{peak}$, flight metabolic rate.
Each individual was measured either at 26°C or at 32°C.
Bold indicates significance.

a large insulated plywood chamber that was heated with an electric heater and equipped with a fan to stabilise the temperature. The average temperatures inside the respirometry jar were 32.5°C (s.d.=1.28, min.=28.9°C, max.=35.0°C) during the measurements of RMR, and 32.9°C (s.d.=0.90, min.=30.8°C, max.=35.1°C) during the measurements of $MR_{peak}$.

### Genotyping

DNA was extracted from the wing samples taken during the marking of butterflies on the day of eclosion (see above). The *Pgi* genotype was characterised as a single nucleotide polymorphism (SNP) in the coding region of the *Pgi* gene, using the methods described for this species (Orsini et al., 2009).

### Statistical analyses

Factors affecting metabolic rate in the different experiments were analysed with ANCOVA using Proc Mixed in SAS 9.1. Backward selection was used to eliminate clearly nonsignificant factors ($P$>0.10) from the initial model that contained all two-way interactions and squared terms. A nonsignificant main effect was retained in the model if an interaction containing that term was significant. When the model contained quadratic terms type I sum of squares were used.

The effect of temperature on RMR was calculated as the $Q_{10}$ value, indicating the increase in metabolic rate with an increase in temperature of 10°C. In the first experiment $Q_{10}$ was calculated from the two temperature treatments using the average RMR for the respective treatments. In the second experiment, a linear regression was used to obtain the predicted RMR values from both ends of the temperature range and these values were used to calculate $Q_{10}$.

## RESULTS

The three possible base pair combinations (genotypes) at SNP AA111 are AA, AC and CC. The AA111 AC and CC genotypes correspond closely to the f-allele in previous allozyme studies (Haag et al., 2005; Saastamoinen, 2007). The frequency of the CC genotype is very low in the Åland metapopulation (Orsini et al., 2009), hence no CC homozygotes were found in the first experiment. With one exception, the CC homozygotes in the second experiment were Chinese and Estonian individuals. The reason for the very low frequency of the CC homozygotes in the Åland population is unclear, but it may be related to linkage with a deleterious recessive mutation (Orsini et al., 2009).

In the first experiment, the resting metabolic rate (RMR) was influenced by body mass and measurement temperature (Table 1). RMR for a given body mass was about twice as high at 32°C as at 26°C (Fig. 1A), yielding the $Q_{10}$ value of 2.6. Neither time of the day nor *Pgi* genotype had any significant effect on RMR, nor were any interactions significant (Table 1).

The peak flight metabolic rate ($MR_{peak}$) was positively correlated with body mass but the measurement temperature had only a weak and statistically nonsignificant effect on $MR_{peak}$ (Table 1). Instead, there was a strong effect of the *Pgi* genotype on $MR_{peak}$ in both temperature treatments. An average-sized AC heterozygote had ~45% higher $MR_{peak}$ than an AA homozygote (Fig. 1B). The effect of *Pgi* became stronger with increasing body mass, as indicated by the near-significant genotype by body mass interaction (Table 1).

In the second experiment, the pupal metabolic rate ($MR_{pupa}$) was influenced by the mass of the eclosed butterfly, temperature and a nonlinear time effect with a peak in the early afternoon (Fig. 2A–C, Table 2). *Pgi* genotype had no significant main effect, though there was a weak interaction suggesting that AC and CC individuals may have higher pupal metabolic rates in low temperatures than AA individuals (Fig. 2). Adult RMR was affected by the same factors as $MR_{pupa}$: body mass, temperature and time of the day (Fig. 2D–F, Table 2), but *Pgi* genotype had no effect on RMR. The $Q_{10}$ calculated for the range of temperatures from 29°C to 35°C was 2.1.

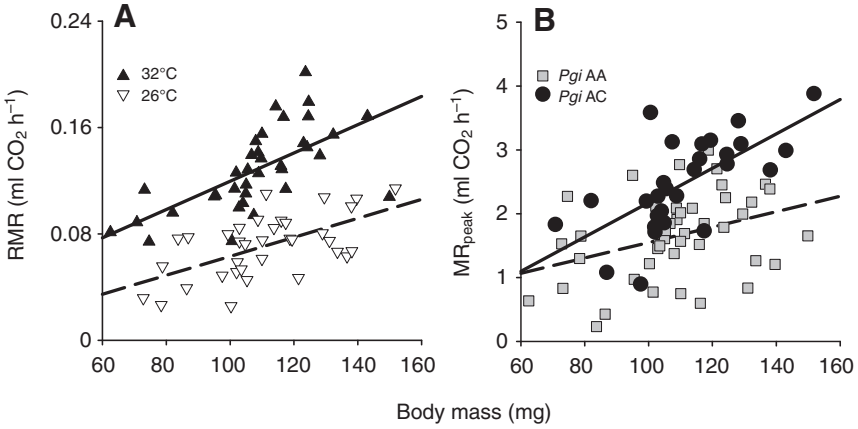


Fig. 1. (A) Resting metabolic rate (RMR) of adult butterflies measured at 26°C and at 32°C. The horizontal axis gives the wet adult body mass. *Pgi* genotype had no effect on RMR. (B) Peak flight metabolic rate ($MR_{peak}$) plotted against wet body mass. Grey squares and the dashed line represent *Pgi* AA111 AA homozygotes, black dots and the solid line *Pgi* AA111 AC heterozygotes. The temperature treatment had no significant effect.

Table 2. Factors affecting pupal metabolic rate, adult resting metabolic rate and adult peak metabolic rate in flight

| | $MR_{pupa}$ | RMR | $MR_{peak}$ |
|---|---|---|---|
| Body mass | $F_{1,64}$=4.71; **$P$=0.0336** | $F_{1,66}$=4.20; **$P$=0.0446** | $F_{1,64}$=0.00; $P$=0.9549 |
| Temperature | $F_{1,64}$=8.23; **$P$=0.0056** | $F_{1,66}$=10.52; **$P$=0.0019** | $F_{1,64}$=3.62; $P$=0.0615 |
| *Pgi* | $F_{1,64}$=0.36; $P$=0.7014 | $F_{1,66}$=1.17; $P$=0.3177 | $F_{1,64}$=0.42; $P$=0.6559 |
| Time | $F_{1,64}$=7.71; **$P$=0.0072** | $F_{1,66}$=1.07; $P$=0.3051 | $F_{1,64}$=0.01; $P$=0.9143 |
| $Time^2$ | $F_{1,64}$=5.42; **$P$=0.0231** | $F_{1,66}$=3.60; $P$=0.0622 | $F_{1,64}$=2.37; $P$=0.1285 |
| Temp × *Pgi* | $F_{1,64}$=2.41; $P$=0.0978 | $F_{1,66}$=0.36; $P$=0.6981 | $F_{1,64}$=5.46; **$P$=0.0065** |

$MR_{pupa}$, pupal metabolic rate; RMR, adult resting metabolic rate; $MR_{peak}$, adult peak flight metabolic rate.
The measurement temperatures ranged from 24°C to 27°C in pupae, from 29°C to 35°C in resting adults, and from 31°C to 35°C in flying adults.
Bold indicates significance.

The peak flight metabolic rate was not affected by the body mass of the adult butterfly (Table 2). There was a positive main effect of measurement temperature but no significant *Pgi* genotype main effect. However, there was a highly significant genotype × temperature interaction due to a linear increase of $MR_{peak}$ with temperature in AA homozygotes, whereas in AC heterozygotes the highest metabolic rates were reached in low measurement temperatures (Fig. 2H). CC homozygotes showed an intermediate response to temperature. $MR_{peak}$ showed a nonlinear trend with the time of the day, but this effect was not statistically significant.

To examine the consistency of the genotypic effect on $MR_{peak}$ in different temperatures the data from the two experiments were pooled. Estonian and Chinese individuals were omitted from this dataset because the molecular structure of the *Pgi* gene may differ among the populations. Adult mass had a clear effect on $MR_{peak}$ ($F_{1,105}$=11.81, $P$=0.0008). AC heterozygotes were metabolically superior over a broad range of temperatures ($F_{1,105}$=17.04, $P$<0.0001; Fig. 3). Temperature had a marginal effect in the dataset ($F_{1,105}$=3.81, $P$=0.0536), but the genotype–temperature interaction was evident in the higher measurement temperatures as explained above (Table 2).

The uncorrected RMR and $MR_{peak}$ were weakly correlated in the two experiments, largely due to both being dependent on body size. To take into account the effects of body size, measurement temperature, time of the day and *Pgi* genotype and its interaction with temperature, residuals from the respective models were used. No significant correlation between the residual RMR and residual $MR_{peak}$ was found in the first experiment, even when the two temperature treatments were analysed separately. In the second experiment there was a significant positive correlation ($r$=0.3129, $n$=73, $P$=0.007), with linear regression explaining 9.8% of the variance in residual $MR_{peak}$.

## DISCUSSION

### Effects of body mass, temperature, *Pgi* genotype and their interactions

The body mass of an individual was positively correlated with its metabolic rate, in agreement with the existing literature (Chown and Nicolson, 2004; Kleiber, 1947; Schmidt-Nielsen, 1984). The relationship between body mass and metabolic rate is one of the most fundamental biological generalisations, though it also remains somewhat controversial (Chown et al., 2007; Downs et al., 2008; Niven and Scharlemann, 2005; Suarez et al., 2004). The present results contribute an interesting twist to the quest for a common scaling factor of metabolic rate by body mass: here the relationship between $MR_{peak}$ and body mass appears to be genotype specific, with a steeper slope for the AC heterozygotes than for the AA homozygotes (Fig. 1B). Most of the literature deals with the relationship between resting metabolic rate and body mass, while measurements of maximum metabolic rate are less common. The present finding suggests that the factors affecting the maximum metabolic rate are more complex than those affecting the resting metabolic rate.

Unexpectedly, adult body mass had no effect on $MR_{peak}$ in the second experiment. This result is most likely due to variation in adult mass, which was measured on the day of eclosion. Newly eclosed individuals vary in the rate of meconium clearance, and some individuals may therefore have weighed more than what their actual body mass was during the measurement of the metabolic rate. A subset of individuals was weighed after the measurement and among these individuals a significant positive correlation between $MR_{peak}$ and post-measurement body mass was evident ($t_{57}$=2.37, $P$=0.021). Because the butterflies were used for other purposes following the experiment, no dry mass or masses of separate body parts were obtained.

As expected, temperature had clear effects on metabolic rates, especially on RMR. The $Q_{10}$ values for RMR in the first and the second experiment were 2.6 and 2.1, respectively. These values indicate that temperature dependence of RMR in the Glanville fritillary is comparable with that in other insects (Chown and Nicolson, 2004; Downs et al., 2008).

The effect of temperature on $MR_{peak}$ was different from that on RMR. In the first experiment, there was no significant difference in $MR_{peak}$ between the two temperature treatments. It thus appears that the flight metabolic rate is essentially independent of temperature between 26 and 32°C. However, in the second experiment, when the measurement temperatures exceeded 33°C, temperature had a genotype-specific effect on $MR_{peak}$ (Fig. 3). In the range from 31 to 35°C, *Pgi* AA homozygotes showed a positive relationship between $MR_{peak}$ and temperature, whereas in AC heterozygotes the relationship was negative. In other words, AC heterozygotes are the metabolically superior genotype in low to moderate temperatures, but AA homozygotes perform better in the highest temperatures. The effect was the same in the full dataset consisting of Finnish, Estonian and Chinese butterflies (Fig. 2H) and in the subset of Finnish butterflies (Fig. 3).

A similar interaction between *Pgi* genotype and temperature has previously been reported for flight metabolism and dispersal rate in the field in the Glanville fritillary (Niitepõld et al., 2009). Both *Pgi* genotypes (the AA homozyotes and the AC heterozygotes) appear to follow a nonlinear though dissimilar reaction norm of metabolic rate in relation to temperature. Such a pattern may arise from biochemical, biomechanical or behavioural reasons, or as a combination of all of them (Harrison and Roberts, 2000). Inverted U-shaped relationships between temperature and performance have been reported in the power output of flight muscles of a winter-flying moth (Marden, 1995a) and in the force production of tethered honeybees (Coelho, 1991) and dragonflies (Marden, 1995b). In honeybees, flight metabolic rate seems to be lower in high ambient temperatures than in moderate temperatures (Harrison and Fewell, 2002; Harrison et al., 1996; Woods et al., 2005).

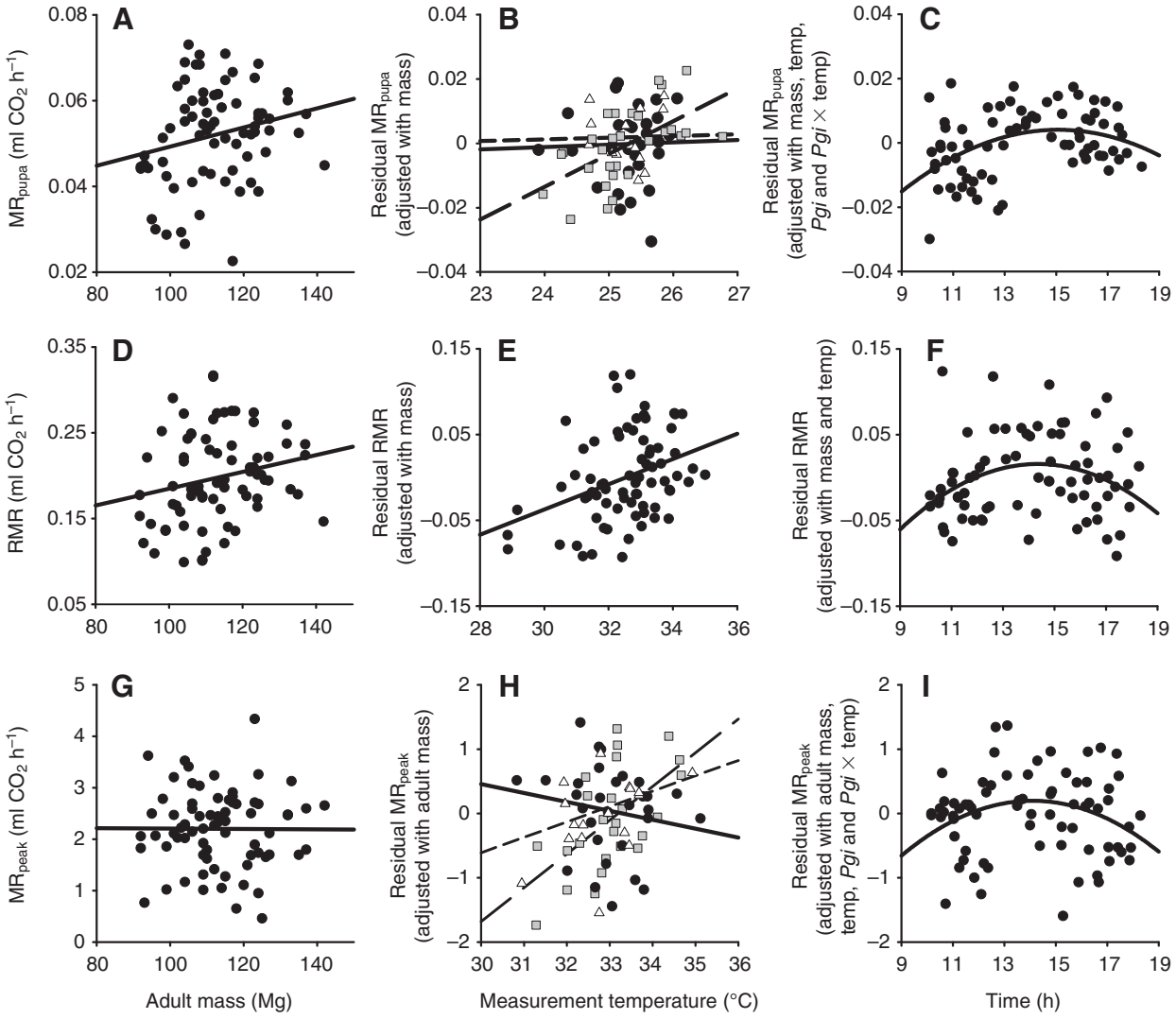


Fig. 2. Effects of adult body mass, measurement temperature, time of measurement and *Pgi* genotype on pupal metabolic rate (A–C), adult resting metabolic rate (D–F) and peak flight metabolic rate (G-I). (A,D,G) The rate of $CO_2$ emission plotted against body mass. (B,E,H) Residual mass-independent metabolic rates plotted against measurement temperature. B and H show interactions between *Pgi* genotype and temperature. Grey squares and the long dashed line represent the genotype AA, black circles and the solid line represent AC, and white triangles and the short dashed line represent the CC genotype. (C,F,I) The effect of the time of day on residual metabolic rates (adjusted for mass, temperature and, when significant, for the genotype by temperature interaction).

Individuals with different variants of the PGI enzyme have been shown to differ in thermal sensitivity in North American *Colias* butterflies (Watt, 1977; Watt et al., 2003) and in willow beetles (Dahlhoff and Rank, 2000; Dahlhoff and Rank, 2007). The differences in organismal performance have been traced to variation in enzyme kinetics and thermal stability: homozygous PGI enzyme variants with high kinetics are sensitive to temperature, whereas variants with low activity are thermally stable (Watt, 1977). Heterozygous enzyme variants combine high activity with good thermal stability. In the present study, in the range from 26 to 33°C, temperature had a very limited effect on flight metabolism but the difference between the *Pgi* genotypes was highly significant. This temperature range is relevant for northern temperate butterflies, which live in low ambient temperatures and are hence dependent on solar radiation to increase their body temperature. The actual body or thorax temperatures were not measured in this study, but in the beginning of the measurement the thorax temperature must have corresponded closely to the ambient air temperature.

The measurement temperatures in the present study match with thoracic surface temperatures of female Glanville fritillaries caught in flight in the field (Saastamoinen and Hanski, 2008). Over a range of typical ambient temperatures the thorax surface temperature, recorded using a thermal image camera, was on average 30.1°C, rarely higher than 33°C, and maximally 35°C (Saastamoinen and Hanski, 2008). I therefore conclude that under natural environmental conditions the *Pgi* AC individuals benefit from their higher flight metabolic rate compared with AA homozygotes. On hot days the relationship may be reversed, but even then heterozygotes may benefit by being able to initiate activity earlier in the day when temperatures are still lower (Saastamoinen and Hanski, 2008).

In resting butterflies the metabolic rate increased linearly with temperature. *Pgi* had no effect on RMR and there was no interaction with temperature. These results suggest that the effect of *Pgi* genotype is due to restrictions on the maximum capacity of the enzyme function. At rest, all metabolic pathways function at very

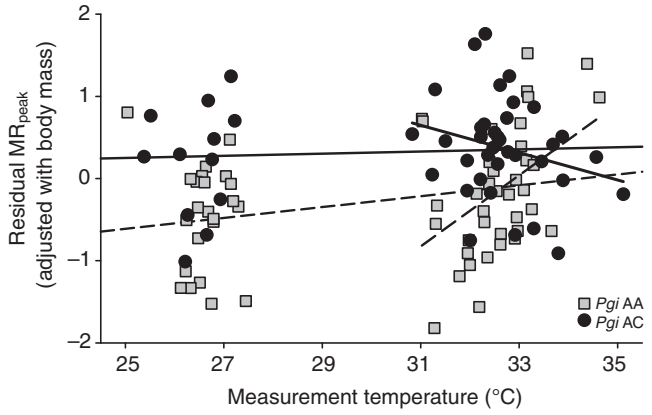


Fig. 3. Peak flight metabolic rate (adjusted for body mass) plotted against measurement temperature in a pooled dataset containing the Finnish individuals from the two experiments. Grey squares and the dashed lines represent AA111 AA homozygotes, and black dots with the solid lines represent the AC heterozygotes. The long regression lines were fitted to the full dataset, while the short regression lines were fitted only to the data for higher temperatures and show an interaction between genotype and temperature ($F_{1,69}$=10.31, $P$=0.002).

low and stable rates and hence no differences between the enzyme forms are likely to occur (Watt and Dean, 2000).

The metabolic rate of pupae increased with temperature. The absolute values of $MR_{pupa}$ were directly comparable with resting metabolic rates of adult butterflies measured in similar temperatures (Fig. 1A, Fig. 2A). Contrary to RMR, $MR_{pupa}$ appeared to be affected by an interaction between temperature and *Pgi* genotype. The interaction, though not quite significant at 5% level, resembled the one found for $MR_{peak}$, with a linear increase in metabolic rate with temperature in AA homozygotes but no increase with temperature in AC heterozygotes (Fig. 2B).

Dahlhoff et al. (Dahlhoff et al., 2008) have reported a significant PGI × temperature interaction in the RMR of the willow beetle *Chrysomela aeneicollis*. In their experiment, individuals were measured at two temperatures, 20°C and 36°C. At the lower temperature there were no significant differences between the PGI genotypes (electromorphs), but the homozygotes that are most commonly found in warm environments had slightly lower metabolic rates than the alternative homozygotes and the heterozygotes. In the higher temperature, close to the field-measured maximum, the metabolic rate of the warm-environment specialist genotype was significantly higher than those of the two other genotypes (Dahlhoff et al., 2008). These results suggest that one genotype is indeed metabolically adapted to warm environments, while the others may perform better at lower temperatures.

The time of the day influenced metabolic rates. Individuals reached the highest metabolic rates during early afternoon. This effect was strongest in pupae but the pattern was similar also in adults. Diel patterns in resting metabolic rates have previously been reported in other Lepidopteran species, both in pupae (Crozier, 1979) and adult individuals (Canzano et al., 2006). In the first experiment in this study, time of the day had only a weak positive and statistically nonsignificant effect on RMR and no effect on $MR_{peak}$. This may be due to conditions experienced by the individuals prior to the measurements, perhaps already during the larval stages. The rearing conditions were different in the two experiments, and the diel cycle was more natural in the second experiment.

### Correlations between resting and flight metabolic rates

Basal metabolic rate (BMR) and resting or standard metabolic rates are among the most commonly recorded physiological variables in animals. In mammals and birds, basal metabolic rate represents the maintenance cost of the physiological machinery and correlates with the maximum metabolic capacity (White and Seymour, 2004). BMR can therefore be used as an indicator of metabolic capacity and it has been demonstrated to vary according to conditions requiring high metabolic capacity and endurance (Broggi et al., 2007; Nilsson, 2002). To what extent are different levels of metabolism correlated in the Glanville fritillary butterfly?

The relationship between RMR and $MR_{peak}$ was examined under three conditions: at low temperature, at optimal temperature, and across a range of temperatures. In the first experiment with two treatments, both RMR and $MR_{peak}$ were positively correlated with body mass but there was no mass-independent correlation between RMR and $MR_{peak}$. The resting metabolic rate had thus no predictive value for the mass-independent metabolic rate in flight. In the second experiment there was a significant positive mass-independent correlation between RMR and $MR_{peak}$, though the correlation explained less than 10% of variation in $MR_{peak}$. $MR_{pupa}$ did not correlate at all with $MR_{peak}$, and the correlation between $MR_{pupa}$ and RMR was weak and clearly nonsignificant.

The poor predictive power of resting metabolic rates for maximum metabolic rate may partly be due to measurement error and measurements in different temperatures; more controlled conditions could have yielded more reliable results. However, a correlation should be robust if it is to be used for predictive purposes. Clearly, measuring directly the flight metabolic rate rather than the RMR is necessary when one is interested in flight-related processes in this and probably other butterflies.

The absence of clear correlation between RMR and $MR_{peak}$ implies only a small general cost of high metabolic capacity. This means that individual butterflies can invest in flight ability and reach high levels of energy expenditure in flight without having to maintain a high metabolic rate at rest. In mammals and birds such a cost seems to be greater, as high maximum metabolic capacity is reflected in high resting metabolic rate (Bennett and Ruben, 1979; Dutenhoffer and Swanson, 1996).

The ratio of flight metabolic rate over resting metabolic rate was relatively small in this study. Flying insects can show a 100-fold or even greater increase in metabolic rate (Bartholomew and Casey, 1978; Chown and Nicolson, 2004), whereas in this study the average increase was 13- to 30-fold. The scale of the increase was negatively affected by temperature: in low temperatures the increase was greater due to the strong dependence of RMR on temperature. The highest increases in metabolic rate from rest to flight were 59-fold at 26°C and 49-fold at 32°C in the first experiment, and 21-fold in the second experiment, measured at 32.4°C. The low increase in the second experiment was caused by the high levels of RMR; there were no differences in $MR_{peak}$ among the experiments. The high levels of RMR may be due to higher physiological activity possibly influenced by the different conditions under which butterflies were maintained in the two experiments.

The values reported here are not fully comparable with factorial scopes reported in the literature because the measurement temperatures were high in order to enable flight. Endothermic insects, such as hawkmoths (Bartholomew and Casey, 1978), can be measured at lower temperatures than butterflies, thus resulting in higher factorial scopes. $MR_{peak}$ was not corrected for the time lag in $CO_2$ being transported from the respirometer to the analyser, which lowers the measured peak value (Bartholomew et al., 1981).

Methodological issues are unlikely to be the only reasons for the relatively low increase in metabolic rate in the present experiment. It seems reasonable to conclude that flight is relatively cheap in butterflies with large wing areas, low wing loadings and low wingbeat frequency compared with other insects with narrower wings, higher wing loadings and higher wingbeat frequency. Measurements of other butterfly species such as the powerful fliers *Inochis io* and *Vanessa cardui* have also yielded only 10- to 20-fold increase in metabolic rate (K.N., unpublished data), suggesting that the present results for the Glanville fritillary may be general for butterflies and other insects with low wingbeat frequency.

## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| BMR | basal metabolic rate (the minimum metabolic rate of homeothermic vertebrates) |
| $MR_{peak}$ | peak metabolic rate |
| $MR_{pupa}$ | pupal metabolic rate |
| RMR | resting metabolic rate (the temperature-dependent minimum metabolic rate of ectothermic animals) |
| SNP | single nucleotide polymorphism |

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