Resting metabolic rate does not predict peak metabolic rate in the Glanville fritillary butterfly

Running title: Metabolic rates in a butterfly

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Abstract

Peak metabolic rate reflects maximal performance and may have direct fitness consequences, whereas resting metabolic rate (RMR) represents the maintenance cost of the whole animal. These traits may be linked, which has significant implications for the evolution of both traits. In vertebrates, a positive correlation between resting metabolic rate and aerobic capacity has been proposed to explain the origin of endothermy. However, as studies on the relationship between resting metabolic rate and aerobic capacity have focused on vertebrates, we know much less about these traits in ectothermic insects. I measured RMR in the Glanville fritillary butterfly (*Melitaea cinxia*) using two configurations: one optimized for measuring flight metabolic rate, the other optimized for resting metabolic rate. The relationship between RMR and body mass was similar for the two configurations. Body mass explained 82% of the variation in RMR when it was measured using the "flight" configuration at 32°C, and 91% when using the "rest" configuration at 23°C. The Q_{10} coefficient calculated based on the two RMR measurements was 2.8. Mass-independent RMR was positively correlated between measurements obtained using the two instrument configurations. However, neither measure of RMR was correlated with peak metabolic rate, which indicates that RMR cannot be used as a surrogate measure for aerobic capacity in the Glanville fritillary. Ectothermic insects may be able to combine high metabolic capacity with no apparent increase in maintenance cost. Even though RMR is among the most frequently measured physiological variables, it may have limited predictive power when it comes to questions related to activity or aerobic capacity, or in the case of butterflies, flight performance.

Box 1: Glossary

BMR: Basal metabolic rate, the metabolic rate of an endothermic, homeothermic vertebrate that is inactive, postabsorptive, adult, nonreproductive and measured during the inactive period of the day in a thermal environment where the animal uses a minimum amount of energy for thermoregulation.

Peak MR: Peak metabolic rate, in the present study, the highest rate of CO_2 production during flight. A likely proxy for aerobic capacity in butterflies.

Q_{10} coefficient: Factor describing the increase in metabolic rate with a 10-degree C increase in temperature.

RMR: Resting metabolic rate, the metabolic rate of an inactive endotherm or ectotherm measured at a specific temperature.

RMRcool: In the present study, resting metabolic rate in postabsorptive, nonreproductive Glanville fritillaries measured at 23°C during the inactive period of the day.

RMRwarm: In the present study, resting metabolic rate in postabsorptive, nonreproductive Glanville fritillaries measured at 32°C during the active period of the day.

SMR: Standard metabolic rate, the metabolic rate of an ectothermic animal that is inactive, postabsorptive, adult, nonreproductive and measured during the inactive period of the day at a specific temperature. Often used in the context of ectothermic vertebrates.

Introduction

Resting metabolic rate (RMR) is the energy expenditure rate of a quiescent, postabsorptive animal and represents the maintenance cost of the physiological machinery. In life-history ecology, allocation of resources among various processes related to growth, survival and reproduction is critical for determining fitness (van Noordwijk and de Jong 1986; Boggs 2009; Lailvaux and Husak 2014). As RMR determines the minimum amount of energy needed for supporting the biological functions of the individual, it sets the foundation for the entire energy budget. Variation in RMR reflects different life-history strategies, and there is considerable interest in to what extent it can be used to predict other behaviorally important or fitness-related traits (Reinhold 1999; White and Seymour 2004; Biro and Stamps 2010; Careau and Garland 2012; Metcalfe et al. 2016; Arnold et al. 2021). A key assumption is that RMR is connected with metabolic phenotype or personality, which may explain differences in performance in a given environment (Careau et al. 2008). Indeed, RMR has been linked to variation in survival or fitness in many taxa, although such effects may be positive or negative and often depend on the environment (Burton et al. 2011). The term RMR can be used for describing maintenance metabolic rate in both endotherms and ectotherms (see Box 1 for exact definitions), whereas basal metabolic rate (BMR) is usually reserved for endothermic vertebrates (McNab 1997) and standard metabolic rate (SMR) for ectothermic vertebrates. In vertebrates, a positive connection between BMR and maximal aerobic capacity has been proposed to explain the origin of endothermy (Bennett and Ruben 1979; Hayes and Garland 1995). The aerobic capacity model postulates that endothermy evolved due to a mechanistic link between aerobic capacity and BMR, as selection acted on the former trait (Nespolo and Roff 2014; Nespolo et al. 2017). Correlations between RMR and various measures representing the upper end of the metabolic spectrum such as aerobic capacity, summit metabolic rate (M_{sum}, cold-induced maximum metabolic rate in endothermic vertebrates, usually in birds), and daily energy expenditure (DEE) have been examined empirically both at the intraspecific and interspecific level, and the general pattern appears to be that RMR and maximum metabolic rate are positively correlated in vertebrates (Auer et al. 2017). In their meta-analysis Auer et al.(2017) found larger effect sizes at the interspecific level than at the intraspecific level, and that the relationships between RMR and M_{sum} or DEE were more consistently positive than the relationship between RMR and aerobic capacity (VO_2max). There are, however, some mixed results, such as no significant correlation between mass-independent BMR, M_{sum} and maximum metabolic rate in several bird species at the intraspecific level (Swanson et al. 2012). The lack of correlation between RMR and maximum metabolic rate may reflect the fact that different organs contribute to metabolism at different activity levels (Weibel 2002). In addition, physiological adaptations such as brown adipose tissue in small mammals may result in different patterns among taxonomic groups (Auer et al. 2017).

As most of the studies on the connection between RMR and aerobic capacity have been performed in the context of the evolution of endothermy, much less is known about these traits in ectotherms, and even less in insects. Flying insects reach extremely high mass-specific metabolic rates (Suarez 2000), and flight capacity is critical for many insects, as flight allows exploring and exploiting resources in three dimensions over large spatial scales. Flight is used for migration and dispersal, and flight allows carrying out tasks such as mate location, oviposition, foraging and escaping from predators (Van Dyck and Baguette 2005). Due to the high energetic cost, there may exist selection pressures against high flight capacity or flight capability (Roff 1994; Zera and Denno 1997). Insect flight appears to be powered by aerobic metabolism (Harrison and Lighton 1998), suggesting that measurements of peak metabolic rate may closely represent aerobic capacity. In support of this, experimental manipulations have shown that Lepidoptera did not increase their peak metabolic rate when weights were added to their bodies (Marden et al. 2008). Among insects, both RMR and flight metabolic rate scale positively with body mass (Niven and Scharlemann 2005), which is in accordance with the general rule of a positive relationship between metabolic rate and body mass found among all animals (Glazier 2010; Gillooly et al. 2017), but we still lack understanding of the interdependence of the two traits. Studies on butterflies, a well-studied group of wing-monomorphic insects that use flight for practically all activities, have shown that flight metabolic rate is affected by the population of origin (Zhan et al. 2014; Kvist et al. 2015; Van Dyck and Holveck 2016; Tenger-Trolander et al.

2023), genotype (Haag et al. 2005; Marden et al. 2013), environmental conditions (Niitepõld et al. 2009; Mattila 2015; Fountain et al. 2016; Wong et al. 2016), mitochondrial performance (Niitepõld et al. 2022), and dietary conditions (Marden et al. 2008; Lebeau et al. 2016; Niitepõld et al. 2022). Nevertheless, in the Glanville fritillary butterfly (*Melitaea cinxia* L.) attempts to find positive correlations between RMR and flight metabolic rate provided mixed results (Niitepõld 2010). In addition, variation in RMR did not explain any significant proportion of the variation in the distance flown by Glanville fritillaries that were tracked in the field using a harmonic radar (Niitepõld et al. 2009).

Unlike endotherms that have a thermoneutral zone, defined as the range of temperatures where the amount of energy needed for thermoregulation (heat production or cooling) is at its lowest, ectotherms do not have a clear temperature point where measurements of metabolic rate should be done. As there is a positive relationship between RMR and body temperature, depicted for example by the Q_{10} coefficient (Chown and Nicolson 2004), the selected measurement temperature has practical, behavioral and physiological implications. Room temperature is an often-used choice but most likely due to practical rather than biological reasons. A more informed approach is to use experimental methods to identify the preferred temperature for the species (Pough and Gans 1982). Experimental assessments suggest that preferred temperatures (Tpref) in ectotherms are often lower than physiologically optimal temperatures (Topt), possibly reflecting adaptations to thermally variable environments and other environmental factors (Woods et al. 2015; Crickenberger et al. 2020; Hui et al. 2022). Measurements of flight metabolic rate on the other hand require experimental temperatures that allow ectothermic insects to fly, and body temperatures of flying insects are often significantly higher than ambient temperatures due to thermoregulation such as metabolic heat production or basking (Harrison and Roberts 2000; Mattila 2015). In the case of butterflies, body temperatures of flying individuals tend to be in the range of 25 to 39°C, and optimal body temperatures for flight are often in the upper part of the range (Watt 1968; Heinrich 1986; Tsuji et al. 1986; Van Dyck and Matthysen 1998; Saastamoinen and Hanski 2008; Mattila 2015). Measurements of RMR at a high temperature in conjunction with measurements of flight metabolic rate have revealed interesting dynamics in the maintenance costs of butterflies exposed to different stressors

(Niitepõld et al. 2014; Niitepõld and Boggs 2015; Niitepõld 2019), but we do not know how measurements of RMR at conditions optimized for flight compare to more conventional measurements of RMR at room temperature.

Here, I performed an experiment using Glanville fritillary butterflies and measured RMR at two temperatures, as well as flight metabolic rate. There were two specific aims: first, to validify measurements of RMR done at a temperature corresponding to body temperatures of active butterflies by comparing the measurements against RMR measurements done at 'gold standard' conditions (room temperature, small measurement chamber, early morning) to establish if RMR represents a metabolic phenotype that is robust across a range of temperatures. Second, I wanted to see, if either measurement of RMR predicted individual variation in flight metabolic rate, i.e. can RMR be used as a proxy for aerobic capacity in an ectothermic butterfly?

Material and Methods

Rearing

The Glanville fritillaries originated from the Åland Islands in Southwest Finland. The parent generation was collected as larvae in the field in the autumn of 2015. The parents were reared in the laboratory and released in a large outdoor cage in 2016. The eggs were collected, and the larvae were reared on Petri dishes with fresh leaves of the host plant *Plantago lanceolata* available *ad libitum*. The larvae entered diapause and were kept at 4°C until January. Diapause was broken by exposing the larvae to light and higher temperature. The larvae were reared in the laboratory under 12 h light / 12 h dark conditions, and the temperature cycled between 28°C at midday and 15°C at night. Fresh leaves of *Plantago lanceolata* were always available. When individuals pupated, they were transferred to an individually marked plastic cup with a mesh cover. After emergence, each individual was sexed, weighed and individually marked with a felt tip pen on the hindwings. Males and females were placed in separate large cylindrical cages (40 by 50 cm). The cages were kept in a brightly lit room during the day and the butterflies could gain flight practice. Food was provided in the form of 20% honey-water absorbed in a sponge. The total sample consisted of 24 males and 14 females.

Measurement of resting metabolic rate at 23°C

In the evening of the first full day after emergence, butterflies were transferred into individual plastic cups and placed in a dark cupboard without access to food. Early next morning, before daylight, each individual was placed in a 110 ml respirometry chamber that was kept in the dark during the measurement of resting metabolic rate. A maximum of seven individuals could be measured in each trial. Drierite (W.A. Hammond, Xenia, OH, USA) and Magnesium perchlorate (Alfa Aesar, Karlsruhe, Germany) were used to dry the incoming air, and CO_2 was scrubbed using Medisorb (GE Healthcare, Chalfont St. Giles, UK) and Ascarite II (Thomas, Swedesboro, NJ, USA). A Sable Systems RM-8 multiplexer (Sable Systems International, Las Vegas, NV, USA) was used to direct the dried and CO_2-free air through one chamber at a time. The flow rate was kept at 300 ml min^{-1} with the help of a Sable Systems SS-4 pump and a Sierra Instruments 840 mass-flow controller (Sierra Instruments, Monterey, CA, USA). The CO_2 production rate of each butterfly was measured twice for the duration of 5 minutes using a Li-Cor 7000 infrared H_2O/CO_2 analyzer (LI-Cor Biosciences, Lincoln, NE, USA). The last recording was used for analyses and the metric 'RMRcool' was calculated from the mean CO_2 production rate during the final two minutes of the recording. Individuals spent between 30 to 90 minutes in the chamber prior to the last recording, depending on the number of individuals in the trial and the order of chambers. Standard equations were used to convert CO_2 concentrations to rates (Lighton 2008). The mean measurement temperature was 23.0 ± SD 0.9°C. After the measurement, the individual was weighed in a glassine envelope using a Mettler Toledo XS 105 balance (Mettler Toledo, Greifensee, Switzerland; readability 0.01 mg), given water from a piece of moist cotton wool, and gently placed in an individual plastic cup.

Measurement of resting metabolic rate at 32°C and flight metabolic rate

The butterfly was placed into a 1-l measurement chamber that was covered with a dark cloth. The chamber was kept in a temperature-controlled cabinet with an open front. The mean temperature across RMR measurements was 31.5±0.69°C. After a ca 25-minute wait period, the recording of CO_2 production was started and two minutes of stable resting metabolic rate was measured. If the individual became restless during the measurement, it was given additional time to settle, and the recording was continued until a steady baseline was reached. In the analysis, the mean of 90 seconds of steady CO_2 production rate was used for the calculation of 'RMRwarm'. Once RMR had been recorded, the dark cloth was removed, and the chamber was exposed to light. After 30 seconds, the chamber was shaken using rapid yet precise hand movements with the intention of stimulating flight by flipping the butterfly in the air. Every time the butterfly attempted to land, the chamber was shaken again. If the butterfly hovered in the air, the chamber was moved only gently. The procedure was repeated for a total of seven minutes. At the end of the experimental period the chamber was covered again, the CO_2 concentration in the chamber was allowed to return to the baseline, and the measurement was terminated. The butterfly was removed from the chamber and weighed with a Mettler Toledo balance (see above). In the analysis, two parameters were extracted: the highest rate of CO_2 production (Peak MR) and the total volume of CO_2 emitted during the flight experiment (total CO_2 production). Peak MR was typically reached during the first minutes of the flight trial and is likely to reflect aerobic capacity. The total volume of CO_2 contains a behavioral component as it is affected by the willingness to fly.

Statistical analyses

All metabolic rates and body masses were $\log_{10}$-transformed to facilitate examining metabolic scaling (Glazier 2021). I used generalized linear models to examine factors that affected resting metabolic rate and flight metabolic rates. The models contained body mass, sex, measurement temperature, and the interaction between body mass and sex as independent effects. Temperature was included as a covariate to account for variation in temperature among measurements in the same treatment, and the body mass by sex interaction term was included for detecting potential sex-specific differences in the relationship between metabolic rate and body mass. I examined correlations between RMR and flight metabolic rates using Pearson Product-Moment correlation analysis. In these analyses, instead of the raw metabolic rates, I used mass-independent metabolic rates. Mass-independent metabolic rates were obtained by extracting the residuals from linear models thus accounting for variation in body mass. For this purpose, I reran the models presented above with only significant variables included in the model. Body mass was included in all models and temperature was included in the models with RMR as the dependent variable. As there was no overlap in the body masses of males and females, and visual inspection of the data suggested that male and female data points did not necessarily follow the same regression line, I performed the correlation analyses for males and females separately, and for both sexes pooled together. All analyses were performed with SAS Studio (SAS Institute, Cary, NC, USA).

I calculated Q_{10} temperature coefficients for resting metabolic rates measured at two temperatures using the formula (RMRwarm/RMRcool)^(10/(T_{warm}-T_{cool})), where T_{warm} is the measurement temperature at which RMRwarm was measured and T_{cool} the temperature at which RMRcool was measured. The Q_{10} coefficient represents the factor by which RMR increases with a 10°C increase in temperature (Chown and Nicolson 2004).

Results

Resting metabolic rate

Resting metabolic rate measured at 23°C (RMRcool) was positively affected by body mass ($F_{1,33} = 52.05$; $P < 0.0001$). Body mass explained 91% of the variation in RMRcool (Fig. 1A). The effect of measurement temperature was not significant ($F_{1,33} = 2.02$; $P = 0.17$). The effect of sex was not significant ($F_{1,33} = 0.10$; $P = 0.76$), and the body mass by sex interaction was also not significant ($F_{1,33} = 0.09$; $P = 0.76$).

Resting metabolic rate measured at 32°C (RMRwarm) was affected by body mass ($F_{1,33} = 23.59$; $P < 0.0001$). Body mass explained 82% of the variation in RMRwarm (Fig. 1B). There was a significant effect of temperature ($F_{1,33} = 5.11$; $P = 0.03$). The effect of sex was not significant ($F_{1,33} = 0.06$; $P = 0.81$), and the body mass by sex interaction was not significant ($F_{1,33} = 0.05$; $P = 0.82$).

The mean Q_{10} coefficient based on RMR measurements at the two temperatures (23°C and 32°C) was 2.8.

Flight metabolic rates

Peak flight metabolic rate was positively affected by body mass ($F_{1,33} = 14.80$; $P = 0.0005$) (Fig. 2A). Body mass explained 22% of the variation in Peak MR. The effect of temperature was not significant ($F_{1,33} = 1.01$; $P = 0.32$). Sex had no significant effect on Peak MR ($F_{1,33} = 0.06$; $P = 0.80$) and the body mass by sex interaction was not significant ($F_{1,33} = 0.00$; $P = 0.98$).

The total volume of CO_2 emitted during the flight experiment was affected by body mass ($F_{1,33} = 14.21$; $P = 0.0006$) (Fig. 2B). Body mass explained 29% of the variation in the total volume of CO_2.Temperature had no significant effect on the total volume of CO_2 ($F_{1,33} = 0.30$; $P = 0.59$). The effect of sex was not significant ($F_{1,33} = 0.01$; $P = 0.91$), and the body mass by sex interaction was not significant ($F_{1,33} = 0.11$; $P = 0.74$).

Correlations between metabolic rates

Mass-independent RMRcool and mass-independent RMRwarm were significantly correlated ($r = 0.44$; $P = 0.005$) (Fig. 3). The residual metabolic rates used in the analysis were extracted from the linear models above and had also been corrected for measurement temperature. In males, the correlation coefficient between mass-independent RMRcool and mass-independent RMRwarm was 0.55 (*P* = 0.005). In the female subsample, there was no significant correlation (*r* = 0.26; *P* = 0.37).

Correlation statistics for mass-independent RMRcool, RMRwarm, Peak MR, and total volume of CO_2 are given in Table 1. In brief, mass-independent RMRcool and RMRwarm were not significantly correlated with mass-independent Peak MR (Figure 4A-D) or with mass-independent total volume of CO_2.

Discussion

The present study compared resting metabolic rates measured at a low temperature in early morning (RMRcool) to measurements performed at a temperature corresponding to the body temperature of an active butterfly during the active phase of the day (RMRwarm). Both measurements confirmed that body mass explains the majority of variation in RMR when individuals are in a postabsorptive state and not actively reproducing or engaging in other energetically intensive activities. Indeed, body mass explained a total of 91% of the variation in RMRcool, indicating that the individuals were in a rather undisturbed state. According to expectations, at the higher measurement temperature, physiological activity increased, and the percentage of variation explained by body mass decreased somewhat to 82%. An effect of temperature was also seen within the high-temperature conditions, as measurement temperature (treated as a covariate) had a positive effect on RMRwarm.

The increase in CO_2 production rate with temperature between the two measurement temperatures was quantified using the Q_{10} coefficient. A Q_{10} value of 2.8 indicated that RMR more than doubled with the increase of 10°C, which is within the typical range for insects (Nespolo et al. 2003; Chown and Nicolson 2004) and close to or somewhat higher than previously measured Q_{10} values in the Glanville fritillary, 2.1 and 2.6, in two different experiments (Niitepõld 2010). Despite the increase in RMR with temperature, there was a significant correlation between mass-independent RMRcool and RMRwarm. The result confirms that both measurements of RMR convey a signal of individual phenotypic variation that is independent of body mass. The finding validates the use of resting metabolic rate recordings that have been measured with the flight configuration at a temperature that is higher than in typical measurements of RMR. That the correlation between RMRcool and RMRwarm was in fact not stronger may depend on the very high proportion of variation explained by body mass in the case of RMRcool, as there simply was not much residual variation left, and part of the biological variation would be masked by measurement error. In addition, as RMR tends to follow temperature in an exponential pattern at sub-optimal temperatures, it is possible that the increase in temperature activated physiological processes that were mostly shut off at the relatively low temperature of 23°C, such as activity of the endocrine system, reproductive maturation or increased mitochondrial proton leak (Schulte 2015). It could therefore be argued that individuals may not have been in the same physiological state between the two measurements, which makes finding a significant correlation even more valuable. The result suggests that RMR is a robust trait that conveys individual phenotypic variation despite poikilothermic butterflies being subjected to considerable variation in body temperature.

Lack of correlation between RMR and peak metabolic rate

No correlations were found between mass-independent resting metabolic rates and flight metabolic rates. Here, the pattern differs from findings in vertebrates where minimum and maximum metabolic rates have often been found to correlate (Auer et al. 2017). However, there tends to be a difference between interspecific and intraspecific studies, such that many intraspecific studies covering amphibians, birds, fish, mammals and reptiles have failed to find a correlation between RMR and aerobic capacity (Auer et al. 2017). For example, RMR was not correlated with aerobic capacity in an ectothermic lizard, the garden skink (*Lampropholis delicata*) (Merritt et al. 2013). In a similar way, a study on RMR and flight metabolic rate in another butterfly, the Speckled wood (*Pararge aegeria*), also found no significant correlation (Van

Dyck and Holveck 2016). These intraspecific studies are in contrast with studies that have compared RMR between taxa that are capable of flight or flightless, where the presence of the flight apparatus is associated with higher RMR (Reinhold 1999). Indeed, the general pattern seems to be that RMR is connected to differences between species, but it is not necessarily correlated with other traits within the species. Not finding a correlation is not surprising in itself, as different organs are responsible for the majority of energy consumption in flight and at rest. In insects, flight muscle tissue is highly active in flight and may use up to 95% of the total oxygen consumed by the individual (Snelling et al. 2012). Future studies in different insect taxa will confirm if the lack of a correlation between RMR and maximum metabolic rates is the norm among insects, but it is worth noting that several differences between vertebrates and insects exist at the physiological level. Among insects, too, one would expect to find physiological differences between taxa that are to certain degree endothermic, such as bees, bumblebees and hawkmoths, and pure ectotherms, such as sun-basking butterflies, as these taxa exhibit strikingly different behaviors (Heinrich 1975, 1986).

In general terms, the apparent decoupling of maximum metabolic rate from RMR, which represents the costs of maintenance, suggests that individual insects can reach high metabolic capacity without an additional energetic cost at rest. Empirical evidence, however, shows that butterflies subjected to repeated flight treatments display chronically elevated RMR (Niitepõld and Boggs 2015; Niitepõld 2019), which may represent increased investment in repair and maintenance and hint that high metabolic expenditure does come with a cost after all. Nevertheless, morphological features such as the tracheal network used for gas exchange in insects may serve as adaptations that allow high energetic throughput when active, while requiring only minimal energetic investments when sedentary. Dynamics related to metabolic enzymes may also differ between rest and flight. For example, in the Glanville fritillary, the *Pgi* locus which encodes the glycolytic enzyme phosphoglucose isomerase has been linked with variation in flight metabolic rate (Haag et al. 2005; Orsini et al. 2009). However, there are no differences between *Pgi* genotypes in RMR (Niitepõld et al. 2009; Niitepõld 2010), suggesting that different physiological processes regulate RMR and flight metabolic rate. Results from heritability experiments, too, point to different mechanisms underpinning variation in minimum and maximum metabolic rates. Flight metabolic rate was found to be significantly heritable while RMR was not (Mattila and Hanski 2014).

While RMR is to a very high degree explained by body mass, Peak MR is inherently more variable. Undoubtedly, the motivation to fly may have an effect on flight performance and the individual may not fly at its absolute maximum level. Nevertheless, measurements of Peak MR and total CO_2 production seem to capture variation in flight performance that carries a biological signal, as seen in previous studies. Flight in the respirometry chamber requires repeated take off bouts, and take off-flight is considered energetically challenging (Berwaerts et al. 2002), suggesting that butterflies fly close to their maximum capacity. We also know that total CO_2 production is correlated with variation in flight performance of female Glanville fritillaries in the field (Niitepõld et al. 2009). Finding out how different measures of flight performance are correlated remains an important question in the context of energetics (Ducatez et al. 2012). As the relationships between energetic traits may not be universal (Careau et al. 2008; Portugal et al. 2016), incorporating behavioral, ecological and evolutionary insight in physiological studies will be beneficial.

Author contributions

K.N. was responsible for all stages from the original idea and experimental work to the preparation of the manuscript.

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Supplementary data

Dataset: Niitepold Melitaea cinxia data

Conflict of interest

No conflict of interest to declare.

Data availability

The data used in the study are available as supplementary material.

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Figure captions

Figure 1A) Resting metabolic rate at 23°C (RMRcool) plotted against body mass. Males are represented as black dots, females open circles. Body mass explained 91% of the total variation in RMRcool. Regression equations are presented for the sexes separately. Body mass explained 58% of the variation in the male subsample, and 64% in the female subsample. The body mass by sex interaction was not significant. B) Resting metabolic rate at 32°C (RMRwarm) plotted against body mass. Body mass explained 82% of the variation in RMRwarm in the pooled sample, and 32% and 57% in males and females, respectively. The body mass by sex interaction was not significant.

Figure 2A) Peak metabolic rate plotted against body mass Glanville fritillaries. Body mass explained 22% of the variation in Peak MR in the pooled sample. Body mass explained 29% of the variation in the male subsample (black dots), and 39% in females (open circles). B) Total volume of CO_2 emitted during the 7-minute flight experiment plotted against body mass. Body mass explained 29% in the variation in the pooled sample, and 27% and 40% in males and females, respectively.

Figure 3) The relationship between mass-independent RMRcool and mass-independent RMRwarm. The correlation was significant ($r = 0.44$; $P = 0.005$). Black squares represent males, open squares females.

Figure 4) The relationship between mass-independent RMRcool and mass-independent Peak MR in A) males and B) females. There was no significant correlation in the pooled sample, and no correlation when the two sexes were analyzed separately. C) The relationship between mass-independent RMRwarm and mass-independent Peak MR in males. D) The relationship between mass-independent RMRwarm and mass-independent Peak MR in females. No significant correlations were found between these traits in the pooled sample or in each sex.

Tables

Table 1. Correlation coefficients between mass-independent resting metabolic rates and mass-independent flight metabolic rates. No significant correlations were found.

		Peak MR			Total CO_2 production	
	Pooled	Males	Females	Pooled	Males	Females
RMRcool	*r* = -0.006 *P* = 0.97	*r* = 0.006 *P* = 0.98	*r* = -0.02 *P* = 0.95	*r* = 0.07 *P* = 0.66	*r* = 0.20 *P* = 0.35	*r* = -0.13 *P* = 0.67
RMRwarm	*r* = -0.04 *P* = 0.797	*r* = 0.12 *P* = 0.57	*r* = -0.36 *P* = 0.20	*r* = 0.02 *P* = 0.90	*r* = 0.22 *P* = 0.31	*r* = -0.43 *P* = 0.12

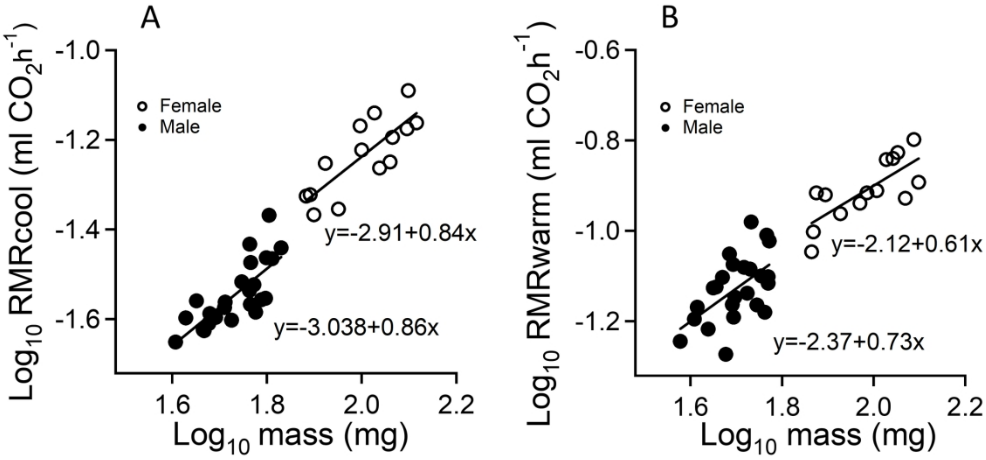

Figure 1A) Resting metabolic rate at 23°C (RMRcool) plotted against body mass. Males are represented as black dots, females open circles. Body mass explained 91% of the total variation in RMRcool. Regression equations are presented for the sexes separately. Body mass explained 58% of the variation in the male subsample, and 64% in the female subsample. The body mass by sex interaction was not significant. B) Resting metabolic rate at 32°C (RMRwarm) plotted against body mass. Body mass explained 82% of the variation in RMRwarm in the pooled sample, and 32% and 57% in males and females, respectively. The body mass by sex interaction was not significant.

180x90mm (300 x 300 DPI)

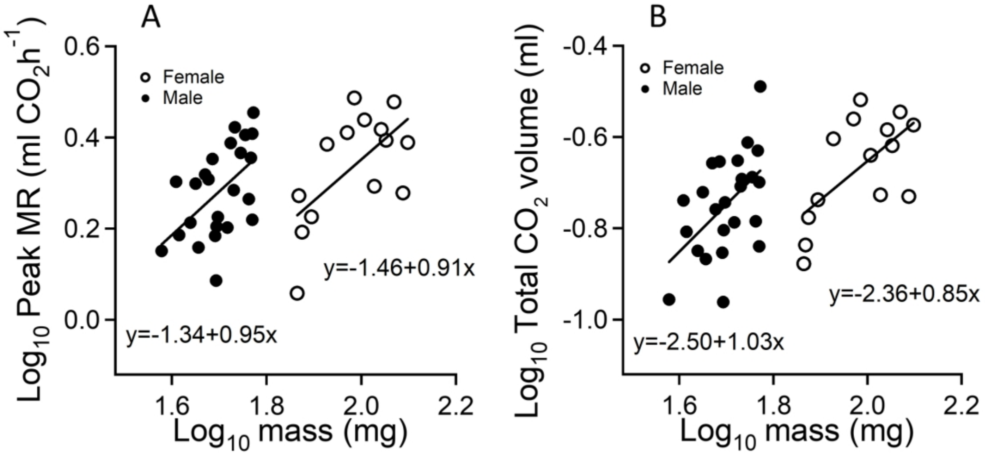

Figure 2A) Peak metabolic rate plotted against body mass Glanville fritillaries. Body mass explained 22% of the variation in Peak MR in the pooled sample. Body mass explained 29% of the variation in the male subsample (black dots), and 39% in females (open circles). B) Total volume of CO2 emitted during the 7-minute flight experiment plotted against body mass. Body mass explained 29% in the variation in the pooled sample, and 27% and 40% in males and females, respectively.

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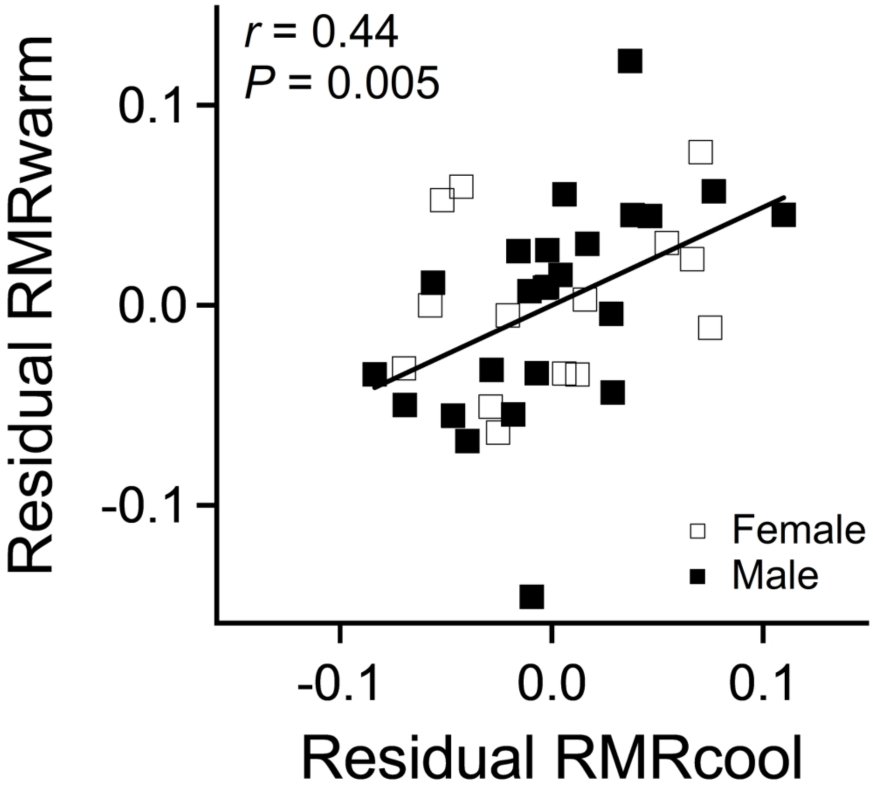

Figure 3) The relationship between mass-independent RMRcool and mass-independent RMRwarm. The correlation was significant (r = 0.44; P = 0.005). Black squares represent males, open squares females.

179x159mm (300 x 300 DPI)

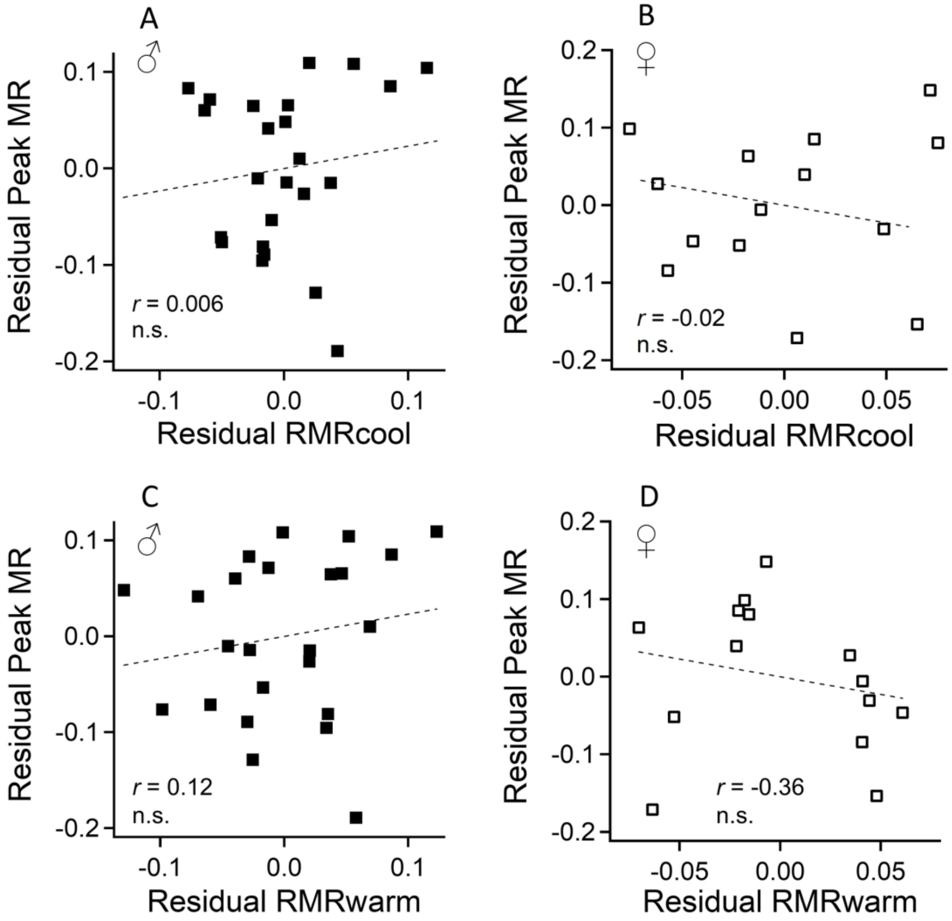

Figure 4) The relationship between mass-independent RMRcool and mass-independent Peak MR in A) males and B) females. There was no significant correlation in the pooled sample, and no correlation when the two sexes were analyzed separately. C) The relationship between mass-independent RMRwarm and mass-independent Peak MR in males. D) The relationship between mass-independent RMRwarm and mass-independent Peak MR in females. No significant correlations were found between these traits in the pooled sample or in each sex.

180x170mm (300 x 300 DPI)

ID	Sex	PeakMR	Log10PeakMR	TotalCO2	Log10TotalCO2	Temp	RMRwarm	Log10RMRwarm
1	Male	1.65852	0.21972	0.14477	-0.8393	31.5242	0.07917	-1.1014
2	Male	1.21931	0.08611	0.10927	-0.9615	30.8237	0.0843	-1.0742
3	Male	1.53443	0.18595	0.15578	-0.8075	30.5045	0.06782	-1.1686
4	Male	1.60331	0.20502	0.15711	-0.8038	30.8558	0.06443	-1.1909
5	Male	2.54499	0.40569	0.20526	-0.6877	30.9987	0.07949	-1.0997
6	Male	1.59411	0.20252	0.16335	-0.7869	30.8881	0.08305	-1.0807
7	Male	1.8407	0.26498	0.16433	-0.7843	30.6878	0.06609	-1.1799
8	Male	1.44184	0.15892	0.1357	-0.8674	31.0024	0.07516	-1.124
9	Male	2.08352	0.3188	0.22021	-0.6572	30.8948	0.07893	-1.1028
10	Male	1.52875	0.18434	0.14015	-0.8534	30.465	0.06867	-1.1632
11	Male	1.63446	0.21337	0.1415	-0.8492	30.8208	0.06065	-1.2172
12	Male	2.64482	0.4224	0.20336	-0.6917	30.8494	0.10466	-0.9802
13	Male	1.98968	0.29878	0.19016	-0.7209	30.6559	0.07479	-1.1262
14	Male	1.68032	0.22539	0.18071	-0.743	30.569	0.07137	-1.1465
31	Male	2.00977	0.30315	0.18244	-0.7389	31.3065	0.0638	-1.1952
32	Male	2.44318	0.38796	0.22305	-0.6516	31.3543	0.0728	-1.1379
33	Male	2.03403	0.30836	0.1745	-0.7582	31.0204	0.05332	-1.2731
34	Male	2.84916	0.45472	0.32438	-0.4889	31.2144	0.09497	-1.0224
35	Male	2.26725	0.3555	0.2346	-0.6297	31.2509	0.09788	-1.0093
36	Male	1.92536	0.28451	0.19587	-0.708	31.2323	0.0823	-1.0846
37	Male	1.41637	0.15118	0.11071	-0.9558	30.9516	0.057	-1.2441
38	Male	2.32528	0.36648	0.24442	-0.6119	30.3661	0.06858	-1.1638
39	Male	2.25493	0.35313	0.22197	-0.6537	31.5566	0.08888	-1.0512
40	Male	2.56214	0.4086	0.20003	-0.6989	31.4443	0.07664	-1.1155
138	Female	1.9634	0.29301	0.1876	-0.7268	31.7522	0.14382	-0.8422
139	Female	2.47609	0.39377	0.24062	-0.6187	32.5762	0.14899	-0.8268
140	Female	2.74615	0.43872	0.22907	-0.64	31.7592	0.12271	-0.9111
141	Female	3.00758	0.47822	0.28504	-0.5451	31.7054	0.11811	-0.9277
142	Female	1.55773	0.19249	0.16728	-0.7766	31.9918	0.1214	-0.9158
143	Female	2.4271	0.38509	0.24901	-0.6038	32.0158	0.10916	-0.9619
144	Female	1.14363	0.05829	0.13253	-0.8777	31.3345	0.0899	-1.0462
152	Female	2.61584	0.41761	0.26049	-0.5842	32.0708	0.14467	-0.8396
154	Female	1.87218	0.27235	0.14568	-0.8366	31.4148	0.09946	-1.0024
156	Female	1.68274	0.22602	0.1831	-0.7373	31.5285	0.12023	-0.92
157	Female	2.5735	0.41052	0.27518	-0.5604	31.6971	0.11512	-0.9388
158	Female	1.89776	0.27824	0.18614	-0.7302	31.425	0.15919	-0.7981
159	Female	2.44945	0.38907	0.2669	-0.5737	31.3218	0.12806	-0.8926
160	Female	3.06824	0.48689	0.30328	-0.5182	30.8785	0.12137	-0.9159

TempRMR	Mass	Log10 Mass	RMRCool Mass	Log10RMR CoolMass	RMRcool2	Log10RMR cool2	RMRctemp2
31.899	58.86	1.7698	62.88	1.7985125	0.034459	-1.462697	22.335
31.6226	49.33	1.6931	58.28	1.7655195	0.033612	-1.473506	22.3203
30.4038	41.22	1.6151	42.51	1.6284911	0.025294	-1.596982	22.3173
31.1198	49.46	1.6943	53.12	1.7252581	0.025009	-1.601904	22.2886
30.6729	56.87	1.7549	61.4	1.7881684	0.027754	-1.556674	22.3264
31.4711	52.11	1.7169	57.95	1.7630534	0.029105	-1.536032	22.0611
30.5036	57.86	1.7624	58.23	1.7651468	0.027118	-1.566742	22.1756
31.9231	45.3	1.6561	47.55	1.6771505	0.024572	-1.609559	22.0575
31.6427	46.77	1.67	47.81	1.6795187	0.025857	-1.587422	22.0584
30.1354	49.13	1.6913	49.16	1.6916119	0.025348	-1.596056	22.0653
30.8176	43.55	1.639	46.29	1.6654872	0.02378	-1.623788	22.0458
31.3354	54.04	1.7327	67.58	1.8298182	0.036241	-1.4408	22.0615
31.4623	44.66	1.6499	46.5	1.667453	0.023672	-1.625765	22.1541
30.3338	49.76	1.6969	51.35	1.7105404	0.026678	-1.573847	22.0645
31.1757	40.62	1.6087	44.78	1.6510841	0.027605	-1.559012	22.9461
31.4753	52.97	1.724	59.7	1.7759743	0.026038	-1.584392	22.5455
31.6802	47.56	1.6772	51.5	1.7118072	0.027436	-1.561679	22.5958
31.5777	59.15	1.772	63.73	1.8043439	0.042848	-1.368069	22.527
31.763	58.42	1.7666	64.67	1.8107029	0.034278	-1.464985	22.5569
31.6887	53.74	1.7303	55.82	1.7467898	0.030442	-1.516527	22.9633
31.163	37.85	1.5781	40.47	1.6071332	0.022339	-1.650936	22.981
30.9092	55.6	1.7451	59.23	1.7725417	0.029979	-1.523183	22.5144
32.0686	48.46	1.6854	58.01	1.7635029	0.036925	-1.432679	22.5882
31.6748	58.85	1.7697	62.67	1.7970597	0.027979	-1.553168	22.5293
31.6371	106.6	2.0278	109.11	2.0378646	0.054611	-1.26272	24.0542
33.2422	112.84	2.0525	116.15	2.0650192	0.0639	-1.194499	24.3628
31.8867	101.6	2.0069	106.39	2.0269008	0.0725	-1.139662	24.173
31.7196	117.3	2.0693	124.6	2.095518	0.0668	-1.175224	24.3261
32.2562	74.96	1.8748	79.24	1.8989445	0.043	-1.366532	24.1362
32.5239	84.66	1.9277	89.29	1.9508028	0.04423	-1.354283	24.047
31.4108	73.23	1.8647	77.84	1.8912028	0.047636	-1.322065	24.0536
32.9344	110.15	2.042	114.76	2.0597905	0.056312	-1.249399	24.064
31.5248	73.83	1.8682	76.37	1.8829228	0.047283	-1.325295	24.0567
32.5586	78.48	1.8948	83.82	1.9233477	0.055897	-1.252612	24.0554
32.4433	93.39	1.9703	100	2	0.059889	-1.222653	24.3586
31.0663	122.46	2.088	125.41	2.0983322	0.081279	-1.090022	24.0458
31.8056	125.33	2.0981	130.6	2.1159432	0.0688	-1.162412	24.3076
31.1864	96.67	1.9853	99.22	1.9965992	0.0678	-1.16877	24.2801