Metabolic rate in *Drosophila*: sexual dimorphism, natural selection, and genetic covariances with mass and locomotor activity

By Mathieu Videlier

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Biology department
Faculty of Science
University of Ottawa

ABSTRACT

A key endeavour in evolutionary physiology is to understand the causes and consequences of individual variation in metabolic rate, including its genetic basis and the selection that acts on it. In diecious species, males and females often differ in their reproductive strategies and this can generate sex-specific selection and the possibility of an evolutionary conflict between the sexes. Males and females may therefore manage their energy budgets differently to achieve these different goals, generating sex-specific selection on metabolic rate and the potential for intralocus sexual conflict. Little attention, however, has been given to the potential for such metabolic conflict. In my thesis, I addressed this by focusing on standard metabolic rate (SMR), the energy invested in the somatic maintenance, which constitutes a major component of an individual's energy budget. Most studies on the evolution of metabolic rate have focused on endotherms likely because metabolic measurements are easier in larger animals. I took advantage of a high-throughput flow-through respirometry system that allows individual-level measurements of a large number of small insects, to study the SMR in the little insect *Drosophila melanogaster*. I used this system first to estimate the repeatability of the resting metabolic rate, RMR (SMR required individuals to be non-reproductive, but some individual were intentionally mated to quantify the effect of this), and to identify factors contributing to its among-individual variation including body mass, sex, and reproductive status. I also demonstrated that the among-individual phenotypic correlation between RMR and locomotor activity varied depending on time of the day and sex, suggesting fundamental difference in how males and female manage their energy budget. Second, I examined the covariance between SMR and relative fitness to estimate the phenotypic selection on SMR in males and females, and I developed some novel multivariate approaches to better account for trait-specific covariates compared to past methods. There was some evidence that selection differed between males and females, although this occurred in a portion of phenotypic space that was non-overlapping between the sexes. Strong collinearity between SMR and body mass also hampered the ability to separate selection gradient on these two traits despite substantial sample sizes. Third, I used a quantitative genetic breeding design to provide insight into the genetic architecture of SMR, body mass and general locomotor activity and how it differed between sexes. I detected additive genetic variances for those traits in both sexes with substantial difference between males and females in their genetic architecture. The genetic architecture also revealed that much of the genetic variance was shared among sexes with positive and strong cross-sex genetic correlations, indicating the potential for sexual conflict in this population. Overall, my results provide further support to the possibility of sex-specific energy management that may lead to an intralocus sexual conflict in this population. Quantifying sex-specific selection at the genetic level will be an important next step in this work.

Un des principaux objectifs de la physiologie évolutive est de comprendre les causes et les conséquences de la variation individuelle du taux métabolique, comprenant sa base génétique et la sélection qui agit dessus. Chez les espèces dioïques, les mâles et les femelles diffèrent souvent dans leurs stratégies de reproduction, ce qui peut générer une sélection spécifique au sexe et la possibilité d'un conflit évolutif entre les sexes. Les mâles et les femelles peuvent donc gérer différemment leur budget énergétique pour atteindre ces différents objectifs, ce qui génèrera une sélection spécifique au sexe sur le taux métabolique et la possibilité d'un conflit sexuel dit intralocus. Cependant, peu d'attention a été accordée à la possibilité d'un tel conflit métabolique. Dans ma thèse, j’aborde ce problème en me concentrant sur le taux métabolique standard (SMR), l'énergie allouée dans le maintien somatique, qui constitue une composante majeure du budget énergétique d'un individu. La plupart des études sur l'évolution du taux métabolique se sont concentrées sur les espèces endothermiques, probablement parce que les mesures métaboliques sont plus faciles chez ces grands animaux. J'ai donc profité d'un système de respirométrie à flux continu à haut débit qui permet des mesures au niveau individuel d'un grand nombre d'individu, pour étudier le SMR chez le petit insecte *Drosophila melanogaster*. J'ai d'abord utilisé ce système pour estimer la répétabilité du taux métabolique au repos (RMR, qui est étroitement lié au SMR car certains individus n'étaient plus vierges) et aussi pour identifier les facteurs contribuant à la variation entre les individus, tel que la masse corporelle, le sexe et l'état reproducteur. J'ai également démontré que la corrélation phénotypique intra-individuelle entre le taux métabolique au repos et l'activité locomotrice variait selon l'heure de la journée mais aussi entre les sexes, ce qui suggère une différence fondamentale dans la manière dont les mâles et les femelles gèrent leur budget énergétique. Ensuite, j'ai examiné la covariance entre le SMR et la valeur adaptative relative pour estimer la sélection phénotypique sur le SMR chez les femelles et les mâles. De plus, j'ai aussi développé une nouvelle approche multivariée pour mieux tenir compte des covariances spécifiques aux traits par rapport aux méthodes antérieures. Il a été mis en évidence que la sélection différait entre les femelles et les mâles, bien que cela se produise dans une partie de l'espace phénotypique qui ne se chevauche pas entre les sexes. La forte colinéarité entre le SMR et la masse corporelle a également entravé la capacité à séparer le gradient de sélection sur ces deux traits malgré la taille d'échantillon importante. Troisièmement, j'ai utilisé une approche de génétique quantitative pour évaluer l'architecture génétique du SMR, de la masse corporelle et de l'activité locomotrice générale et comment elle diffère entre les sexes. J'ai détecté des variances génétiques additives pour les traits chez les deux sexes, avec des différences substantielles entre les mâles et les femelles dans leur architecture génétique. Celle-ci a également révélé qu'une grande partie de la variance génétique était partagée entre les sexes avec des corrélations génétiques entre les sexes positives et fortes, ce qui indique le potentiel d’un conflit sexuel dans cette population. Dans l'ensemble, mes résultats confirment la possibilité d'une gestion de l'énergie spécifique au sexe qui pourrait conduire à un conflit sexuel intralocus dans cette population. La quantification de la sélection spécifique au sexe au niveau génétique sera une prochaine étape importante de ce travail.

PREFACE

The work in the Chapter 2 is a slightly modified version of the following paper, co-authored by Howard D. Rundle and Vincent Careau (Department of Biology , University of Ottawa):

Videlier, M., H. D. Rundle, and V. Careau. 2019. Sex-specific among-individual covariation in locomotor activity and resting metabolic rate in *Drosophila melanogaster*. American Naturalist 194: E164–E176.

I took the lead role in all stages of this work, including conceiving the experimental design, analysing the model, interpreting the results, and writing the manuscript, with input from Howard D. Rundle and Vincent Careau.

The work in the Chapter 3 is a slightly modified version of the following paper, co-authored by Vincent Careau, Howard D. Rundle (Department of Biology , University of Ottawa) and Alastair J. Wilson (Centre for Ecology and Conservation, University of Exeter):

Videlier, M., V. Careau, A. J. Wilson, and H. D. Rundle. 2020. Quantifying selection on standard metabolic rate and body mass in *Drosophila melanogaster*. Evolution (in press).I took the lead role in all stages of this work, including conceiving the experimental design, collected the data. analysing the model, interpreting the results, and writing the manuscript, with input from and Vincent Careau, Alastair J. Wilson, and Howard D. Rundle .

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“The mystery of life isn't a problem to solve, but a reality to experience.”

— Frank Herbert, Dune.

“No result is a result.”

— Source unknown

“ A difference in significance is not a significant difference.”

— Source unknown

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How to start! It is been a very long journey for me.

Firstly, I would like to acknowledge that I completed my PhD on the unceded territory of the Algonquin people, whom I respectfully thank for hosting me on their ancestral lands.

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CHAPTER 1: INTRODUCTION

Sexual conflict

Sexual conflict arises from the divergence of evolutionary interests of males and females that result from their distinct roles in reproduction (Chapman et al. 2003; Bonduriansky 2010; Hosken et al. 2019) which are themselves rooted ultimately in the evolution of anisogamy (Parker 1979; Arnqvist and Rowe 2005; Schärer et al. 2012). Sexual conflict generates sex-specific selection on shared traits such that an adaptive response in a trait can occur because it increases fitness in one sex, but this may come with a fitness cost for the other sex. Indeed, sex-specific selection is often observed in nature (Cox and Calsbeek 2009; Singh and Punzalan 2018) which can emerge in response to new physical or social conditions (Chapman 2006; Bonduriansky 2010; Pennell and Morrow 2013) and is often hypothesized to occur due to the presence of sexual selection in males that is absent in females (Arnqvist and Rowe 2005; Bonduriansky and Chenoweth 2009).

Depending on the genetic architecture of a targeted trait, two different forms of sexual conflict can be described: interlocus and intralocus (Bonduriansky 2010; Pennell and Morrow 2013). Interlocus sexual conflict occurs when the targeted trait has a distinct genetic basis in males and females. In interlocus sexual conflict, traits arise from the interaction of the sexes, with mating rate being a classic example. In this case, selection can favour loci that increase a male's reproductive success (via an increased mating rate) relative to competitor males, even if such loci reduce female fitness. At the same time, selection through females can favour other loci that increase fitness relative to other females, for instance by reducing their susceptibility to male-induced harm by decreasing mating rate. An evolutionary “arms race” between sexes can therefore result from the antagonistic co-evolution of sex-specific traits (Holland and Rice 1998; Arnqvist and Rowe 2005; Perry and Rowe 2015). Another classic example are seminal fluid proteins in male *Drosophila melanogaster*. Seminal fluid proteins decrease the probability of females remating and increase female short-term reproductive rates, both of which benefit males, but with a toxic side effect for females such as a reduction in longevity (Rice 1996; Chapman 2001; Crean et al. 2016). To counterbalance this cost, females rapidly adapt in terms of increased avoidance of males and stronger resistance to mating (Wigby and Chapman 2005).

I am interested in the other form, namely intralocus sexual conflict, as its prevalence is not well understood. Intralocus conflict occurs when the shared trait has the same genetic basis in males and females (Bonduriansky and Chenoweth 2009; Van Doorn 2009). In this case, selection is sexually antagonistic with the distinct fitness optima between sexes and alleles that increase fitness in one sex may decrease it in the other sex. An evolutionary “tug-of-war” between the sexes can thus emerge in which adaptation of one sex impedes that of the other (Bonduriansky and Chenoweth 2009; Van Doorn 2009). Intralocus sexual conflict can be resolved via the evolution of sexual dimorphism such that phenotypes in males and females can achieve their sex-specific fitness optima. Sexually dimorphic traits are thus strong candidates for those that have experienced past, and potentially ongoing, intralocus conflict.

The resolution of intralocus conflict is hampered by the shared genetic basis of the trait in the two sexes, and the extent of this evolutionary constraint can be quantified via the intersex genetic correlation (r_{fm}) for the trait (Lande 1980; Cheverud et al. 1985). r_{fm} is a standardized quantitative genetic measure of the additive genetic covariance of the trait in males and females and reflects the potential for independent evolution of the trait in the two sexes (Bonduriansky and Chenoweth 2009). In quantitative genetics, additive genetic effects are the primary determinant of the resemblance between relatives and govern the response to selection in a population (Falconer 1962). A positive r_{FM} indicates a positive correlation of additive effects in males and females such that selection on one sex will produce a correlated response in the trait in the same direction in the other sex. For traits with no history of sexually-antagonistic selection, r_{FM} is expected to be at or near one, indicating a shared genetic basis in the two sexes (Poissant et al. 2010).

A strong positive intersexual genetic correlation for a trait “Z” (i.e. $r_{fm\,(Z)}$) that is subject to sexually antagonistic selection will generate a strong negative intersexual genetic correlation for fitness (w) (i.e. $r_{fm\,(w)}$), indicating that alleles increasing fitness in one sex tend to decrease fitness in the other (Bonduriansky and Chenoweth 2009). Persistent sexually antagonistic selection should favour mechanisms that lower the intersexual genetic correlation for the trait ($r_{fm\,(Z)}$), thereby weakening the previously negative $r_{fm\,(w)}$ by allowing males and females to evolve toward their sex-specific optima (Bonduriansky and Chenoweth 2009). These mechanisms can include gene duplication, (Gallach and Betrán 2011; Wyman et al. 2012; Perry 2018), sex specific transcription (Rice 1984), and epigenetic processes such as genomic imprinting on autosomal chromosomes (Day and Bonduriansky 2004) or sex-specific splicing (Pennell and Morrow 2013). During the resolution of the conflict, as dimorphism of the trait “Z” evolves, $r_{FM(Z)}$ will decrease below unity and $r_{fm\,(w)}$ will become less negative. Values of both genetic correlations upon resolution of conflict (if achieved) are likely to vary depending on several factors including the strength and form of selection and the mechanism by which $r_{FM(Z)}$ was reduced (Bonduriansky and Chenoweth 2009). Sexual dimorphism is common in populations and is interpreted as a signature of past conflict, although whether the conflict is entirely or only partially resolved is an empirical question of current interest (Cox and Calsbeek 2009).

A comprehensive understanding of the potential for ongoing conflict requires estimates of the intersexual genetic correlation for the trait and for fitness, as well as sex-specific selection gradients. One of the first estimation of intralocus sexual conflict of fitness involved a laboratory-adapted population of *Drosophila melanogaster*, with a negative $r_{\mathrm{fm}\,(\mathrm{w})}$ for reproductive fitness in adults but a positive correlation for juvenile survival, thus suggesting the presence of a sexual conflict over reproduction in adults in this population (Chippindale et al. 2001). Observations of negative $r_{\mathrm{fm}\,(\mathrm{w})}$ have subsequently been found in other *Drosophila* populations (Pischedda and Chippindale 2006; Delcourt et al. 2009; Innocenti and Morrow 2010; Punzalan et al. 2014; Collet et al. 2016; Duffy et al. 2019) and other insects such as beetles (Berger et al. 2014*b*). Moreover, intralocus sexual conflict is not an artefact of laboratory conditions, as it has been observed in wild populations including deer (Foerster et al. 2007) and birds (Brommer et al. 2007). Although a negative $\mathrm{r}_{\mathrm{FM(w)}}$ indicates the existence of an intralocus conflict, it does not identify the shared trait involved in the sexual conflict. To date, only a few traits have been examined within the context of intralocus sexual conflict. Perhaps not surprisingly, those traits are classic dimorphic traits such as body mass (Mainguy et al. 2009; Stulp et al. 2012), wing size (Tarka et al. 2014), mandibula size (Harano et al. 2010), development time (Lewis et al. 2011), testosterone (Garver-Apgar et al. 2011; Mills et al. 2012; Mokkonen et al. 2012; Iserbyt et al. 2015), diet (Reddiex et al. 2013; Rapkin et al. 2017), immune defense (Svensson et al. 2009) and traits under sexual selection such as locomotion activity (Long and Rice 2007; Berger et al. 2014*a*), coloration (Charmantier et al. 2017; Cox et al. 2017) and pheromones (Ingleby et al. 2014).

The divergent evolutionary interest of the sexes is expected to lead to the evolution of different life history strategies. Sexually dimorphic life history traits are therefore strong candidates as targets of intralocus sexual conflict. Surprisingly, intralocus sexual conflict over metabolic rate has never been examined, despite the fundamental role of metabolic rate in life-history evolution. An individual's energy budget is likely to be strongly related to their reproductive strategy, and hence fitness, and so males and females with distinct reproductive strategies are expected are likely to differ in how they manage their energy budget. Furthermore, metabolic rate may underlie the trade off between longevity and reproduction. For example, a live fast-die young strategy may be associated with a high metabolic rate, whereas maintaining a slower rate of reproduction over a longer lifespan may be associated with a reduced metabolic rate, although it has more recently been suggested that this is an overly simplistic expectation . In addition, metabolic rate has been observed to differ between sexes, and is correlated with other traits such as locomotion, behaviour, and immune system, at the phenotypic and genetic level (Réale et al. 2010). Given the above consideration, metabolism is a prime candidate for intralocus sexual conflict. As the above discussion highlights, an inference of intralocus sexual conflict over metabolic rate requires estimating the intersexual genetic correlation for the trait, as well as sex-specific selection gradients.

Metabolic rate

Energy – the capacity or power to perform physiological work — fuels biological processes. To yield energy, organisms produce adenosine triphosphate (ATP) (Kleiber 1961) via anerobic (fermentation) or aerobic (oxidative reaction within the mitochondria) processes (or via anaerobic respiration in rare bacteria). ATP turnover within the cell is a key requirement to induce biological processes at larger scales such as muscle activation and locomotion. Accordingly, energy expenditure has become a fundamental consideration in ecology and evolution (Chown and Gaston 1999; Ricklefs and Wikelski 2002; Brown et al. 2004; Clarke 2006). Energy expenditure in a given time – i.e. metabolic rate – can be measured via respirometry using CO_2 production, or O_2 consumption, as an indirect proxy (Bourcquet and Lavoisier 1777; Lighton 2008*a*). In ectotherm species, standard metabolic rate (SMR) represents the minimum level of energy turnover required for sustaining life, and is usually measured on resting, non-growing, nonreproductive, and post-absorptive individuals (Rolfe and Brown 1997; Hulbert and Else 2004; Careau et al. 2014). Because metabolic rate in ectotherms is strongly influenced by ambient temperature (Clarke and Fraser 2004; White et al. 2006; Schulte 2015), SMR must be recorded at a given specified temperature. One of the major factors influencing SMR or BMR (basal metabolic rate; the analogous of SMR in endotherms) is body mass (White 2011; White and Kearney 2013). However, even after controlling for body mass, there remains substantial variation among individuals and species in SMR (reviewed in White and Kearney 2013). Explaining sources of variation in "mass-residual" SMR has been, and is still, a key challenge in integrative organism biology (Hulbert and Else 2004; Careau et al. 2014).

Countless comparative studies have investigated correlates of mass-residual variation in SMR at the inter-specific and inter-population levels. For example, it has been shown that "mass-residual" metabolic rate correlates with intrinsic factors such as mitochondria activity (Polymeropoulos et al. 2011; Salin et al. 2015) , aspects of body composition such as fat mass (Cunningham 1982; Johnstone et al. 2005), and relative size of metabolically active organs like the liver (Steyermark et al. 2005). Interspecific variation in SMR is also correlated with extrinsic factors like temperature (Nespolo et al. 2003; Terblanche et al. 2005; DeVries and Appel 2013; DeVries et al. 2013; Auer et al. 2018), altitude (Chown and Gaston 1999; Rourke 2000), and food regime (Van Leeuwen et al. 2012; Auer et al. 2015, 2016*b*; Ayayee et al. 2018). These results suggest that SMR can be shaped by natural selection as species and populations adapt to different environments.

More recently, research has focused on the causes and consequences of inter-individual variation in SMR (White and Kearney 2013; Pettersen et al. 2018, but see Bennett 1988) Studying individual variation can lead to a better understanding of the relationship between SMR and other ecologically-relevant traits such as performance or behaviour (Réale et al. 2010; Immonen et al. 2018). Several studies have shown consistent (i.e., repeatable) inter-individual differences in SMR or BMR (Nespolo and Franco 2007; White et al. 2013), which is a prerequisite for follow-up quantitative genetics studies (Falconer 1962). Several studies have reported significant narrow-sense heritability (h^2; the proportion of phenotypic variance due to additive genetic variance) for BMR in bird and mammal populations (Sadowska et al. 2005; Rønning et al. 2007; Tieleman et al. 2009; Wone et al. 2009; Careau et al. 2011; Bushuev et al. 2012; Boratyński et al. 2013; Mathot et al. 2013). However, there are only a small number of heritability estimates (narrow-sense or broad-sense) available for SMR or other metabolic rate traits in ectotherms populations including from snakes (Garland and Bennett 1990), aphids (Carter et al. 2012), crickets (Rantala and Roff 2006; Nespolo et al. 2007; Ketola and Kotiaho 2009), snails (Bruning et al. 2013; Nespolo et al. 2014), butterflies (Mattila and Hanski 2014), cockroaches (Schimpf et al. 2013), and *Drosophila* (Montooth et al. 2003; Jumbo-Lucioni et al. 2010; Castañeda and Nespolo 2013; Alton et al. 2017). The paucity of heritability estimates for metabolic rate in ectotherms is probably due, at least in part, to the logistical challenge of performing measurements on multiple individuals with low metabolic rate and possibly small size. Moreover, it is possible that strong selection has eroded additive genetic variance, leading to nonsignificant h^2 estimates (Mousseau and Roff 1987).

Several studies in birds and mammals have shown that natural selection can act on BMR (Blackmer et al. 2005; Boratyński et al. 2010; Zub et al. 2014; Nilsson and Nilsson 2016) or on RMR (‘resting metabolic rate’; similar to SMR but one of the conditions is violated such that individuals may be growing or digesting food) (Jackson et al. 2001; Larivée et al. 2010; Careau et al. 2013). Fewer studies have tested whether SMR is under selection in ectotherms (Álvarez and Nicieza 2005; Artacho and Nespolo 2009; Bartheld et al. 2015). For example, in terrestrial juvenile snails, SMR is negatively associated with survival, with a standardized selection gradient of -0.106 (Artacho and Nespolo 2009). The link between metabolism and fitness is therefore poorly understood and may also be complex, as positive or negative selection may be expected based on two distinct energy management models (Burton et al. 2011; Careau and Garland 2012; Portugal et al. 2016).

First, the allocation model predicts negative selection on SMR because the energy invested in maintenance (SMR) cannot be allocated to competing traits such as activity or reproduction. In this case, selection may favour a lower SMR to provide more energy to allocate to other competing demands. By contrast, in the performance model SMR is seen as the “engine”, reflecting the maintenance costs of the metabolic machinery required to sustain a given level of activity and reproduction. The performance model therefore predicts positive selection on SMR as larger metabolic machinery will provide more energy to allocate to various fitnessenhancing traits. Moreover, another way to examine the different energy management models is to explore the correlations between SMR, the energy cost of general activity, and the total energy budget. For the allocation model, the total energy budget is fixed, resulting in a trade-off between SMR and the energy cost of the general activity and hence a negative correlation between these traits. For the performance model, SMR correlates positively with activity, reflecting the "idling" aspect of SMR within a dynamic total energy budget. Of course, these two models are crude simplifications of energy budgets with only two main components: maintenance metabolism vs. non-basal energy expenditure. However, just as maintenance metabolism can be broken down into multiple components (e.g., digestive, muscular, respiratory, cardiovascular systems, etc.), non-basal energy expenditure can also be partitioned into different energy demands including reproduction, growth, and digestion. As additional components are added, models become more complex and predictions become difficult. In addition, energy invested into the general activity can be broken down between the different components such as foraging, exploration, aggression, territoriality, and locomotion. Each of these has a distinct energy cost and may correlate differently with SMR (Mathot and Dingemanse 2015; Mathot et al. 2019) and thus further complicates our understanding of the different energy management models.

An interesting possibility is that the allocation and performance models may apply differently to females and males, which could underlie sexually antagonistic selection and hence intralocus sexual conflict. In general, sexual dimorphism in metabolic rate and SMR has been observed in various species including humans (Cunningham 1982; Arciero et al. 1993; Sandboge et al. 2012), birds (Droge et al. 1991; Stuber et al. 2015), amphibians (Finkler et al. 2003; Rogers et al. 2007; Dalton et al. 2014; Ducret et al. 2020), crustaceans (Glazier et al. 2016), spiders (Watson and Lighton 1994; Shillington 2005; Walker and Irwin 2006), beetles (Rogowitz and

Chappell 2000; Arnqvist et al. 2017), wasps (Tomlinson and Phillips 2015) and *Drosophila* (Arking et al. 1988; Giesel et al. 1989; Berrigan and Hoang 1999; Piper et al. 2014). Sexual dimorphism in metabolic rate may have arisen from selection favouring different strategies of energy allocation due to the divergent life history of males and females. In spiders for example, metabolic rate is higher in males and this might reflect higher energetic demands of escaping females after mating (Shillington 2005). In crickets , metabolic rate is also higher in males compared to females, possibly to support the important energetic demand induced by male-male combat and/or sexual displays such as calling effort (Kolluru et al. 2004). Moreover, metabolic rate is linked to fecundity. For females, fitness is often driven by the balance between number and quality of eggs and metabolic rate varies with ovary activation and hence egg production (Davey 1993). In addition, metabolic rate has been shown to be higher for mated compared to virgin females (Giesel et al. 1989; DeVries et al. 2015; Arnqvist et al. 2017), consistent with an increased energetic demand of reproduction. Finally, metabolic rate is a major component of the pace-of-life syndrome (POLS; (Réale et al. 2010). The pace-of-life syndrome suggests the correlated evolution of metabolic rate and other traits, such as behaviour and performance, which would explain genetic correlations among these traits and their integration along a slow-fast life-history continuum. However, sexual dimorphism in ‘position’ along the slow-fast life-history continuum has been shown (Royauté et al. 2018; Tarka et al. 2018) , suggestive of past sex-specific selection and the potential of ongoing sexual conflict (Hämäläinen et al. 2018; Immonen et al. 2018). Individual traits contributing to POLS have been observed to be under intralocus sexual conflict including body mass (Mainguy et al. 2009; Stulp et al. 2012), locomotory activity (Long and Rice 2007; Berger et al. 2014*a*) and immune defense (Svensson et al. 2009). As the discussion above highlight, sexual conflict over SMR seems likely but this has not, to the best of my knowledge, been addressed to date.

Objectives

I am interested in understanding intraspecific variation in SMR, including its h^2 (of which there are few estimates) and, ultimately, how selection acts on it, whether it differs between males and females, and whether it is subject to intralocus sexual conflict. The optimal approach to inferring conflict (or its absence) would be to quantify both SMR and fitness in a large number of pedigreed individuals, allowing me to estimate genetic covariance matrices (and hence r_{fm}), phenotypic and genetic selection gradients, and predicted responses to selection in SMR in both males and females (Stinchcombe et al. 2014). *D. melanogaster* is a good model species for this sort of investigation because they have a short generation time, which is advantageous to breeding designs (Roff and Mousseau 1987), and various phenotypes, including comprehensive measures of lifetime fitness, can be obtained from many replicate individuals. Metabolic studies have been performed in this species (Khazaeli et al. 2005; Lighton 2008*b*; Bharucha 2009; Charette et al. 2011; Rajan and Perrimon 2013), but to date challenges associated with measuring individual metabolic rate in such a small organism have prevented large scale quantitative genetic studies.

Therefore, in chapter 2 (my first data chapter), I begin more generally by taking repeated measures of individual metabolic rate for 232 males and 245 females using a high-throughput flow-through respirometry. This allows me to estimate repeatability of individual-level SMR and to identify key factors that influence its variation (which may inform appropriate designs for further studies). Individuals were also repeatedly measured for their general locomotor activity, providing insight into key sources of variance, in addition reflecting a proxy of the non-basal energy expenditure. Doing so also allows me to estimate the correlation between SMR and locomotor activity to gain insight into sex-specific energy management strategies in *Drosophila*. Then, in chapter 3, I measure SMR, body mass and lifetime reproductive success of 515 males and 522 females. I take advantage of a laboratory population with a defined life cycle to obtain comprehensive and relevant measures of fitness. By foregoing a breeding design, I can estimate linear and non-linear selection on SMR and body mass in a very large number of males and females, and I can test for sexually antagonistic selection. Finally, in chapter 4, I present results from a quantitative genetic breeding design with over 1230 females and 1252 male measured either for SMR or locomotor activity. The goal is to estimate sex-specific matrices of additive genetic (co)variances for these traits (i.e. $\mathbf{G}_f$ and $\mathbf{G}_m$), as well as the matrix of cross-sex genetic covariances within and between traits, known as the **B** matrix. This provides insight into genetic constrain arising from any shared genetic basis of these traits. Unfortunately, statistical, and conceptual issues surrounding the inclusion of male and female fitness into the analyses was beyond the scope of my thesis, but I return to this issue when discussing future directions in my General Discussion.

Subsequent data chapters were written as stand-alone manuscripts for publication in peer-reviewed journals. As such, there is some repetition of background and minor variation in formatting. Contributions by myself and co-authors are addressed at the beginning of each chapter.

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CHAPTER 2: Sex-specific among-individual covariation in locomotor activity and resting metabolic rate in *Drosophila melanogaster.*

This chapter has been published in *American Naturalist* 2019 in collaboration with Vincent Careau (VC) and Howard D. Rundle (HDR). I designed the experiment with the help of VC and HDR. I collected the data. I analyzed the data with inputs from VC and HDR, and all authors wrote the manuscript.

Introduction

All biological processes require energy, and because metabolic rate (MR) quantifies the energy expenditure of an organism over time, it is a fundamental measure of an organism's physiology. Understanding sources of variation affecting MR is therefore a key endeavour in evolutionary physiology. One of the major components of organisms' energy expenditure is their standard metabolic rate (SMR), defined for ectotherms as the lowest MR of an adult, post-absorptive, non-reproductive, and inactive individual measured at a specified ambient temperature. SMR has been measured in numerous ectotherms including fish (Salin et al. 2015), amphibians (Louppe et al. 2018), molluscs (Naya et al. 2011), small insects (DeVries et al. 2013), and other arthropods (Schimpf et al. 2012). It is well established that SMR scales with body mass at the inter- and intra-specific levels (Niven and Scharlemann 2005; White and Kearney 2013). Yet, after accounting for body mass, substantial among-individual variation in SMR (or basal MR for endotherms) often remains within a population/species (Burton et al. 2011).

Residual variation in SMR may relate to investment in other energy-demanding activities or processes (e.g., reproduction, locomotion, digestion). Various models have been proposed to describe how individuals manage their energy budget and explain the resulting covariances (or lack thereof) among energy demanding traits (Ricklefs et al. 1996; Speakman 1997; Nilsson 2002). The performance model posits that increased investment in non-resting energy expenditure (e.g., activity and reproduction) requires a larger metabolic machinery, the maintenance costs of which increase SMR. In the performance model, SMR is seen as the "idling cost" of the engine that is required to sustain high levels of energy expenditure associated with activity and reproduction. By contrast, in the 'independent' model of energy management, increased non-resting expenditure is dissociated from maintenance costs and hence SMR due to the lack of a mechanistic link between them (Careau and Garland 2012). Finally, the allocation model assumes a restricted energy budget that creates a direct trade-off, and hence a negative association, between resting and non-resting energy expenditure (Careau and Wilson 2017). Empirical support exists for all three models as various relationships have been observed between SMR (or basal MR for endotherms) and other energy-demanding traits (e.g., locomotion, aggressiveness, exploration, reproduction, growth; Burton et al. 2011; Careau and Garland 2012). However, phenotypic correlations were not partitioned in many studies (see below), which may contribute to the heterogeneity in the MR- activity relationships across taxa and contexts and between the sexes.

In insects, males and females often differ in whole-organism SMR (Burggren et al. 2017), which is not surprising given prevalent sexual size dimorphism (Leimar et al. 1994; Teder and Tammaru 2005; Blanckenhorn et al. 2007). However, sex differences in SMR sometimes persist after conditioning on body mass (Tomlinson and Phillips 2015; Arnqvist et al. 2017). This suggests that there are other phenotypic differences between males and females that contribute to dimorphism in SMR. These differences may include energy-expensive activities like locomotion and other behaviours that arise from the contrasting life histories of the sexes. Locomotor activity in insects, most notably flying can be energy demanding (Kammer and Heinrich 1978; Dickinson and Lighton 1995). While walking may only elevate MR by 5-10%, in species that walk a lot this may represent a non-trivial component of the non-resting energy budget (Berrigan and Lighton 1994; Berrigan and Partridge 1997). The relationship between locomotor activity and SMR (the resting component of the energy budget) is thus of interest. Testing for the relative effect of sex on SMR and locomotor activity, as well as their relationship, may provide insight into the different energy management models and shed light on how RMR might coevolve with behaviour and life-history strategies (Réale et al. 2010; Burggren et al. 2017).

Direct and indirect measures of individual SMR in small insects like *Drosophila* have proven challenging to date because their low metabolism yields levels of heat production and CO_2 enrichment that are difficult to quantify precisely (Burggren et al. 2017; Fiorino et al. 2018). With respect to indirect measures, a common solution to this problem has been stop-flow respirometry, whereby an individual is sealed in a chamber for a period long enough for CO_2 enrichment to be measurable with precision (Van Voorhies et al. 2004; Khazaeli et al. 2005; Jensen et al. 2014; Messamah et al. 2017; Stahl et al. 2017). However, stop-flow measurements can be inaccurate because activity may vary over the measurement period (Lighton and Halsey

2011). This problem is especially severe in insects like *D. melanogaster* which generally remain quiescent for short periods only (Greenspan et al. 2001) and increase their activity as they starve (Yang et al. 2015). To minimise bias introduced by activity during measurement, it is necessary to use flow-through respirometry coupled with motion detectors to simultaneously measure instantaneous CO_2 production and locomotor activity (Lighton and Halsey 2011). This way, it is possible to identify periods of inactivity and corresponding low MR estimates representing SMR. In the absence of activity measurements, discontinuous CO_2 emission is sometimes used to indicate that an insect is at rest (Matthews and White 2011). However, this is problematic because not all species exhibit discontinuous gas exchange (Marais et al. 2005).

An additional challenge when testing for a link between SMR and locomotor activity is that both traits typically show moderate repeatability (Bell et al. 2009; White et al. 2013). Therefore, the phenotypic correlation (r_P) between SMR and activity will not only reflect the among-individual covariance, but will also be influenced by the within-individual covariance (Dingemanse and Dochtermann 2013). The among-individual correlation (r_ind) reveals the strength of the relationship between two traits resulting from additive genetic and/or permanent environment sources of covariance, while a within-individual correlation (r_e) can arise from correlated measurement error and/or correlated plasticity of the trait at the individual or population level (Careau and Wilson 2017). In many cases, r_ind and r_e are different such that focussing on r_P alone can be misleading (Careau and Wilson 2017). Properly partitioning r_P into r_ind and r_e requires repeated measurements of multiple individuals, meaning a high-throughput measurement system is needed.

We address these issues in a small insect (*D. melanogaster*) by separately and repeatedly measuring individual variation in MR and locomotor activity (walking) in both males and females. Using high-throughput flow-through respirometry (with concomitant activity monitoring), along with separate activity monitors, we performed repeated paired measurements of MR and locomotor activity outside of respirometry on male and female *D. melanogaster*. Between the first and second measurement, half of the individuals were maintained in isolation whereas the others were housed with an opposite-sex partner and allowed to mate. As a result, one of the criteria for measuring SMR (non-reproductive) was intentionally violated in half of our subjects in their second measurement, and therefore we refer to our measurements as "resting metabolic rate" (RMR). In doing so, our experimental protocol allowed us to not only evaluate how MR and activity were affected by body mass and sex, but also reproductive status. After taking body mass, sex, and reproductive status into account, we partitioned r_P between RMR and activity into r_{ind} and r_e in males and females separately, and for activity levels at different periods of the day; this revealed important sex and time differences in how RMR and activity covaried.

Materials and Methods

Study animals and husbandry

A stock population was established in February of 2016 from a large sample of a laboratory-adapted population of *D. melanogaster* that had originally been collected from Dundas, ON, Canada in 2006 (MacLellan et al. 2012). This new stock was maintained with discrete, non-overlapping generations in 64 vials at 25°C, 50% relative humidity, and with a 12L:12D photoperiod (lights turn on/off at 7am/pm respectively) on a standard cornmeal-based food (90g/L cornmeal, 100g/L turbinado sugar, 40g/L yeast and 12g/L agar).

The life cycle of our population included a 4-day ‘mating phase’ that took place in an environment (bottles) that featured reduced density and increased spatial complexity compared to standard *Drosophila* maintenance techniques, followed by a 1-day laying phase in a standard vial for females. The mating phase occurred in standard 8 oz polypropylene round-bottom culture bottles (Fisherbrand, Pittsburgh, PN, USA) filled with 75 ml of food (fig. S2.1; figs. S2.1– S2.3 are available online or in the annexe). The surface of the food was divided into six approximately equal sectors by inserting opaque plastic barriers into the food, preventing individuals on the surface of the food in one sector from seeing those in another sector. To further increase spatial complexity, two coiled pipe cleaners also protruded from the bottom of the foam plug into the interior space of the bottle. Together with the reduced adult density (10 males and 10 females), this mating environment provides individuals with places to hide (e.g., to avoid unwanted sexual attention) and may result in the expression of a richer repertoire of sexual and other behaviours (e.g., search effort and territory defense in males; escape behaviours and choice among multiple food patches for feeding and egg laying in females). Each generation when adult offspring eclosed, all individuals from the 64 vials were mixed and then 640 individuals of each sex were randomly chosen and evenly distributed among 64 bottles (i.e. 10 females and 10 males per bottle). After four days in these bottles, males were discarded, and surviving females were evenly transferred among 64 standard glass culture vials (28.5 mm x 95 mm containing 10 mL food and with abundant live yeast sprinkled on the top) for egg laying. Females were allowed to lay eggs for 24 h, after which they were discarded. Adult offspring were subsequently collected from these vials 9 days later and the above mating protocol was repeated to create the next generation.

Experimental design

The experiment was conducted in blocks of 64 individuals (32 of each sex). Flies were collected from the stock population as virgins (3-5 h after eclosing) and stored separately by sex in standard food vials. All flies were subsequently individually measured twice for locomotor activity and twice for MR, with half of the individuals being housed individually between the two sets of measurements and the other half housed with an opposite-sex individual with which they could mate. Starting 4-6 hours after flies were collected as virgins, locomotor activity was recorded for 24 hours, after which individuals were directly transferred into a chamber in which MR was measured over 9 hours overnight. After respirometry, each fly was weighed to the nearest 0.001 mg on an MX5 Microbalance (Mettler Toledo, Columbus, OH, USA) and then transferred to a standard food vial either individually or together with an opposite sex individual from the same block. After 36 hours in these vials, all individuals were measured again for locomotor activity, then MR overnight, followed finally by another body mass measurement. Eight blocks were performed for a total of $N = 520$ individuals. The loss of a few individuals (natural and accidental deaths and escapees) reduced the final sample size to $N = 496$.

Locomotor activity measurement

Locomotor activity outside of respirometry was measured using two DAM2 activity monitors (Trikinetics, Waltham, MA, USA), each capable of measuring 32 separate flies. For each set of measurements, 64 flies were anesthetised with CO_2 and then individually and randomly placed in separate polycarbonate tubes (65 mm long, 5 mm diameter). Tubes were filled at one end with 2 cm of food and capped with a rubber stopper. At the opposite end, tubes were plugged with 1.5 cm of cotton to allow gas exchange. Locomotor movement was detected by a single infrared beam that bisected each tube. The monitors were housed in a separate incubator (with environmental conditions matching those of the stock) that was left undisturbed for the duration of the recording. The activity monitors were connected to a computer running DAM System 303X software (Trikinetics, Waltham, MA, USA) which recorded the total number of infrared beam breaks in 5 min bins separately for each tube (i.e., fly).

Recordings started at 21:00 and lasted for 24 hours. The first 2 hours of data were discarded to allow acclimation to the experimental conditions. Overall activity for each fly was calculated as the average number of movements in a 5-min bin during the remaining 22 hours (ACT_{avg}). As typical for *D. melanogaster* (Helfrich-Förster 2000; Ferguson et al. 2015), locomotor activity followed a bimodal pattern with peaks occurring immediately after changes in lighting (Fig. 2.1). Accordingly, for analysis we also partitioned locomotor activity into four separate components corresponding to different specific periods of the day (i.e. peak or not peak, lights on or off; Fig. 2.1). We extracted average activity (1) during the hour after the lights were switched on, which corresponds to the morning peak (ACT_{mp}), (2) during the hour after the lights are switched off, which corresponds to the evening peak (ACT_{ep}), (3) between the two peaks when the lights are on (ACT_{day}), (4) between the two peaks when the lights are off (ACT_{night}). Overall, we collected n = 956 activity measurements from N = 496 individuals.

Metabolic rate measurement

CO_2 production (VCO_2) of individual flies was measured using a 64-chamber flow-through respirometry system, housed in an incubator with a regulated temperature of 25°C (±1°C). The system consists of four separate units, each comprised of a differential CO_2 analyser (Li-Cor7000, Li-Cor Biosciences, Lincoln, NE, USA) and a 16-channel flow management, data acquisition, and signal processing system (MAVEn; Sable Systems International, North Las Vegas, NV, USA). Each MAVEn incorporates a flow-distribution manifold, a main board (flow measurement, regulation, and control plus data acquisition and signal processing), and an activity board (sensors for activity, ambient temperature, humidity, and light intensity). All CO_2 analysers were calibrated simultaneously at the beginning and midway through the experiment with pure nitrogen (zero) and spanned with certified gas at 14 ppm CO_2. Under normal operation, a constant stream of dry CO_2-free air produced by a PG14L purge gas generator (Peak scientific, Glasgow, Scotland) was split into four different streams and pushed through the reference cell of each CO_2 analyser (Cell A). Afterwards, each air stream was humidified by flowing through Nafion tubing (du Pont de Nemours and Company, Wilmington, DE, USA) submerged in distilled water. The re-humidified air stream was then fed into the flow-distribution manifold where it was physically split into 17 streams (one for each of the 16 chambers and one for the baseline), of which only the baseline was actively regulated at a flow rate of 20 $ml \cdot min^{-1}$. The approximately equivalent flow rates in the non-baseline channels (range: 15 to 25 $ml \cdot min^{-1}$) were maintained by means of matched flow resistances based on micro-orifice flow restrictors. A second mass flow meter on the MAVEn's main board measured the actual flow rate of each selected air stream before it was automatically directed through the measurement cell (Cell B) of the CO_2 analyser.

Flies were gently placed without anaesthesia into individual chambers made of clear plastic tubes (40 mm high by 6 mm diameter). Measurements were performed for 9 hours overnight, between 21:00 and 7:00, which correspond to the period of lowest activity in this population (Fig. 2.1). Each MAVEn was set to measure each chamber for 120 seconds (dwell time) with baseline measurements (120 seconds) taken every 16 chambers (i.e., interleave ratio = 16), resulting in a 34 min time cycle (i.e., each fly was measured 16 times for 2 min every 34 min). The chambers were placed on the MAVEn activity board above three infrared activity sensors per chamber that constantly recorded movement during the measurements.

All data transformation and extraction were done in ExpeData (Sable Systems). The raw activity channels (one per chamber) were transformed into an index of activity by first calculating the cumulative sum of the absolute difference between adjacent samples and then by differentiating the resulting channel vs. time (equivalent to calculating the slope of the cumulative activity vs. time). The CO_2 trace (one for all of the 16 chambers for a given MAVEn unit) was corrected for drift using multiple baseline correction measures and corrected for a 15 second lag with the activity channels. VCO_2 was then calculated by multiplying flow rate by the fractional concentration of CO_2. Although O_2 consumption was not measured, precluding the quantification of the respiratory quotient, small organisms like *Drosophila* do not consume enough O_2 to significantly bias VCO_2 measurements (Lighton 2008*a*). Moreover, *Drosophila* rely mostly on carbohydrates, such that their respiratory quotient does not substantially deviate from 1 in normoxia (Van Voorhies 2009).

The first 40 seconds of each measurement was discarded to allow the system to fully equilibrate after changing between chambers. From the remaining 80 seconds of each measurement period, we extracted the lowest 20 second continuous bout of VCO_2 using the "nadir" function in ExpeData. In addition to the average of the lowest 20 seconds, we also extracted the average flow rate, water pressure, temperature, light intensity, and activity (ACT_{20}). We also extracted the average activity over the 20 seconds immediately prior to the VCO_2 measurement (ACT_{20p}). For each respirometry run, the lowest of the 16 extracted VCO_2 values was selected per individual (Fig. S2.2). Because one of the criteria for measuring SMR (non-reproductive) was violated for half of our individuals in the second set of measurements, we collectively refer to our measures as RMR instead of SMR. Sample size for RMR was slightly lower than for general activity because one unit failed to record data in the second measurement of the third block, eliminating 16 observations. One unit was also incorrectly calibrated mid-way through the experiment such that all 158 observations from it were excluded, thereby yielding $n = 800$ observations from $N = 477$ individuals overall.

Statistical analyses

Data for analyses have been deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.8pp0nv1; Videlier et al. 2019). All continuous variables were standardised to a mean of 0 and a variance of 1. ACT_{20} and ACT_{20p} were log-transformed before standardisation. Sex was treated as a binary variable (female = 0, male = 1), as was reproductive status (virgin = 0, mated = 1) and test sequence (first measurement = 1, second measurement = 2). Sex, sequence, and reproductive status were centered by subtracting their respective means from every observation to facilitate the interpretation of main effects in the presence of interactions (Schielzeth 2010). Analyses were conducted in ASReml- R (Butler et al. 2007) and were organized in two distinct parts.

First, we ran separate univariate mixed models to test for the influence of body mass, sex, and reproductive status on RMR and on each of the five activity variables (ACT_{avg}, ACT_{mp}, ACT_{day}, ACT_{ep}, ACT_{night}). All models included body mass, sex, reproductive status, and test sequence as fixed effects, as well as all two-way interactions among these except the test sequence × reproductive status interaction which was not be included since no individuals were mated in the first measurement. We also tested for possible three-way interactions; none were significant ($P > 0.07$ in all cases), so we restrict our presentation to two-way interactions. Fixed effects were tested using a conditional Wald *F*-statistic and the denominator degrees of freedom were determined following Kenward and Roger (1997). Additional covariates were included for RMR to account for variation caused by nuisance variables: temperature, flow rate, water vapour, light intensity, and direct activity during and immediately prior to the RMR measurement (ACT_{20} and ACT_{20p}). All univariate mixed models included block and individual identity as random effects. For RMR, we added another random effect to account for the non-independence of the 16 measurements with a given unit on a given day (i.e., separate levels for each day × unit combination). For RMR and the five activity variables, we calculated repeatability (R) conditioned on the fixed and random effects in each model (Dingemanse and Dochtermann 2013).

In the second part of the analysis, we fit bivariate mixed models to quantify the relationship between RMR and activity at the among- and within-individual levels. Accordingly, all bivariate mixed models included RMR and one independent measure of activity as response variables. Models included the same random and fixed effects as the univariate models above. Correlations between RMR and activity at the among-individual (r_{ind}) and within-individual (i.e. residual, r_e) levels were estimated by specifying a 'corgh' structure within (G and R) matrices.

We used profile likelihoods from nadiv package (Wolak 2012) in R v.3.4.4 (R Core Team 2018) to calculate approximate 95% confidence intervals (CIs) for all correlations. Phenotypic correlations were estimated using repeatabilities and partitioned correlation estimates (Careau and Wilson 2017). Finally, we repeated each bivariate mixed model while specifying a heterogeneous correlation structure according to sex, providing separate variance and correlation estimates for males and females. Significance of the sex-specific correlations was evaluated as above using 95% CI's.

Results

RMR: Effect of activity during the metabolic measurements

For a given individual, RMR was selected as the lowest VCO_2 value from among the 16 separate measurements collected over the 9 hours respirometry run (from 21:00 to 7:00 the next day); these tended to occur later during the run (i.e., between 4:00 and 7:00; Fig. S2.2A). Activity was also recorded during the RMR extraction period, both immediately before and during the metabolic extraction (ACT_{20p}, ACT_{20}). Both ACT_{20p} and ACT_{20} tended to be low, and were often zero, at the lowest VCO_2 value, although some flies were moderately but regularly active throughout the entire respirometry run such that some activity occurred even during their lowest VCO_2 measurement (Fig. S2.2B). As expected, this remaining activity was positively correlated with MR (Table S2.2A, Fig. S2.2C; tables S2.1–S2.3). Although such activity is an unwanted source of variation in our RMR measurements, ACT_{20} and ACT_{20p} were included as covariates in all subsequent models analysing variation in RMR to statistically account for the effect of activity during respirometry. Results presented below remained qualitatively unchanged when analyses were repeated after removing all RMR observations with $ACT_{20} > 0.76$ (the value at which the effect of ACT_{20} on RMR became nonsignificant; Fig. S2.3).

RMR: Mass, sex and reproductive effects

As expected, RMR increased with body mass (Fig. 2.2A) and was also significantly higher in mated compared to virgin individuals (Fig. 2.2B). The effect of reproductive status did not vary by sex or with body mass, as indicated by the non-significant two-way interactions (Table 2.1, pt. A). Although RMR did not differ between males and females when accounting for body size (Table 2.1, pt. A), there was a significant sex × body mass interaction, such that RMR increased at a faster rate with body mass in males than females (Table 2.1, pt. A, Fig. 2.2A). After accounting for all these sources of variation, the repeatability ($R \pm$ SE) of RMR was 0.342 ± 0.055 (see Table S2.1 for variance components).

Locomotor activity: Sex and reproductive effects

Outside of the metabolic measurements, mated flies were significantly more active than virgin flies and activity also decreased significantly with increasing body mass (Table 2.1, pt. B). Males and females did not differ significantly in average activity (Table 2.1, pt. B) and none of the two-way interaction among these effects were significant.

Partitioning locomotor activity into four separate periods of the day revealed substantial differences between males and females, and between mated and virgin individuals, during all periods except the non-peak night (Fig. 2.1; Table 2.2). Males were significantly more active than females during the morning and evening peaks but were significantly less active than females during the day. In term of reproductive status, mated females were significantly more active than virgin females and males (mated and virgin) during the day, generating a significant sex × reproductive status interaction. There was also an effect of reproductive status during the evening peak, whereby mated flies were less active than virgins for both males and females. After accounting for these sources of variation, the repeatability of average activity was $R = 0.581 \pm 0.031$ and values were similar for its component parts (Table S2.1).

Relationship between RMR and locomotor activity

There was a weakly positive yet significant phenotypic correlation between RMR and average activity outside of respirometry (ACT_{avg}; estimate [95% CI]; $r_P = 0.08$ [0.06, 0.10]; Table 2.3). This correlation was stronger and again significant at the among-individual level ($r_{ind} = 0.26$ [0.13, 0.39]; Table 2.3), indicating that individuals with a higher RMR also tended to be more active. Average activity and RMR were not correlated at the within-individual level (Table 2.3).

Partitioning locomotor activity into components during different times of the day revealed variation in its relationship with RMR. At the phenotypic level, all activity variables remained weakly yet positively correlated with RMR, with the highest point estimate occurring for activity during the night ($r_P = 0.11$ [0.18, 0.13]; Table 2.3). Again, correlations were stronger at the among-individual level and ranged from 0.20-0.26, except for activity during evening peak when it was weaker and non-significant (0.08). At the within-individual level, all correlations between RMR and activity were non-significant except for a positive association with activity during night (Table 2.3).

Sex-specific correlations between RMR and locomotor activity

In males, there were significant and positive among-individual correlations between RMR and average activity, as well as with activity during the four components of the day (Table S2.3, Fig. 2.3). By contrast, in females only the among-individual correlation between RMR and evening peak activity was significant, and it was negative (r_{ind} = -0.44 [-0.70, -0.20]). Sex-specific correlations are significantly different (i.e. 95% CIs were non-overlapping; Table S2.3, Fig. 2.3) only for the relationship between RMR and the two peak activity periods. For the evening peak, the sex-specific among-individual correlations were both moderately strong (|0.44-0.46|), but of opposite sign, likely contributing to the non-significant among-individual correlation at the population level (see above; Fig. 2.3; Table 2.3). Within-individual correlations were non-significant in most cases (Table S2.3).

Discussion

We measured locomotor activity and RMR twice in a large number (> 400) of male and female *D. melanogaster* to quantify sources of variation affecting these traits and understand their covariation. In our population, RMR and locomotor activity were influenced by body mass, reproductive status, sex, and interactions among some of these. Accounting for these effects, the repeatabilities of RMR and average activity were moderate and significant, reflecting consistent among-individual differences in both traits. The correlation between RMR and average activity was positive at the among-individual level (r_{ind} = 0.26) but was sex- and time-specific. In females, among-individual correlations were either non-significant or negative when considering activity over different periods of the day but were consistently and significantly positive in males. Results remain qualitatively unchanged when excluding measurements involving flies that were unusually active during RMR estimation. Because RMR and locomotor activity are substantial components of an individual's energy expenditure, differences in their covariance between males and females suggests fundamental differences in how the sexes manage their energy budget.

Source of variation in RMR

Consistent with past studies in *Drosophila*, females had higher RMR than males (1.33-fold greater on average; Fig. 2.2). When conditioned on body mass, however, RMR did not differ significantly between the sexes (Table 2.1A). Interestingly, the RMR-body mass relationship did differ between males and females, with males showing a steeper slope than females (Fig. 2.2A). A steeper slope could arise if, compared to females, males disproportionally invest in energetically more expensive tissues as they gain mass. For example, it is known that sexes differ in lipid content (Parisi et al. 2011), midgut length and activity (Fear and Oliver 2016), water content (Chippindale et al. 2009; Parkash et al. 2014), and musculature (Taylor and Knittel 1995), but whether these differentially scale with body mass in males and females has, to our knowledge, not been addressed. These potential sexual differences in the RMR-body mass relationship should be further examined using a wider range of body sizes and greater overlap between males and females.

RMR was also affected by reproductive status such that mated individuals had higher RMR than virgins in both sexes (Table 2.1A). This is consistent with previously observed differences in daily energy expenditure in *D. melanogaster* (Piper et al. 2014). In our case, the opportunity to mate was associated with a change in social environment, so the resulting impact on RMR could be the product of reproduction and/or social effects. Reproduction induces major physiological changes in *Drosophila*, some of which may be costly and could thus elevate RMR. RMR in our experiment was measured at least 24 hours after the period during which individuals could mate, so any underlying effect on RMR must have persisted for a substantial period of time. *Drosophila* females are known to increase egg production for at least five days following mating (Manning 1962). In males, mating induces sperm replacement and resynthesis of accessory gland proteins (Herndon et al. 1997) as well as modification in the head of the expression of genes associated with behaviour (Ellis and Carney 2010), although the time course of these changes and their associated energetic costs are not fully known.

Source of variation for locomotor activity

Consistent with several past studies (Helfrich-Förster 2000 and references therein), locomotion showed a bimodal pattern through the day, with morning and afternoon peaks associated with switches in lighting. When activity was partitioned into different components reflecting the morning and evening peaks and periods between, the factors affecting it varied (Table 2.2). The differing life histories of males and females may underlie this heterogeneity (Martin et al. 1999; Ferguson et al. 2015). For instance, males were more active than females during both peaks; this could reflect increased investment in mate acquisition during periods when females are most sexually receptive. Outside of the peaks during daylight hours, females were more active than males and mated individuals were more active than virgins. Moreover, sex and reproductive status interacted such that mated females were particularly active during the non-peak day. A similar pattern has been observed in other studies (Ferguson et al. 2015 and references therein) and, as previously suggested, could reflect increased activity in females associated with egg production, egg laying, and/or behavioural avoidance of male sexual harassment.

Repeatability of RMR and locomotor activity

Quantifying among-individual differences in MR can be challenging because it is affected by numerous intrinsic and extrinsic factors and is technically difficult to measure, most notably in small insects. Failing to control for such factors can bias estimates of repeatability. For instance, if locomotor activity is repeatable, as our results suggest (R, $\mathrm{ACT}_{\mathrm{avg}} = 0.581 \pm 0.031$), then failing to control for activity during metabolic measurements would inflate the repeatability of MR. The solution involves longer-term and concomitant monitoring of instantaneous MR and locomotor activity, allowing RMR to be estimated during periods of lowest activity. However, this is time-consuming and exacerbated by the need for repeated measures of individuals to partition components of variation. Here, we used a novel, multi-channel flow-through respirometry system to measure RMR twice in over 400 individuals. Our results revealed a moderate and reasonably precise estimate of the repeatability of 0.33 ± 0.06 for RMR. Importantly, given that two-thirds of the variation in RMR occurs within individuals, the phenotypic correlation between RMR and activity is unlikely to provide a good proxy for the among-individual correlation.

Relationship between activity and RMR

Although there was a weak phenotypic correlation between RMR and overall locomotor activity, the noticeably stronger among-individual correlation indicates that the low within- individual correlation was attenuating the phenotypic relationship. These pooled estimates across sexes and activity components (i.e., peak vs. non-peak periods) also obscured substantial heterogeneity in this RMR-activity relationship. Further partitioning by activity periods (i.e., ACT_{mp}, ACT_{day}, ACT_{ep}, ACT_{night}) revealed fairly consistent and positive correlations in males across all times, whereas the correlation in females was weak and non-significant in three activity periods and significant but negative in the fourth (Fig. 2.3). To date, rare examples of sex-specific relationship between RMR and behaviour have been observed in rodents (Lantová et al. 2011; Šíchová et al. 2014) with opposite correlations between proactive behaviours and RMR in males and females. Another study in birds found a significant negative correlation between breathing rate and nest defense in males but not in females (Krams et al. 2014).

The contrasting relationships between RMR and activity in males versus females implies that sexes differ in how they manage their energy budget. In males, the positive and consistent among-individual correlation between RMR and activity supports the “performance” model in which increased RMR is a cost associated with the metabolic machinery needed for other energy-demanding activities. In *Drosophila*, males can increase their reproductive success by increasing their mating frequency (Bateman 1948; Hall 1994). To achieve more matings a male may have to allocate more energy to costly behaviours such as searching for and/or pursuing potential mates, courting females, and fighting with other males, all of which should lead to increased maintenance costs reflected by a higher RMR. Such behaviours may be more common in our population compared to other *Drosophila* lab populations because its complex mating environment was specifically designed to allow the expression of a richer repertoire of sexual and other behaviors (see fig. S2.1). Recent experiments have shown that such environmental complexity alters sexual selection and sexual conflict (MacLellan et al. 2010; Yun et al. 2017; MacPherson et al. 2018; Malek and Long 2019).

In females, the general absence of an RMR-activity association is consistent with the “independent” model of energy management in which maintenance costs and non-resting expenditure are dissociated. This suggests that the organs required to sustain activity do not contribute to RMR, or in other words that RMR and activity are free to vary independently within the female energy budget. In contrast to males, which actively pursue and court females during reproduction, female activity associated with reproduction is likely more dependent on resource acquisition (both foraging and locating egg-laying sites). These female-specific reproductive behaviours may require different muscle structures and/or neuronal circuits (Dickson 2008; Nojima et al. 2018) that are independent of RMR. Our lower density and structurally complex mating environment (Fig. S2.1) may also allow females to avoid male harassment by hiding instead of via more energy expensive resistance and escape behaviours.

The one exception to the lack of an RMR-activity association in females was a significant negative correlation between RMR and activity during the evening peak, which is instead consistent with an “allocation” model in which resting and non-resting energy expenditure trade off. A potential explanation could involve large variation in another component of the energy budget that generates a trade-off between activity and RMR, but only during the evening peak. In contrast to males, *Drosophila* females maximize fitness by investing primarily in gamete production, which may impose a constraint on a female’s energy budget. The reasons why this negative relationship only occurred during the evening peak is unclear, but it implies that females must be engaged in some activity that relates differently to RMR than do activities performed at other times of the day. Unlike the morning peak which occurs after lights switch on, the evening peak occurs entirely in the dark. Behavioural studies that track diurnal patterns of investment in various energy-demanding activities (locomotion, foraging, reproduction, etc.) would be useful in understanding the proximal and ultimate causes of this temporal variation in females.

In summary, sexual dimorphism in the RMR-activity covariance suggests fundamental differences in how males and females manage their energy budgets. Whether this sexual dimorphism in RMR-activity covariance is an adaptive response to divergent life histories is an intriguing question. Quantifying sex-specific selection on RMR and activity will be important to addressing this. Activity in our case was measured on single flies in small chambers, so the extent to which this serves as a reasonable proxy for activity under conditions normally experienced in these populations (i.e., mixed-sexed social groups in bottles) will also be important to determine.

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Table 2.1. Sources of variation in (**A**) resting metabolic rate (RMR) and (**B**) locomotor activity in a lab-adapted population of *D. melanogaster*. Parameters are from separate univariate mixed models that included fixed effects of body mass, sex, reproductive status, and all possible two-way interactions between these. Bold values denote significance ($P < 0.05$). Shown are estimate of each fixed effect with their standards errors (SE), denominator degrees of freedom (df_{den}; numerator $df = 1$ in all cases), conditional Wald-F statistic, and P-values.

	Source	Estimate	SE	df_{den}	F	P
(**A**) Resting Metabolic Rate	Intercept	0.07	0.09	8.8	0.62	0.452
	Sex	0.20	0.07	488.1	1.11	0.293
	Body mass	**0.69**	**0.04**	**518.2**	**440.60**	**<0.001**
	Reproductive status	**0.17**	**0.04**	**654.3**	**20.78**	**<0.001**
	Sex × Body mass	**0.25**	**0.06**	**522.5**	**15.18**	**<0.001**
	Sex × Reproductive status	0.07	0.13	656.1	0.27	0.602
	Body mass x Reproductive status	0.02	0.06	670.3	0.12	0.734
(**B**) Average activity (ACT_{avg})	Intercept	-0.03	0.09	21.0	0.13	0.722
	Sex	-0.12	0.15	521.4	0.91	0.341
	Body mass	**-0.15**	**0.08**	**434.6**	**4.22**	**0.040**
	Reproductive status	**0.23**	**0.07**	**700.6**	**10.34**	**0.001**
	Sex × Body mass	-0.07	0.15	648.5	0.23	0.633
	Sex × Reproductive status	-0.36	0.26	689.3	1.92	0.167
	Body mass x Reproductive status	0.12	0.13	684.0	0.92	0.338

Table 2.2. Sources of variation in locomotor activity during (**A**) morning peak (ACT$_{\text{mp}}$), (**B**) non-peak day (ACT$_{\text{day}}$), (**C**) evening peak (ACT$_{\text{ep}}$), and (**D**) non-peak night (ACT$_{\text{night}}$). See Table 2.1 for details.

	Source	Estimate	SE	df_{den}	*F*	*P*
(**A**) Activity during the morning peak (ACT$_{\text{mp}}$)	Intercept	-0.14	0.09	15.6	2.27	0.152
	Sex	**0.40**	**0.14**	**532.6**	**20.44**	**<0.001**
	Body mass	**-0.23**	**0.07**	**494.2**	**4.32**	**0.038**
	Reproductive status	0.02	0.08	826.5	0.05	0.828
	Sex × Body mass	**-0.34**	**0.14**	**586.7**	**6.11**	**0.014**
	Sex × Reproductive status	-0.14	0.28	819.5	0.25	0.615
	Body mass x Reproductive status	-0.06	0.13	815.9	0.18	0.670
(**B**) Activity during the day (ACT$_{\text{day}}$)	Intercept	-0.01	0.08	27.6	0.01	0.946
	Sex	**-0.74**	**0.14**	**417.5**	**37.83**	**<0.001**
	Body mass	**-0.23**	**0.08**	**294.3**	**13.94**	**<0.001**
	Reproductive status	**0.37**	**0.07**	**733**	**25.87**	**<0.001**
	Sex × Body mass	-0.01	0.14	610.8	<0.01	0.951
	Sex × Reproductive status	**-0.72**	**0.26**	**723.6**	**7.81**	**0.005**
	Body mass x Reproductive status	0.02	0.12	719.1	0.04	0.849
(**C**) Activity during the evening peak (ACT$_{\text{ep}}$)	Intercept	-0.08	0.08	25.9	0.99	0.329
	Sex	**0.87**	**0.14**	**425.5**	**63.28**	**<0.001**
	Body mass	0.05	0.07	319.1	2.78	0.097
	Reproductive status	**-0.17**	**0.08**	**790.0**	**4.67**	**0.031**
	Sex × Body mass	-0.17	0.14	580.7	1.6	0.206
	Sex × Reproductive status	0.46	0.27	782.5	2.95	0.086
	Body mass × Reproductive status	0.08	0.13	778.8	0.33	0.565
(**D**) Activity during the night (ACT$_{\text{night}}$)	Intercept	0.05	0.12	12.5	0.19	0.669
	Sex	0.32	0.15	572.8	2.79	0.095
	Body mass	0.07	0.08	577.1	0.1	0.753
	Reproductive status	-0.05	0.08	816	0.26	0.611
	Sex × Body mass	0.13	0.15	596	0.81	0.368
	Sex × Reproductive status	0.01	0.29	808.1	<0.01	0.99
	Body mass × Reproductive status	0.13	0.14	804.2	0.88	0.349

Table 2.3. Phenotypic (r_{P}), among-individual (r_{ind}), and within-individual (r_e) correlations between resting metabolic rate (RMR) and different activity variables in a lab-adapted population of *D. melanogaster*. Correlations are presented with their 95% of confidence intervals (CI) and bold denotes significance (i.e. CI's that do not include zero).

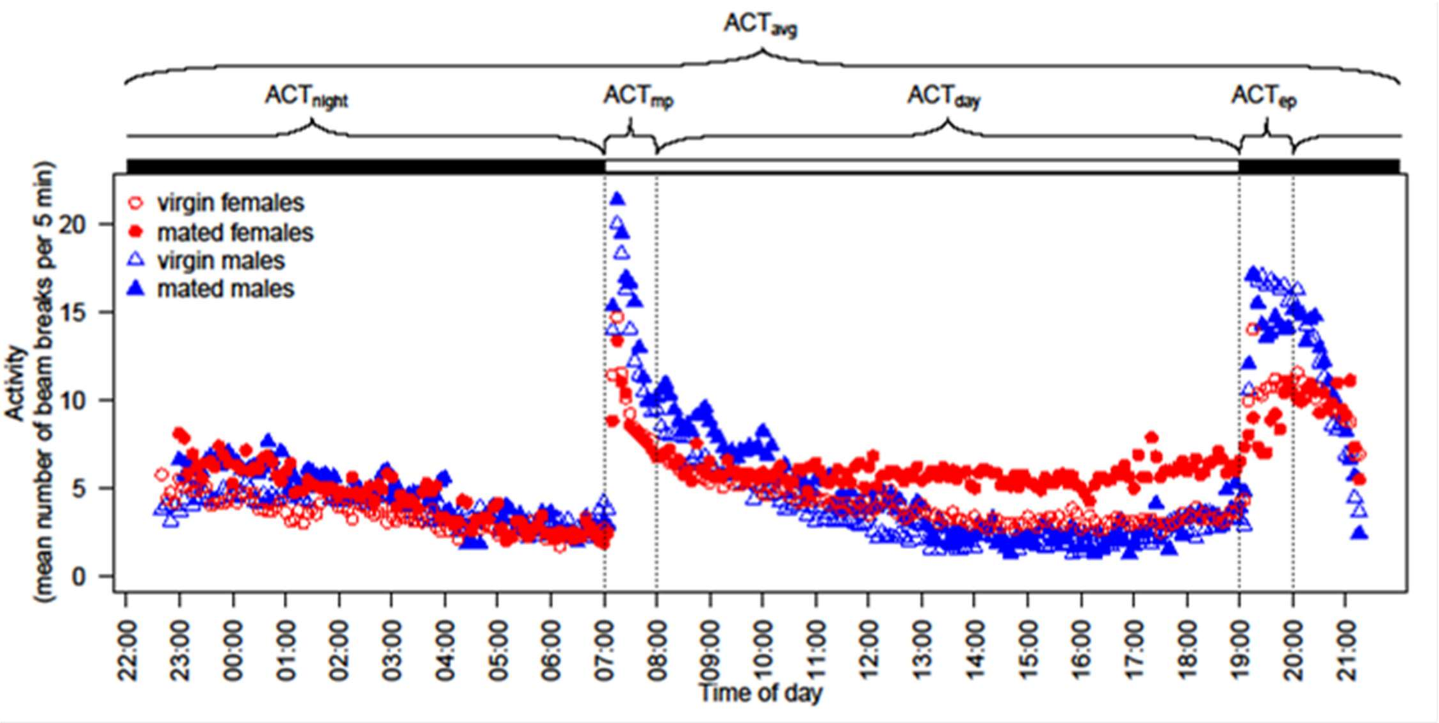
Models	r_{P} (95% CI)	r_{ind} (95% CI)	r_e (95% CI)
RMR × $\mathrm{ACT}_{\mathrm{avg}}$	**0.08 (0.06, 0.10)**	**0.26 (0.13, 0.39)**	0.05 (-0.03, 0.13)
RMR × $\mathrm{ACT}_{\mathrm{mp}}$	**0.02 (0.01, 0.03)**	**0.21 (0.06, 0.37)**	-0.06 (-0.14, 0.02)
RMR × $\mathrm{ACT}_{\mathrm{day}}$	**0.05 (0.04, 0.06)**	**0.20 (0.07, 0.33)**	-0.01 (-0.08, 0.07)
RMR × $\mathrm{ACT}_{\mathrm{ep}}$	**0.01 (0.007, 0.013)**	0.08 (-0.07, 0.22)	-0.02 (-0.10, 0.06)
RMR × $\mathrm{ACT}_{\mathrm{night}}$	**0.11 (0.08, 0.13)**	0**.26 (0.11, 0.41)**	**0.15 (0.07, 0.23)**

Figure legends

Fig. 2.1. Daily variation in locomotor activity (mean number of beam breaks per 5 min; *N* = 496) in male (blue triangles) and female (red circles) *D. melanogaster* that were either virgin (open symbols) or mated (filled symbols). Black and white boxes show when incubator lights were on or off. There are two majors peak of activity occurring immediately after switching from lights on (7:00) and off (19:00). For analysis, activity was subdivided into different parts of the day consisting of the morning peak (ACT_{mp}), the non-peak day (ACT_{day}), the evening peak (ACT_{ep}), and the non-peak night (ACT_{night}).

Fig. 2.2. Resting metabolic rate ($\mu l\ CO_2 \cdot min^{-1}$) as a function of (**A**) wet body mass (mg) and (**B**) reproductive status (virgin vs. mated) in *D. melanogaster*. Body mass-RMR relationship is shown separately for males (blue triangles) and females (red circles) with corresponding linear regressions. RMR is conditioned on wet mass when testing the effect of reproductive status.

Fig. 2.3. (**A**) Among-individual correlations (r_{ind}) with ± 95% confidence intervals (CI) between resting metabolic rate (RMR) and the different activity variables (ACT_{avg}, ACT_{mp}, ACT_{day}, ACT_{ep} and ACT_{night}; see Fig. 1). Blue triangles denote males and red circles females. Black and white boxes show the lighting status (white vs. black = lights on vs off; grey = mix of both) when the activity variable was extracted. Individual estimates (best linear unbiased predictors ± SE) were extracted from the bivariate model to illustrate the contrasting relationships between RMR and ACT_{ep} in (**B**) males and (**C**) females.


ACT$_{avg}$
ACT$_{night}$
ACT$_{mp}$
ACT$_{day}$
ACT$_{ep}$
virgin females
mated females
virgin males
mated males
Activity (mean number of beam breaks per 5 min)
0
5
10
15
20
22:00
23:00
00:00
01:00
02:00
03:00
04:00
05:00
06:00
07:00
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13:00
14:00
15:00
16:00
17:00
18:00
19:00
20:00
21:00
Time of day

Fig. 2.1.

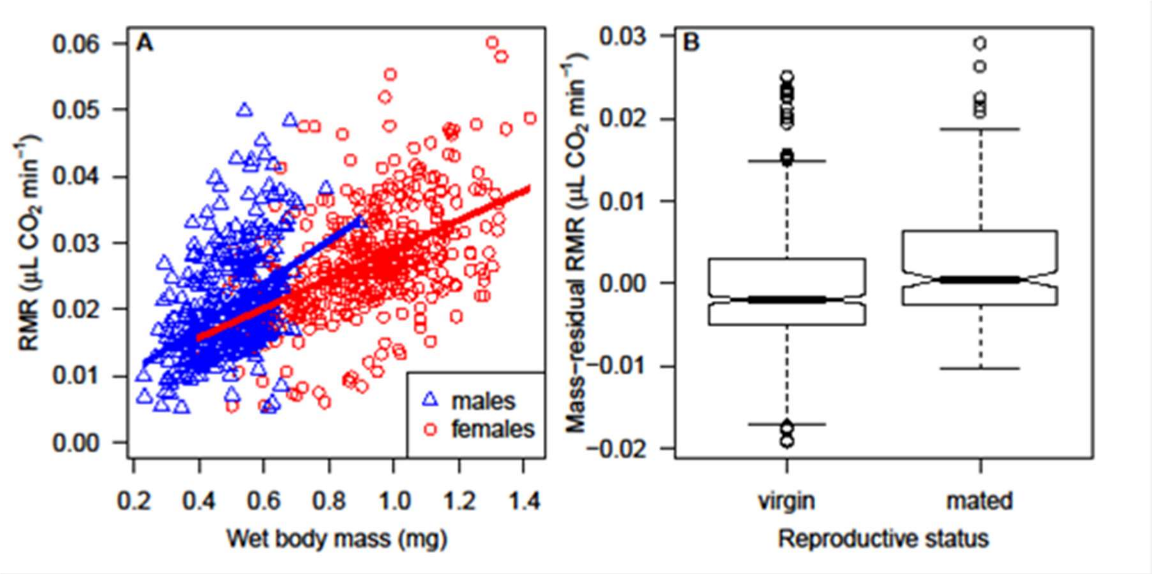
A
B
0.06
0.05
0.04
0.03
0.02
0.01
0.00
RMR (µL CO$_2$ min^{-1})
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Wet body mass (mg)
males
females
0.03
0.02
0.01
0.00
−0.01
−0.02
Mass−residual RMR (µL CO$_2$ min^{-1})
virgin
mated
Reproductive status

Fig. 2.2.

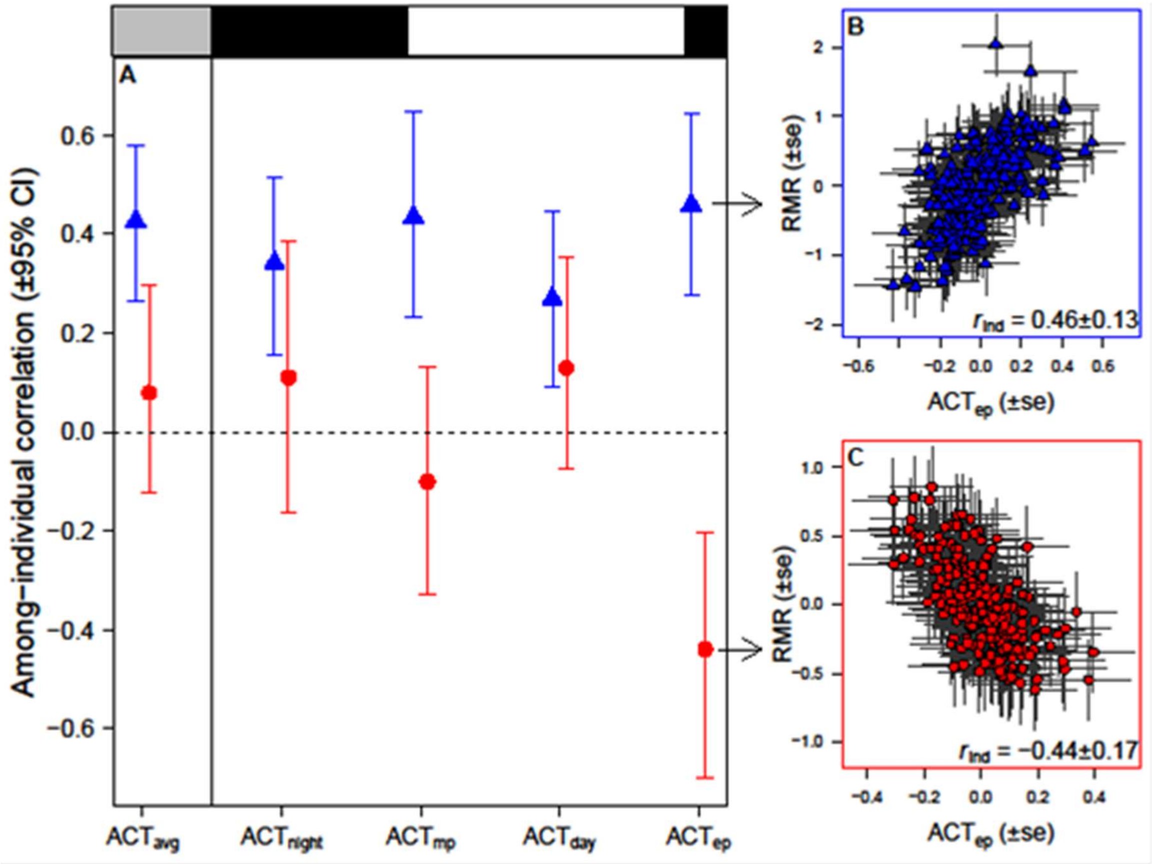
A
Among−individual correlation (±95% CI)
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
ACT$_{avg}$
ACT$_{night}$
ACT$_{mp}$
ACT$_{day}$
ACT$_{ep}$
B
RMR (±se)
2
1
0
−1
−2
−0.6
−0.2
0.0
0.2
0.4
0.6
ACT$_{ep}$ (±se)
r_{ind} = 0.46±0.13
C
RMR (±se)
1.0
0.5
0.0
−0.5
−1.0
−0.4
−0.2
0.0
0.2
0.4
ACT$_{ep}$ (±se)
r_{ind} = −0.44±0.17

Fig. 2.3.

Chapter 2: Supplementary information

Table S2.1. Random-effect variance components from separate univariate mixed models of resting metabolic rate (RMR) and activity variables. Individual identity was included as a random effect to quantify the among-individual variance (V_{ind}). The within-individual variance (V_e) corresponds to the residual variance arising from repeated measures of individuals and that is not explained by individual identity or the fixed effects (shown in Table 2.1). Repeatability was calculated as the ratio: $V_{ind}/(V_{ind} + V_e)$.

Trait	V_{ind}		V_e		Repeatability	
	Estimate	SE	Estimate	SE	Estimate	SE
Resting Metabolic Rate (RMR)	0.046	0.008	0.088	0.008	0.342	0.055
Average Activity (ACT_{avg})	0.534	0.049	0.384	0.025	0.581	0.031
Activity during the morning peak (ACT_{pm})	0.291	0.04	0.503	0.033	0.366	0.041
Activity during the day (ACT_{day})	0.444	0.044	0.394	0.026	0.530	0.034
Activity during the evening peak (ACT_{pe})	0.343	0.041	0.460	0.031	0.427	0.039
Activity during the night ($\text{ACT}_{\text{night}}$)	0.353	0.046	0.559	0.037	0.387	0.040

Table S2.2. Sources of variation in (*a*) resting metabolic rate and (*b*) locomotor activity in a lab-adapted population of *D. melanogaster*. Parameters are from separate univariate mixed models that included fixed effects of body mass, sex, reproductive status, sequence, and all possible two-way interactions among these. The analysis of RMR also included the following fixed effects as nuisance variables: temperature, flow rate, light intensity, vapour water and activity during the same 20 seconds over which RMR was measured and activity over the 20 seconds immediately prior to the RMR measurement. Bold values denote significance ($P<0.05$). Shown are estimate of each fixed effect with their standards errors (SE), denominator degrees of freedom (df_{den}; numerator $df = 1$ in all cases), conditional Wald-F statistic, and P-values.

Source	Estimate	SE	df_{den}	F	P
(*a*) Resting Metabolic Rate (RMR)					
Intercept	0.07	0.09	8.8	0.62	0.452
Sequence	0.12	0.15	53.3	0.63	0.431
Sex	0.20	0.07	488.1	1.11	0.293
Reproductive status	**0.17**	**0.04**	**654.3**	**20.78**	**<0.001**
Body mass	**0.69**	**0.04**	**518.2**	**440.6**	**<0.001**
Sequence × Sex	0.09	0.11	477.7	0.66	0.418
Sequence × Body mass	0.03	0.06	485.5	0.26	0.611
Sex × Reproductive status	-0.07	0.13	656.1	0.27	0.602
Sex × Body mass	**0.25**	**0.06**	**522.5**	**15.18**	**<0.001**
Body mass × Reproductive status	0.02	0.06	670.3	0.12	0.734
Activity during record	**0.18**	**0.02**	**715.8**	**106.8**	**<0.001**
Activity before record	**0.18**	**0.02**	**702.6**	**109.7**	**<0.001**
Temperature	**0.07**	**0.03**	**251.0**	**4.06**	**0.045**
Flowrate	**-0.11**	**0.02**	**691.5**	**30.27**	**<0.001**
Water vapor	**-0.30**	**0.07**	**24.3**	**19.06**	**<0.001**
Light intensity	-0.01	0.02	672.0	0.60	0.439
(*b*) Average activity (ACT_{avg})					
Intercept	-0.03	0.09	21.0	0.13	0.722
Sequence	**0.31**	**0.06**	**580.3**	**33.87**	**<0.001**
Sex	-0.12	0.15	521.4	0.91	0.341
Reproductive status	**0.23**	**0.07**	**700.6**	**10.34**	**0.001**
Body mass	**-0.15**	**0.08**	**434.6**	**4.22**	**0.040**
Sequence × Sex	**0.50**	**0.19**	**567.4**	**7.07**	**0.008**
Sequence × Body mass	0.12	0.09	554.4	1.87	0.173
Sex × Reproductive status	-0.36	0.26	689.3	1.92	0.167
Sex × Body mass	-0.07	0.15	648.5	0.23	0.633
Body mass × Reproductive status	0.12	0.13	684.0	0.92	0.338

Table S2.3. Sources of variation in locomotor activity during (*a*) morning peak, (*b*) non-peak day, (*c*) evening peak, and (*d*) non-peak night. See Table 2.1 for further details.

Source	Estimate	SE	df_{den}	*F*	*P*
(*a*) Activity during the morning peak (ACT_{mp})					
Intercept	-0.14	0.09	15.6	2.27	0.152
Sequence	-0.05	0.06	619.4	0.49	0.483
Sex	**0.40**	**0.14**	**532.6**	**20.44**	**<0.001**
Reproductive status	0.02	0.08	826.5	0.05	0.828
Body mass	**-0.23**	**0.07**	**494.2**	**4.32**	**0.038**
Sequence × Sex	**0.54**	**0.21**	**615.1**	**6.87**	**0.009**
Sequence × Body mass	0.15	0.10	603.3	2.12	0.145
Sex × Reproductive status	-0.14	0.28	819.5	0.25	0.615
Sex × Body mass	**-0.34**	**0.14**	**586.7**	**6.11**	**0.014**
Body mass × Reproductive status	-0.06	0.13	815.9	0.18	0.670
(*b*) Activity during the day (ACT_{day})					
Intercept	-0.01	0.08	27.6	<0.01	0.946
Sequence	**0.35**	**0.06**	**592.2**	**42.15**	**<0.001**
Sex	**-0.74**	**0.14**	**417.5**	**37.83**	**<0.001**
Reproductive status	**0.37**	**0.07**	**733.0**	**25.87**	**<0.001**
Body mass	**-0.23**	**0.08**	**294.3**	**13.94**	**<0.001**
Sequence × Sex	**0.57**	**0.19**	**581.8**	**9.02**	**0.003**
Sequence × Body mass	0.14	0.09	569.3	2.50	0.115
Sex × Reproductive status	**-0.72**	**0.26**	**723.6**	**7.81**	**0.005**
Sex × Body mass	-0.01	0.14	610.8	<0.01	0.951
Body mass × Reproductive status	0.02	0.12	719.1	0.04	0.849
(*c*) Activity during the evening peak (ACT_{ep})					
Intercept	-0.08	0.08	25.9	0.99	0.329
Sequence	-0.26	0.06	53.3	0.63	0.431
Sex	**0.87**	**0.14**	**425.5**	**63.28**	**<0.001**
Reproductive status	**-0.17**	**0.08**	**790.0**	**4.67**	**0.031**
Body mass	0.05	0.07	319.1	2.78	0.097
Sequence × Sex	-0.08	0.20	598.8	0.14	0.708
Sequence × Body mass	0.05	0.10	586.7	0.23	0.632
Sex × Reproductive status	0.46	0.27	782.5	2.95	0.086
Sex × Body mass	-0.17	0.14	580.7	1.60	0.206
Body mass × Reproductive status	0.08	0.13	778.8	0.33	0.565
(*d*) Activity during the night (ACT_{night})					
Intercept	0.05	0.12	12.5	0.19	0.669
Sequence	**0.24**	**0.06**	**618.1**	**13.74**	**<0.001**
Sex	0.32	0.15	572.8	2.79	0.095
Reproductive status	-0.05	0.08	816.0	0.26	0.611
Body mass	0.07	0.08	577.1	0.1	0.753
Sequence × Sex	0.37	0.22	612.6	2.86	0.092
Sequence × Body mass	-0.02	0.11	600.6	0.04	0.839
Sex × Reproductive status	<0.01	0.29	808.1	<0.01	0.990
Sex × Body mass	0.13	0.15	596.0	0.81	0.368
Body mass × Reproductive status	0.13	0.14	804.2	0.88	0.349

Table S2.4. Sex-specific among-individual (r_{ind}) and within-individual (r_{e}) correlations between RMR and various activity variables (Estimate) from bivariate heterogeneous mixed models partitioned by sex. See Table 2.3 for details.

		r_{ind}						r_{e}					
Models	Sex	Estimate	SE	95% CI		LRT		Estimate	SE	95% CI		LRT	
				Lower	Upper	χ^2	*P*			Lower	Upper	χ^2	*P*
RMR × $\mathrm{ACT}_{\mathrm{avg}}$	Male	**0.43**	**0.11**	**0.27**	**0.58**	**12.27**	**<0.001**	0.13	0.08	0.01	0.25	2.4	0.121
	Female	0.08	0.14	-0.12	0.30	0.29	0.593	-0.08	0.08	-0.19	0.04	0.86	0.353
RMR × $\mathrm{ACT}_{\mathrm{mp}}$	Male	**0.43**	**0.15**	**0.23**	**0.65**	**8.68**	**0.003**	-0.12	0.09	-0.24	0.00	2.00	0.158
	Female	-0.10	0.16	-0.33	0.13	0.38	0.539	-0.02	0.08	-0.13	0.09	0.07	0.786
RMR × $\mathrm{ACT}_{\mathrm{day}}$	Male	**0.27**	**0.13**	**0.09**	**0.44**	**4.30**	**0.038**	0.11	0.09	-0.01	0.22	1.55	0.214
	Female	0.13	0.15	-0.08	0.35	0.75	0.385	-0.11	0.08	-0.22	0.00	1.97	0.161
RMR × $\mathrm{ACT}_{\mathrm{ep}}$	Male	**0.46**	**0.13**	**0.28**	**0.64**	**11.63**	**0.001**	-0.08	0.09	-0.20	0.04	0.83	0.363
	Female	**-0.44**	**0.17**	**-0.70**	**-0.20**	**6.52**	**0.011**	0.01	0.08	-0.10	0.12	0.02	0.892
RMR × $\mathrm{ACT}_{\mathrm{night}}$	Male	**0.34**	**0.13**	**0.16**	**0.51**	**5.85**	**0.016**	**0.24**	**0.08**	**0.13**	**0.35**	**8.57**	**0.003**
	Female	0.11	0.19	-0.16	0.38	0.32	0.572	0.05	0.08	-0.06	0.16	0.39	0.535

Fig. S2.1. Side and top view of the complex mating environment for our population of *Drosophila melanogaster*. The environment is made of a polypropylene bottle filled with 75 ml of food. The surface of the food is divided into six approximately equal sectors delimited by opaque plastic barriers inserted into the food. Equal amount of liquid yeast was dropped in each food patch. In addition, two coiled pipe cleaners protrude from the bottom of the foam plug into the interior space of the bottle.

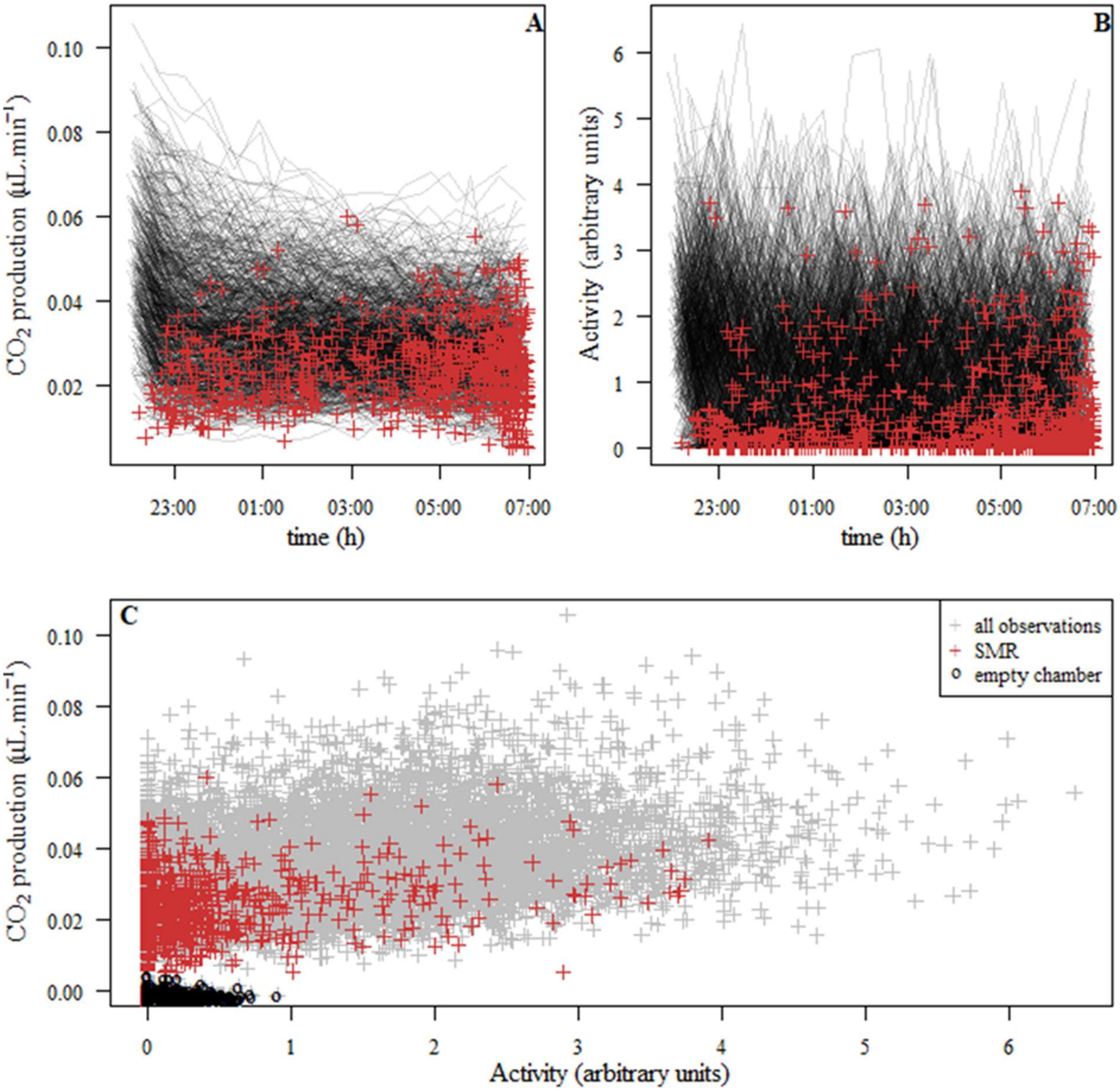

Fig. S2,2. Simultaneous recordings of (**A**) metabolic rate (CO_2 production in µL·min^{-1}) and (**B**) activity (arbitrary units) measured 16 times over 9 hours. Shown are all observations (grey cross) per fly and those corresponding to the value used in calculating resting metabolic rate (RMR; red cross), which tend to occur towards then end of the metabolic trials and at relatively low levels of activity. (**C**) Metabolic rate as a function of activity during measurements. Open black dots denote empty chambers measurement.

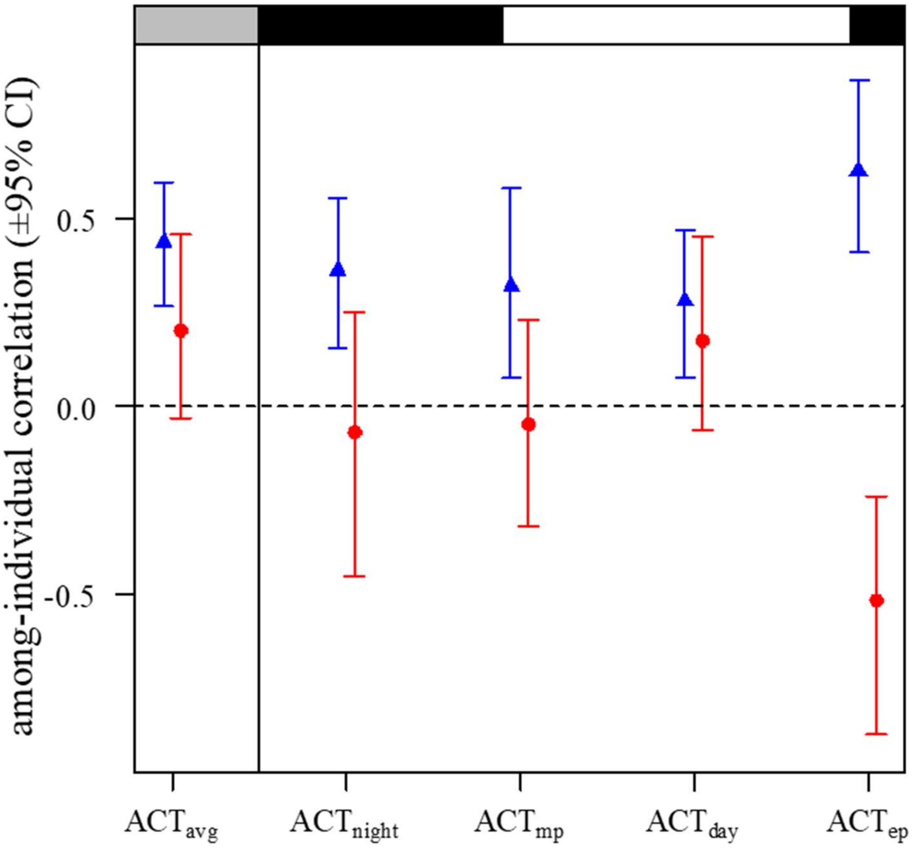

Fig. S2.3. Among-individual correlations (r_{ind}) with ± 95% confidence intervals (CI) for bivariate models of resting metabolic rate (RMR) and the different activity variables (ACT_{avg}, ACT_{mp}, ACT_{day}, ACT_{ep} and ACT_{night}). Those correlations have been quantified from a dataset where ACT_{20} (activity during the RMR measurement) was below 0.76, the value at which the effect of ACT_{20} becomes non-significant (sample size reduced to 233 females and 221 males). Blue triangles denote males and red dots females. Black and white boxes show the lighting status (white vs black = lights on vs off; grey = mix of both) when the activity variable was extracted.

CHAPTER 3 : Quantifying selection on standard metabolic rate and body mass in *Drosophila melanogaster*

This chapter has been published in *Evolution* in 2020, as a collaboration with Vincent Careau (VC), Howard D. Rundle (HDR) and Alastair J. Wilson (AJW). I designed the experiment with the help of VC and HDR. I collected the data. I analyzed the data with input from VC, AJW and HDR, and all authors wrote the manuscript.

Introduction

Metabolic rate reflects the amount of energy that an organism needs to grow, reproduce, and survive. Because resources are limited, organisms must allocate their finite energy to competing demands, which forces allocation trade-offs that ultimately play an important role in shaping life-history strategies. All else being equal, energy allocated to self-maintenance cannot be invested in other energetically demanding traits such as reproduction. However, reproducing at a high rate may necessitate a large metabolic machinery that translate into high maintenance costs. As such, maintenance metabolism is likely to be linked to fitness (Burton et al. 2011), but studies so far have produced inconsistent results (Pettersen et al. 2018) and we therefore lack a good understanding of how selection shapes maintenance metabolism. This is perhaps not surprising given that estimating selection involves challenges such as measuring fitness and maintenance metabolism appropriately in a large number of individuals and parsing the relative contribution of highly collinear variables (e.g., body mass and metabolism) to fitness.

Quantifying fitness is technically challenging yet of utmost importance when studying selection. Lifetime reproductive success of an individual (total number of offspring produced) can be broken down into three main components: survival, fecundity, and reproductive success (pre- and postcopulatory). These components of fitness can vary independently and may relate differently to metabolic rate (Pettersen et al. 2018). For example, a high maintenance metabolism may be beneficial to survival, but uses energy that otherwise could be invested in reproduction. Most estimates of selection on maintenance metabolism have, at best, quantified a portion of a single fitness component such as over-winter survival (Jackson et al. 2001; Artacho and Nespolo 2009; Boratyński et al. 2010; Larivée et al. 2010; Careau et al. 2013; Zub et al. 2014) or output from a single reproductive event (Earle and Lavigne 1990; Stephenson and Racey 1993; Johnston et al. 2007; Hayes et al. 2009; Boratyński and Koteja 2010; Schimpf et al. 2012; Mariette et al. 2015). A small number of studies have attempted to relate metabolic rate to a more comprehensive measure of fitness (Blackmer et al. 2005; Pettersen et al. 2016), but we have limited insight into how total selection acts on this fundamental trait.

Measuring maintenance metabolism can also be challenging as, by definition, it excludes contributions due to activity, growth, and reproduction (Hulbert and Else 2004; Careau et al. 2014). In ectotherms, the “minimum cost of living” is measured as the standard metabolic rate (SMR): the metabolic rate of a resting, post-absorptive, and non-reproductive adult at a specified temperature. Meeting these criteria requires careful methodological considerations and can take time because individuals must be monitored over a sufficient period such that they relax and rest within the confinement of a metabolic chamber. Therefore, the criteria to measure SMR can impose major constraints on achieving sufficient sample sizes to estimate selection with precision. Small insects offer advantages as it is relatively easy to obtain to a large number of individuals, but their low metabolic rate makes it difficult to measure SMR precisely.

An additional challenge in estimating selection on metabolic rate is its strong (positive) collinearity with body mass (White 2011; White and Kearney 2013). Such collinearity can make it difficult to parse the relative strength of selection between these two traits. Collinearity can be alleviated by excluding traits that are not of interest, or by working with principal components (Zuur et al. 2010; Dormann et al. 2013; Chong et al. 2018; Harrison et al. 2019), but such approaches are not particularly useful when all of the correlated traits are of interest (e.g., metabolic rate and body mass are both hypothesized to be under selection). Historically, selection is estimated on SMR after correcting for body mass, usually by taking the residuals of a linear regression of SMR as function of mass (or by dividing SMR by body mass). However, this approach removes variation in SMR due to body mass and it is therefore not possible to estimate selection on the shared variation, nor does it allow correlational selection to be estimated for these traits. A preferable approach is to apply the Lande and Arnold (1983) framework to simultaneously quantify linear and nonlinear selection on both SMR, body mass, and their interaction. The Lande and Arnold (1983) framework is usually done by fitting a multiple linear regression with relative fitness as the response variable and the traits of interest (and their squared terms and second-order interactions for nonlinear selection) as predictors. When doing so, however, it is difficult to account for various nuisance parameters or other covariates that only apply to a subset of the traits without ‘doing statistics on statistics’ (i.e., using residuals from a regression of a trait on its covariates). Such a two-step approach fails to carry forward uncertainty in estimates and can produce statistical artifacts (Garcia-Berthou 2001; Freckleton

2002; Morrissey 2014). A solution to this challenge is to use a multivariate approach to model the variance-covariance matrix between fitness, SMR and body mass while correcting one or more traits for their unique covariates (in the current case for nuisance parameters unique to the estimation of SMR and relative fitness). Standard selection differentials and gradients can then be obtained from the residual covariance matrix (see Methods).

Here, our primary goal is to quantify multivariate selection on SMR and body mass. To do so, we build on the Lande & Arnold (1983) framework, employing multivariate mixed models to better account for trait-specific covariates. In measuring selection on these traits, we take advantage of a high-throughput respirometry system and a laboratory population of *Drosophila melanogaster* that has been evolving under a life cycle that facilitates a comprehensive measure of fitness. In this population, newly emerged adult flies interact for four days in a mating environment at a specific (and fairly low) density, after which females lay eggs for 24h to produce the next generation. Male fitness is therefore the number of offspring they sire during this 4-day period, and female fitness is the number of adult offspring they produce during the 24h window. Our fitness measure therefore includes survival over these four days, fecundity, and reproductive success of the adult, along with the egg-to-adult survival of the resulting offspring they produce. This is a more comprehensive fitness measure than previous studies estimating selection on SMR. The mating environment also features added structural complexity (see Methods), potentially allowing a greater range of sexual behaviours to be expressed compared to standard *Drosophila* populations that are generally maintained at high density in structurally simple environment (i.e., standard fly vials or bottles). For example, male mating success may involve searching for females and/or defending a territory, and female can flee when faced with male courtship, all of which are energetically costly and may thus impact SMR. We have previously shown in this population that SMR is both repeatable and differentially correlated with body mass and activity in males vs. females (Videlier et al. 2019). Here we used the same high-throughput metabolic system to measure SMR, in addition to body mass and fitness, in close to one thousand separate individuals.

Methods

Stock population

A stock population was established in February of 2016 from a large sample of a laboratory-adapted population of *D. melanogaster* that was originally collected in Dundas, ON in 2006 (MacLellan et al. 2012). Since then, this stock has been maintained with discrete, non-overlapping generations at 25°C, 50% relative humidity, and with a 12L:12D photoperiod (lights switch at 7 am/pm) on a standard cornmeal-based food (90 g/L cornmeal, 100 g/L turbinado sugar, 40 g/L yeast and 12 g/L agar). The population life cycle includes a 4-day ‘mating phase’ that takes place in an environment (8 oz. culture bottles) with reduced density (10 males and 10 females/bottle) and increased spatial complexity (i.e., dividers inserted into the food and two coiled piper cleaners inside the bottle) compared to standard *Drosophila* maintenance techniques. Males are discarded after the mating phase and females are allowed to lay eggs for 24 h in standard glass culture vials (28.5 mm x 95 mm). Additional details are provided in Videlier et al. (2019). To create a separate marked ‘competitor’ for use in the fitness assays, in November 2016 a brown eye recessive (*bw*) mutation was introgressed into a copy of the stock population via two rounds of backcrossing. This population was then synchronized with the stock and was maintained in the same way and following the same schedule.

Experimental design

To quantify selection, both metabolic rate and fitness were measured on individual males and females from the stock population under conditions that closely mimicked their normal maintenance routine. The experiment was performed in six temporal blocks over six generations of the stock population, with each block consisting of three separate temporal sub-blocks of 32 males and 32 females each (i.e., one sub-block per day over three days; see below).

Individuals for use in the assay were raised at four different densities by allowing two, five, ten or 15 stock females to lay eggs in a vial for 24 hours (10 females/vial matches the density during normal maintenance). This was done to increase phenotypic variation in size, and potentially SMR, thereby increasing the power to detect selection. A downside of such a phenotypic manipulation is that it creates the possibility of a density-induced fitness-trait covariance that could be mistakenly interpreted as selection (Rausher 1992; Stinchcombe et al. 2002). In our case this appears unlikely (see Fig. S3.1 and Discussion). To increase sample size within each block, virgin collection was performed over three consecutive days corresponding to 8, 9 and 10 days after egg laying, creating three groups corresponding to three different 'days of emergence'. (Nine days after egg laying corresponds to the normal maintenance routine of the stock.) On each day, all newly emerged virgin offspring from the four rearing densities were pooled and then 45 males and 45 females were randomly selected using light CO_2 anaesthesia (in the late morning). These flies were subsequently stored, separately by sex, in three vials of 15 within the same incubator as the stock population. At approximately 19:00, 32 females and 32 males were randomly chosen for metabolic rate measurement overnight (remaining individuals were discarded). The following morning, these individuals were weighed (as described below) and then placed in the complex environment for a three day 'mating phase' together with mutant competitor flies (see below), after which females were transferred to new vials for egg laying. While the stock population normally experiences a 4-day mating phase, we used three days so that when the assay females were subsequently transferred to vials for egg laying, they were of the same age as stock females when they lay eggs during regular maintenance.

Metabolic and body mass measurements

Metabolic rate measurements were performed following Videlier et al. (2019) using a 64-chamber flow-through respirometry system, housed overnight in a separate incubator. The system consists of four separate units, each comprised of a differential CO_2 analyser (Li-Cor7000, Li-Cor Biosciences, Lincoln, NE, USA) and a 16-channel flow management, data acquisition, and signal processing system (MAVEn; Sable Systems International, North Las Vegas, NV, USA). Each MAVEn incorporates a flow-distribution manifold, a main board (flow measurement, regulation, and control plus data acquisition and signal processing), and an activity board (sensors for activity, ambient temperature, humidity, and light intensity). A constant stream of dry, CO_2-free air produced by a purge gas generator (PG14L Peak scientific, Glasgow, Scotland, UK) was split into four different streams, which were pushed through the reference cell of each CO_2 analyser (Cell A). The air stream was then humidified by flowing through Nafion tubing (du Pont de Nemours and Company, Wilmington, DE, USA) submerged in distilled water, and finally was directed into the flow-distribution manifold where it was physically split into 17 streams (one for each of the 16 chambers and one for the baseline), of which only the baseline was actively regulated at a flow rate of 20 $ml \cdot min^{-1}$. The approximately equivalent flow rates in the non-baseline channels (range: 15 to 25 $ml \cdot min^{-1}$) were maintained by means of matched flow resistances based on micro-orifice flow restrictors. A second mass flow meter on the MAVEn’s main board measured the actual flow rate of each selected air stream before it was automatically directed through the measurement cell (Cell B) of the CO_2 analyser.

Before measurement, individuals were chosen randomly from the three sex-specific holding vials and were gently placed, without anaesthesia, separately into chambers made of clear plastic tubes (40 mm high by 6 mm diameter). Females and males were placed in odd and even numbered chambers respectively. Measurements were performed for 12 hours overnight, between 19:00 and 7:00, which correspond to the period of lowest average locomotor activity in this population (Videlier et al. 2019).

Data transformation and extraction were done using ExpeData (Sable Systems International, North Las Vegas, NV, USA). The raw outputs from the activity detectors (one per chamber) were transformed into an index of locomotor activity by first calculating the cumulative sum of the absolute difference between adjacent samples and then by differentiating the resulting channel vs. time (equivalent to calculating the slope of the cumulative activity vs. time). The CO_2 trace (one for all of the 16 chambers in a given unit) was corrected for drift using multiple baseline correction measures and was also corrected for a 15 second lag. CO_2 production (VCO_2) was then calculated by multiplying flow rate by the fractional concentration of CO_2. Considering our sampling scheme (~12 hours respirometry run with a 34 min sampling cycle), each fly was sampled for 120 seconds per sample over a total of 21 separate measurement periods. The first 40 seconds of each measurement was ignored to allow the system to fully equilibrate after changing between chambers. From the remaining 80 seconds we extracted the lowest 20 seconds continuous bouts of VCO_2 using the “nadir” function in ExpeData. In addition to the average of the lowest 20 seconds continuous bout of VCO_2, we also extracted the average flow rate, water vapor, temperature, light intensity, and locomotor activity. We also extracted the average locomotor activity over the 20 seconds immediately prior to the VCO_2 measurement. For each respirometry run, the lowest of the 21 extracted VCO_2 values was selected per individual as their standard metabolic rate (SMR).

The following morning, immediately after each metabolic measurement, body mass was measured by anesthetising individuals with CO_2 and then weighing them to the nearest 0.001 mg with an MX5Microbalance (Mettler Toledo, Columbus, OH, USA) as described in Videlier et al. (2019). After body mass measurements, individuals were transferred into the fitness assay.

Fitness assay

Fitness was measured in a competitive assay in which a single focal individual (male or female), which previously had its metabolic rate and body mass measured, was placed together with nine same-sex *bw* mutant individuals and ten opposite sex *bw* individuals in the same ‘complex’ bottle as used during the stock mating phase. Individuals were allowed to interact and mate for three days, after which males were discarded. In the female fitness assay, the single focal female was then transferred to a new vial with fresh media to lay eggs for 24 hours, while in the male fitness assay we randomly selected eight of the surviving *bw* females and placed them in pairs in four separate vials with fresh media for egg laying for 24 hours. Brown eye mutant individuals for use in these assays were collected at the same time as the focal individuals and prior to use were housed separately by sex in bottles of 50 individuals within the same incubator.

Female fitness was quantified as the total number of offspring emerging from a vial across two counts performed eight and 10 days after egg laying. (Counting twice reduces the chance of missing individuals that die and are lost in the food.) Focal females that died during the mating phase were assigned a fitness of zero. Male fitness was quantified in the same way except offspring were phenotyped for eye color and counted separately (wild-type red eyes indicating they were sired by the focal male, brown eyes indicating they were sired by a *bw* competitor male). Male fitness was the total number of wild-type offspring produced, although results were qualitatively the same if male fitness was calculated as the proportion of offspring sired by the focal male (unpublished results). Given this, we present only results based on the absolute number of wild-type offspring to avoid additional statistical complexity when dealing with proportions. While our measure of fitness will be influenced by variation in egg to adult survival of offspring, such mortality was likely low as larvae were raised at low density so most of the variance in fitness likely originates from differences in survival and fecundity (females) or reproductive success (males; Bateman 1948) of the focal adults themselves.

We attempted to measure all three traits (SMR, mass, and fitness) on 1,088 individuals in 17 blocks (64 individuals per block). However, handling errors, equipment problems, and unexplained deaths reduced sample sizes slightly (Videlier et al. 2020*a*). Individuals with missing values for two of the traits were excluded as they were not informative for estimating covariances (see below). This resulted in a total sample size of 1,037 individuals (515 males and 522 females). Of these, 78 individuals had a missing value for one of the traits but were retained because they are informative for estimating the covariance between the other two traits. Repeating the analyses below after excluding these 78 individuals did not qualitatively alter our conclusions.

Statistical analyses

We estimated selection separately in males and females because body mass is sexually dimorphic and previous work on these populations demonstrated that males and females differ in how SMR scales with body size and how activity and SMR covary (Videlier et al. 2019). We applied a modified Lande and Arnold (1983) framework using multivariate models in ASReml-R (Butler et al. 2018) that allowed us to estimate the covariance between fitness, body mass, and SMR while correcting SMR for nuisance parameters that only apply to it. The model included relative fitness (absolute fitness divided by its mean) and standardised (mean = 0, sd = 1) body mass and SMR as response variables, and an unstructured (co)variance matrix at the residual level. The inclusion of one or more fixed effects on a trait will change its residual variance such that it is no longer one, meaning gradients calculated from this will not be standardized gradients. To address this, SMR and body mass were standardized such that their variances (and hence sd) were one after accounting for relevant fixed effect(s) on each. This was done by dividing each trait not by its variance, but by its residual variance obtained from a first fitting a model using the unstandardized traits and the same fixed effects. To control for block and day effects, we fitted a variable that consisted of a unique combination of block (six levels) and day of emergence (three levels) as a fixed effect fitted to all three variables. Fixed effects of temperature, flow rate, and locomotor activity (both 20 s before and during SMR measurement) were fitted to SMR only. Light intensity and water vapor were not included because preliminary analyses reveal their effect sizes to be very small. For male fitness, the number of *bw* females which were used for 24 hours of egg laying was also fitted as a continuous effect.

Standardized linear selection differentials (*S*) were estimated as the covariance between the traits (SMR and mass) and relative fitness from the unstructured residual variance-covariance matrix in the above model. The vector of standardized linear selection gradients (β) on the traits was then estimated as:

Equation 1. $$\boldsymbol{\beta} = \mathbf{P}^{-1}\boldsymbol{S},$$

where S is a vector of selection differentials (on mass and SMR) and **P** is the 2×2 phenotypic (co)variance matrix of body mass and SMR (Lande and Arnold, 1983). The (co)variances in **P** were taken from the larger 3×3 residual covariance matrix from the multivariate model.

To estimate the nonlinear selection gradients, three new second-order 'traits' were constructed representing the quadratic (mass^2 and SMR^2) and cross-product terms involving mass and SMR (i.e., mass×SMR). These terms were then included, alongside relative fitness, SMR and body mass, in a second multivariate model, yielding a 6×6 phenotypic covariance matrix at the residual level. The same fixed effects applied to SMR were also applied to the second-order terms associated with SMR together with all unique pairwise interactions of these fixed effects. Standardized nonlinear selection gradients (i.e., *γ's*) were estimated as:

Equation 2 $$\boldsymbol{\gamma} = \mathbf{P_2}^{-1}\mathrm{Cov}(w, \mathrm{traits}),$$

where cov(w, traits) is the vector of covariance between relative fitness and the 'traits' (i.e., SMR, mass, SMR^2, mass^2, and SMR×mass) from the unstructured residual variance-covariance matrix and $\mathbf{P_2}$ is the 5×5 phenotypic covariance matrix between SMR, mass, SMR^2, mass^2, and SMR×mass . As for Eq. 1, $\mathbf{P_2}$ was extracted from the full residual covariance matrix from the multivariate model. Like Eq. 1, Eq. 2 is a specific case of the general formula for the least-squares estimates of the partial regression coefficients via matrix algebra (Kendall and Stuart 1973; Morrissey 2014). The partial regression coefficients for the 2nd order terms were retained as estimates of nonlinear selection, while those for mass and SMR (representing linear selection) were discarded as these are taken from the 1st-order model (i.e., Eq. 1; Lande and Arnold, 1983). Quadratic (but not correlational) gradients were doubled (Stinchcombe et al. 2008).

The overall significance of linear and nonlinear selection were separately tested using a model comparison approach (Chenoweth et al. 2013). For linear selection, a likelihood ratio test (LRT) was used to compare the fit of a ‘full’ multivariate model that included relative fitness, body mass, and SMR and that specified an unconstrained residual covariance matrix, with a ‘reduced’ version of the same model in which the covariances between relative fitness and both SMR and body mass were set to zero. For nonlinear selection, the full model included the three second-order terms (i.e., SMR^2, $mass^2$, and SMR × mass) and the reduced model constrained the residual covariances between fitness and the three second-order terms to be zero. To test the significance of the individual selection differentials and gradients (i.e., β’s and γ’s), the appropriate multivariate model was bootstrapped 10,000 times to estimate empirical 95% confidence intervals as the 0.025 and 0.975 quantiles of the distribution of the bootstrapped estimates.

Finally, we analyzed selection separately in males and females for the reasons outlined above but, for completeness, we also compared selection between the sexes. Differences in linear and nonlinear selection between males and females were separately tested using an analogous model comparison approach to that above on a pooled dataset that combined the sexes, treating SMR, mass and fitness in each sex as separate traits. Sex was also included as a fixed effect. The fit of a model with an unconstrained residual covariance matrix was compared with one that specified a ‘reduced’ version in which the covariances between relative fitness and traits (both SMR and body mass for linear selection, and SMR^2, $mass^2$, and SMR × mass for nonlinear selection) were constrained to be the same in males and females. In both models non-estimable covariances (i.e., between traits in opposite sexes) were fixed to zero.

Results

In males, there was evidence of linear selection on SMR and body mass overall (LRT: $\chi^2_{2\text{ df}} = 17.37$, $P < 0.001$; Fig. 3.1A). Selection differentials on both traits were positive and significant (Table 3.1). Selection gradients were of somewhat smaller magnitudes than the differentials and had larger 95% CI's and hence were not significant (Table 3.1). Such a pattern is potentially due to collinearity between body mass and SMR ($r = 0.70$; Fig. 3.1). Finally, there was no evidence of nonlinear selection overall in males (LRT: $\chi^2_{3\text{ df}} = 0.77$, $P = 0.856$; Table 3.2).

In contrast to males, in females there was no evidence of linear selection overall (LRT: $\chi^2_{2\text{ df}} = 5.21$, $P = 0.074$). Linear selection differentials were smaller than in males and, although individually significant for body mass, both selection gradients were weak and non-significant (Table 3.1). There was, however, statistical support for nonlinear selection overall in females (LRT: $\chi^2_{3\text{ df}} = 8.54$, $P = 0.036$; Fig. 3.1B), with two of the three nonlinear selection differentials being significant and the third approaching so (Table 3.2). The estimated gradients suggest that this nonlinear selection arose primarily from stabilizing selection on body mass, but the bootstrapped CI's span zero for the individual gradients, again suggesting collinearity.

Finally, when pooling males and females, the observed difference between the sexes in overall linear (LRT: $\chi^2_{2\text{ df}} = 1.62$, $P = 0.445$) and nonlinear selection (LRT: $\chi^2_{3\text{ df}} = 1.05$, $P = 0.790$) were both non-significant. Consistent with this, the 95% CI's of all linear and nonlinear selection gradients overlap between the sexes (Tables 3.1, 3.2).

Discussion

Estimating selection on physiological traits such as SMR is challenging, most notably in small insects, as it involves precisely measuring metabolic rate and fitness in a large number of individuals. Metabolic rate varies substantially between individuals (Nespolo and Franco 2007; White et al. 2013; Auer et al. 2016*a*), necessitating careful attention to controlling for covariates in the design and analysis. Traditionally, selection on metabolic rate has been estimated while "correcting" for body mass, either by using mass-specific values (i.e., per unit mass) or by taking the residuals from a regression of metabolic rate on mass. However, such approaches are unable to separate the traits under selection (i.e., body mass, SMR or both; Hayes 2001; Hagmayer et al. 2020), they ignore the possibility of correlational selection, and they can involve doing 'statistics on statistics' that can fail to propagate uncertainty and may result in statistical bias (Garcia-Berthou 2001; Morrissey 2014). Measuring fitness can also be challenging and past studies have tended to rely on components thereof. While useful for understanding how selection arises, this can provide biased insight into net selection.

Here, we performed high-throughput respirometry on individuals from a laboratory population of *D. melanogaster* with a life cycle that facilitated comprehensive measures of fitness in both sexes. Our fitness measure integrated adult survival, reproductive success, and fecundity, as well as the viability to adult emergence of resulting offspring, all in an abiotic and social environment that was extremely similar to that which the population was adapted. Using these data, we employed a multivariate modelling approach to estimating linear and nonlinear selection while controlling statistically for nuisance variables specific to each trait. Our results provide evidence of linear selection on body mass and/or SMR in males, and nonlinear selection primarily on body mass in females. Despite substantial sample sizes (515 males and 522 females), the partitioning of selection between these two highly correlated traits remained challenging.

In males, linear differentials on body mass and SMR were both positive and significant, indicating direct and/or indirect selection for increased values of these traits. Selection gradients, which quantify selection on each trait while controlling for the other traits in the model, were of somewhat smaller magnitudes to the differentials and were slightly stronger for mass compared to SMR (Table 3.1). While the individual gradients were not significant based on approximate 95% CI's, they approached so, in particular for mass (i.e., the lower bound of the 95% CI just crossed zero). Notably, the 95% CI's for the gradients are 50% wider than those for the differentials, reflecting increased uncertainty in partitioning selection in the face of a strong correlation between these traits (Fig. 3.1).

With the above caveat in mind, the point estimates of our gradients suggest moderately strong directional selection on body mass and SMR in males (median standardized phenotypic gradients from a review of selection in nature is |0.18|; Kingsolver et al. 2001), and little evidence of nonlinear selection including correlational (i.e., SMR × body mass gradient; Table 3.2). It is therefore worth considering why selection may favour increased values of each these traits independent of the other. For body mass, sexual selection is one possibility if increased mass leads to greater reproductive success. Increased mating success of larger males has sometimes, but not always, been observed in *Drosophila* (e.g., Partridge and Farquhar 1983; Partridge et al. 1987; Santos et al. 1988; Pitnick 1991; Baxter et al. 2018, but see Markow et al. 1996; Bangham et al. 2002). Larger males may also have higher postcopulatory success (Pitnick and Markow 1994; Bangham et al. 2002). Compared to standard *Drosophila* lab stocks, our population was also adapted to a lower density mating environment with added structural complexity. This may provide increased opportunity for males to defend food/egg laying substrates as a way to access females, and larger males tend to have an advantage in such territorial interactions in *Drosophila* (Hoffmann 1987; White and Rundle 2014).

With respect to SMR, increased values correspond to males with higher metabolic maintenance costs, which can be seen as the “idling” cost of an individual’s metabolic machinery. As such, males with higher SMR may have more energy available to allocate to costly behaviours or physiological processes. Why might selection favour this? Again, it is possible that such males have increased mating success if they are better at defending a territory and/or searching for, pursuing, and courting females. These demands may be enhanced in our lower density, structurally complex mating environment in which females can hide and escape male courtship. Indeed, similar manipulations of the mating environment in *D. melanogaster* have been shown to reduce the frequency of sexual interactions and mating, and to increase female feeding rates (Yun et al. 2017; Fig. S1 in Yun et al. 2019). Previous work with the current population also revealed a positive correlation between resting metabolic rate and locomotor activity in males (Videlier et al. 2019), suggesting that individuals that perform more energetically demanding activities tend to have elevated maintenance costs.

In females, nonlinear selection was significant overall, indicating curvature of the fitness surface. This appeared to arise in large part from stabilizing selection on body mass although the individual quadratic and correlational gradients were non-significant (Table 3.2), probably because collinearity will be even more problematic for 2nd-order traits. Nevertheless, the point estimates for body mass was negative and substantially larger than that for SMR or the correlational gradient (Table 3.2). The non-parametric fitness surface supports this and reveals a fitness peak within the upper range of mass values (Fig. 3.1B).

While the fitness surface and selection differentials suggest directional selection for both body mass and SMR over much of the phenotypic range in females (i.e., for trait values below the peak), our estimated gradients indicate that this selection on SMR is largely indirect, arising from its correlation with body mass (i.e., gradients on SMR are weak in Tables 3.1 and 3.2). Why might selection favour increased female body size? Fecundity selection seems likely as there is a strong positive association between body size and egg production in *Drosophila* (Lefranc and Bundgaard 2000; Byrne and Rice 2006). It is less obvious as to why fitness may decline at high body mass, although this could represent a trade-off in energy allocation if the energetic costs of further increases in mass come at the expense of greater investment in fecundity. A recent result in this population suggests the presence of allocation trade-offs in females, as reflected by a negative correlation between resting metabolic rate and locomotor activity at the beginning of the night (Videlier et al. 2019), a time which may correspond to a peak in egg laying (Manjunatha et al. 2008).

At first glance, the contrasting significance of linear vs. nonlinear selection in males vs. females suggests sex-specific selection on these traits. However, these differences were not significant, likely reflecting in part the similarity of the fitness surfaces for overlapping trait values between the sexes (Fig. 3.1; the curvature in females occurs at trait values greater than those observed in males). It is therefore possible that males of a similarly large size would likewise experience reduced fitness, but in the absence of such phenotypes we do not know. Further phenotypic manipulation to generate an even broader range of male phenotypes would be necessary to resolve this. Phenotype manipulations can also be useful in reducing or eliminating collinearity among traits (Sinervo 1990; Campbel 2009), allowing combinations of traits to be created that would otherwise be rare or non-existent. In this case, however, it is unclear how mass could be manipulated independently of SMR. A potential downside of a phenotypic manipulation like density is that it can affect all traits, including fitness, and it therefore creates the possibility of a environmentally- (i.e., density-) induced fitness-trait covariance that can be mistaken for selection (Rausher 1992; Stinchcombe et al. 2002). Increased density slows development and thus delays adult emergence in *Drosophila*. Day of emergence was included as a fixed effect in all our analyses, so to the extent that density and emergence day covary, our analysis accounts for density effects. In addition, neither male nor female fitness varied significantly by day of emergence (Fig. S3.1), strongly suggesting that the selection we observed was not the result of a density-induced fitness-trait covariance.

Lande and Arnold (1983) provide a framework for quantifying selection via multivariate regression but problems arise when unique covariates apply to different traits, including fitness. Here we outlined an approach that allows trait-specific covariates by extracting phenotypic covariance matrices at the residual level from a multivariate model of traits and fitness. Linear selection differentials are given by the covariance between fitness and each standardized trait, and linear selection gradients are estimated as the product of the linear selection differentials and the inverse of a subset of the full phenotypic covariance matrix (**P**) that excludes fitness as a trait (Lande and Arnold 1983). The latter is simply the least-squares estimates of the partial regression coefficients obtained via matrix algebra (Kendall and Stuart 1973), meaning this approach can be extended to estimating nonlinear gradients simply by including the squared traits and their second-order interactions in the multivariate model. This is preferable to eq. 14a in Lande & Arnold (1983), which provides an approximation of the nonlinear gradients under certain assumptions. To our knowledge, this statistical approach to estimating nonlinear selection has not been previously employed.

White et al. (2019) recently put forward correlational selection as an explanation for the widely observed metabolic scaling allometry. For correlational selection to occur, particular combinations of SMR and mass must be advantageous over other combinations and, over time, correlational selection change trait covariance (Sinervo and Svensson 2002). In particular, correlational selection favouring small and large individuals with respectively high and low mass-specific SMR would the give rise to the widely observed sublinear scaling of SMR with mass. Using a simulation approach combined with interspecific data, White et al. (2019) concluded that the scaling allometry between metabolic rate and body mass arose as a consequence of correlational selection on these traits. In our study, however, we did not detect correlational selection on SMR and body mass, but more research is needed to estimate the possibility of non-linear trait-fitness covariance at the genetic level.

Finally, as with any observational selection analyses, confounding effects of environmentally-induced covariances between traits and fitness can be mistaken for selection (Rausher 1992; Stinchcombe et al. 2002). This includes potential effects of density discussed above, but also other unidentified environment variables that could affects traits and fitness. The problem of environmentally-induced covariances can be overcome via a breeding design that estimates selection at the genetic level. Estimating the quantitative genetic architecture of fitness and SMR may also provide a direct test of the possibility of sexual conflict over metabolic rate.

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Table 3.1. Variance-covariance matrix between relative fitness (*w*), standardized standard metabolic rate (SMR), and standardized body mass in A) 515 male and C) 522 female *Drosophila melanogaster* extracted from a 3-trait multivariate model. Selection differentials (*S*) were estimated as the covariance between *w* and the trait of interest (values in red), whereas standardized selection gradients (β) were estimated as $\beta = \mathbf{P}^{-1}S$ (Eq. 1), where *S* is the vector of selection differentials (red values) and **P** is the trait-based phenotypic covariance matrix (blue values). 95% confidence intervals (CI) are based on 10,000 bootstrap estimates. Bold denotes significant values.

	(co)variance matrix			selection differentials			selection gradients		
	w	SMR	Mass	*S*	Lower CI	Upper CI	β	Lower CI	Upper CI
A) males									
w	0.765	0.144	0.154						
SMR	0.144	1.000	0.701	**0.144**	**0.071**	**0.214**	0.071	-0.040	0.179
Mass	0.154	0.701	1.000	**0.154**	**0.077**	**0.225**	0.104	-0.008	0.213
B) females									
w	0.294	0.048	0.056						
SMR	0.048	1.000	0.830	0.048	-0.002	0.094	0.005	-0.084	0.093
Mass	0.056	0.830	1.000	**0.056**	**0.007**	**0.103**	0.052	-0.039	0.142

Table 3.2. Variance-covariance matrix between relative fitness (*w*), standard metabolic rate (SMR), standardized body mass, and the three variables from second-orders of SMR and body mass in A) 515 male and C) 522 female *Drosophila melanogaster* extracted from a 6-trait multivariate model. Nonlinear standardized selection gradients were estimated as $\gamma = \mathbf{P}_2^{-1}$cov(*w*, traits) (Eq. 2), where cov is the vector of covariance between relative fitness (*w*) and traits (red values) and $\mathbf{P}_2$ is the trait-based 5×5 phenotypic covariance matrix (blue values). 95% confidence intervals (CI) are based on 10,000 bootstrap estimates. Bold denotes significant values.

	(co)variance matrix						selection differentials			selection gradients		
	w	SMR	Mass	SMR^2	Mass^2	SMR × Mass	*C*	Lower CI	Upper CI	*γ*	Lower CI	Upper CI
A) males												
w	0.765	0.145	0.154	-0.044	0.023	-0.013						
SMR	0.145	1.001	0.701	0.216	0.230	0.210						
Mass	0.154	0.701	1.000	0.197	0.481	0.304						
SMR^2	-0.044	0.216	0.197	3.909	1.982	2.760	-0.044	-0.196	0.102	-0.036	-0.246	0.152
Mass^2	0.023	0.230	0.481	1.982	3.725	2.712	0.023	-0.126	0.171	0.001	-0.154	0.166
SMR × Mass	-0.013	0.210	0.304	2.760	2.712	2.950	-0.013	-0.132	0.109	-0.005	-0.147	0.145
B) females												
w	0.294	0.048	0.056	-0.077	-0.133	-0.101						
SMR	0.048	1.000	0.830	0.565	0.409	0.469						
Mass	0.056	0.830	1.000	0.454	0.486	0.444						
SMR^2	-0.077	0.565	0.454	3.824	2.516	3.099	-0.077	-0.185	0.022	-0.019	-0.266	0.227
Mass^2	-0.133	0.409	0.486	2.516	3.642	2.993	**-0.133**	**-0.233**	**-0.038**	-0.121	-0.321	0.108
SMR × Mass	-0.101	0.469	0.444	3.099	2.993	3.071	**-0.101**	**-0.194**	**-0.013**	0.024	-0.205	0.233

Figure captions

Figure 3.1. Standard metabolic rate (SMR) as function of wet body mass in A) 515 male and B) 522 female *D. melanogaster*. The contour map (thin-plate spline) shows how predicted relative fitness varies as function of SMR and body mass. Points represent individuals.

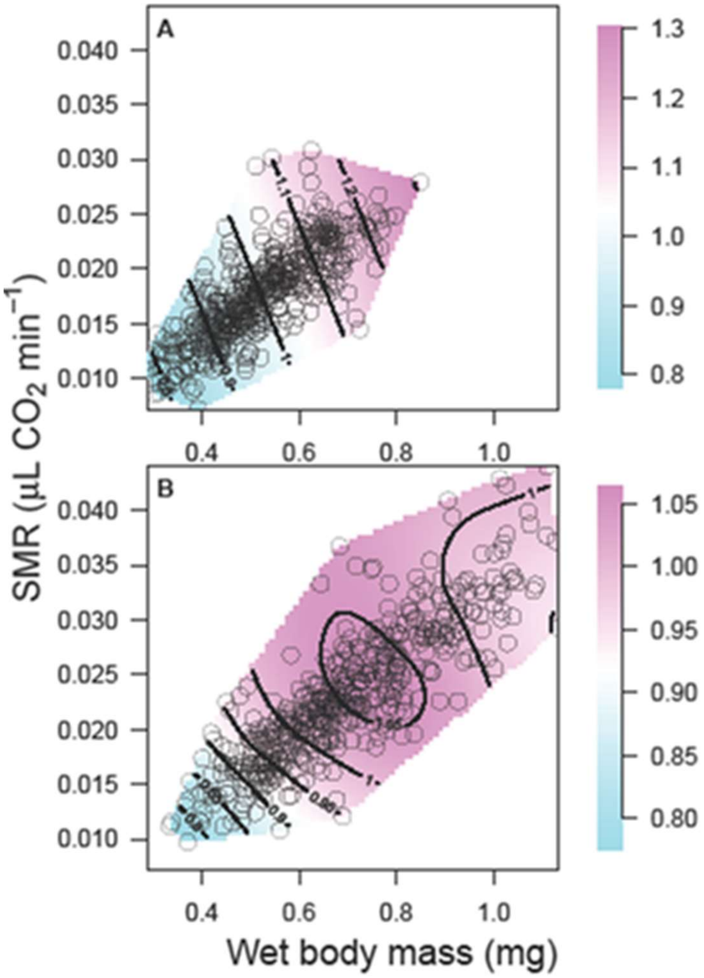
A
B
SMR (μL CO$_2$ min^{-1})
Wet body mass (mg)
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.4
0.6
0.8
1.0
1.3
1.2
1.1
1.0
0.9
0.8
1.05
1.00
0.95
0.90
0.85
0.80

Fig. 3. 1.

Chapter 3: Supplementary information

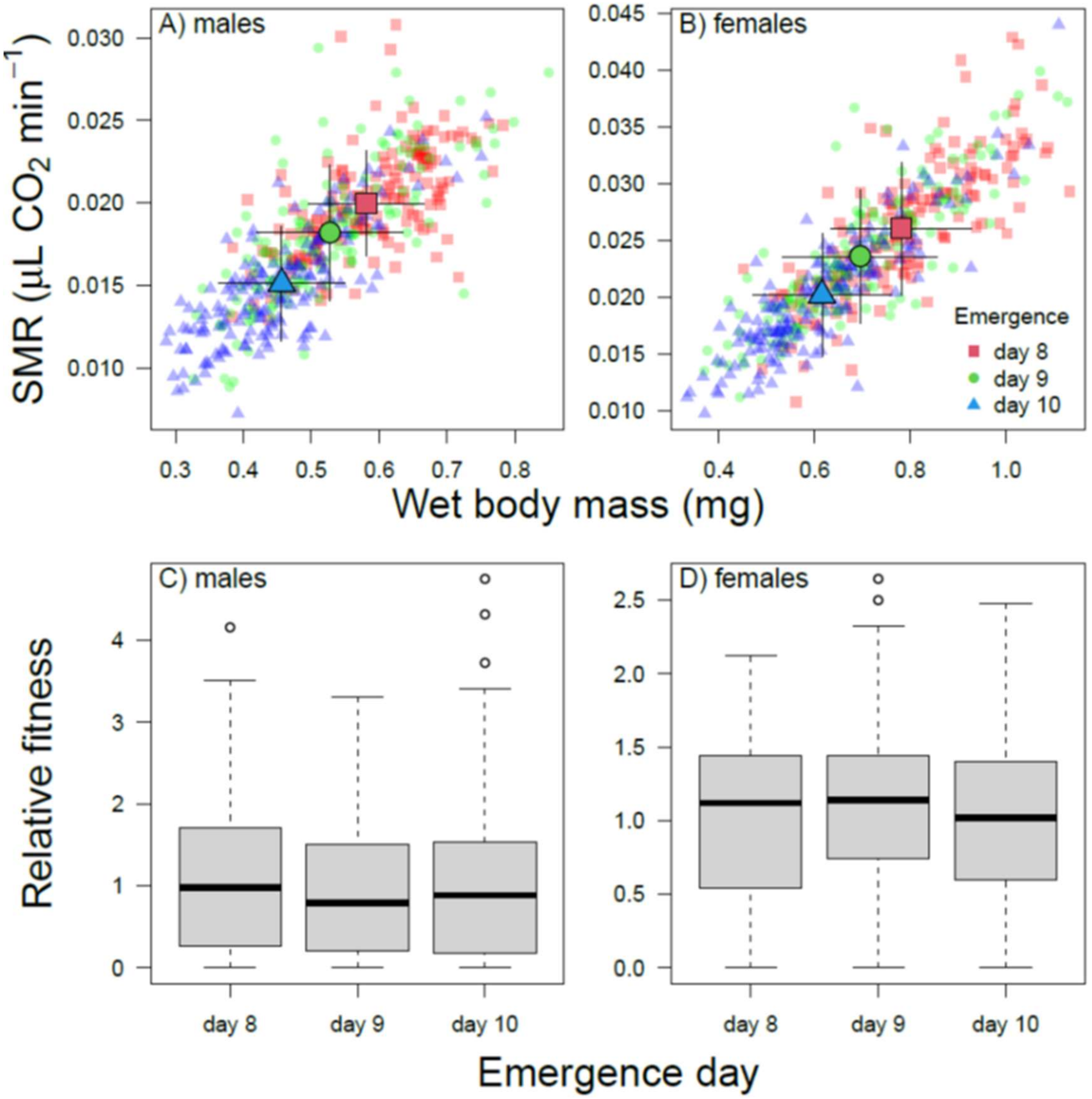

Figure S3.1. Standard metabolic rate (SMR) as function of wet body mass in A) 515 male and B) 522 female *D. melanogaster* that emerged on day 8 (red squares), day 9 (green dots), or day 10 (blue triangles) after egg laying. Relative fitness as function of emergence day in C) males and D) females, showing that neither male nor female fitness varied by day of emergence.

Supplementary information: unpublished results

Difference in selection by day of emergence

Differences in selection among the three days of emergence were tested using a model comparison approach. For linear selection, a likelihood ratio test (LRT) was used to compare a ‘full’ multivariate model that featured three distinct unconstrained residual matrices (one for each day of emergence) with a ‘reduced’ version of the same model in which the covariances between relative fitness and both SMR and mass were constrained to be the same in the three matrices. The same approach was used for nonlinear selection such that the ‘full’ model included the three second-order terms (i.e., SMR^2, mass^2, and SMR × mass) and in the ‘reduced’ model the covariances between relative fitness and three second-order terms were constrained to be the same in the three matrices. The overall significance of linear and nonlinear, and that of the individual selection gradients (β and γ), were tested separately for each day following the same procedure as in the main text when pooling across days. Note that sometimes during the bootstrap a multivariate model would fail to converge because the sample of the data yielded singularities in the average information matrix. When this happened (in 1 out of ~500 iteration when estimating non-linear selection on emergence day 10 only), a new random sample of the data was generated, and the bootstrap process continued until it reached 10,000 iterations.

In males, neither linear nor nonlinear selection varied significantly by day of emergence (LRT: linear $\chi^2_{4\ df} = 5.23$, P = 0.27; nonlinear $\chi^2_{6\ df} = 9.88$, P = 0.13). However, in female linear selection varied significantly by day of emergence (LRT: $\chi^2_{4\ df} = 15.19$, P = 0.004) but nonlinear selection did not ($\chi^2_{6\ df} = 5.99$, P = 0.43). Analyzing linear selection separately by day in females, it was significant overall on day 10 (LRT: $\chi^2_{2\ df} = 14.02$, P = 0.001) but not on the other days (day 8: $\chi^2_{2\ df} = 3.65$, P = 0.16; day 10: $\chi^2_{2\ df} = 2.80$, P = 0.25). For completeness, day-specific linear and nonlinear selection gradients for mass and SMR are given for the overall population and both sexes in Tables S3.2 and S3.3 respectively, in addition to their fitness surface (Fig. S3.2)

Table S3.1. Likelihood ratio tests for the overall presence of selection on standard metabolic rate (SMR) and body mass in males and females and separated by days of emergence (Day 8, 9 and 10) of *Drosophila melanogaster*. For linear selection, the full model with associated Matrix structure was compared to a reduced model that fixed the two covariances (between fitness and SMR and fitness and mass) to 0. For non-linear selection, the full model was compared to a reduced model that fixed the three covariances (between fitness and SMR^2, and fitness and mass^2, and fitness and SMR × mass) to 0. In addition, the likelihood ratio test statistics were used to test if selection differed among days of emergence by comparing an enlarged matrix and the reduced model where covariances are constraint to be the same across day-matrix. Bold denote significant values.

		Linear				Non-linear			
	Reduced Model	Matrix	χ^2	df	P	Matrix	χ^2	df	P
Males	Cov=0	3x3	**17.378**	**2**	**<0.001**	6x6	0.594	3	0.898
Males by Day	CovD1=CovD2=CovD3	9x9	5.228	4	0.265	18x18	9.876	6	0.130
Males Day 8	Cov=0	3x3	1.480	2	0.477	6x6	1.909	3	0.591
Males Day 9	Cov=0	3x3	5.499	2	0.064	6x6	1.143	3	0.767
Males Day 10	Cov=0	3x3	**14.832**	**2**	**0.001**	6x6	3.965	3	0.265
Females	Cov=0	3x3	5.203	2	0.074	6x6	**8.565**	**3**	**0.036**
Females by Day	CovD1=CovD2=CovD3	9x9	**15.192**	**4**	**0.004**	18x18	5.986	6	0.425
Females Day 8	Cov=0	3x3	3.648	2	0.161	6x6	3.146	3	0.370
Females Day 9	Cov=0	3x3	2.796	2	0.247	6x6	**8.395**	**3**	**0.039**
Females Day 10	Cov=0	3x3	**14.018**	**2**	**0.001**	6x6	0.608	3	0.895

Table S3.2. Variance-covariance matrix between relative fitness (*w*), standardized standard metabolic rate (SMR) and body mass in males for each day of emergence A) Day 8, B) Day 9, and C) Day 10 and in females for each day of emergence D) Day 8, E) Day 9, and F) Day 10 extracted from separate 3-trait multivariate models. See Table 3.1 for the calculation methods of selection differentials (S) and selection gradients (β). Bootstrapping with 10,000 iterations was used to calculate the 95% confidence intervals (CI) for S and β estimates. Bold denote significant values.

	(co)variance matrix			selection differentials			selection gradients		
	w	SMR	Mass	S	CI low	CI up	β	CI low	CI up
A) males day 8:									
w	0.648	0.066	0.041						
SMR	0.066	0.743	0.457	0.066	-0.027	0.162	0.088	-0.092	0.278
Mass	0.041	0.457	0.772	0.041	-0.055	0.139	0.001	-0.178	0.172
B) males day 9:									
w	0.852	0.140	0.154						
SMR	0.140	0.783	0.572	**0.140**	**0.024**	**0.247**	0.078	-0.179	0.339
Mass	0.154	0.572	0.791	**0.154**	**0.017**	**0.276**	0.138	-0.139	0.406
C) males day 10:									
w	0.850	0.187	0.231						
SMR	0.187	0.796	0.602	0.078	-0.179	0.339	0.024	-0.238	0.282
Mass	0.231	0.602	0.777	0.138	-0.139	0.406	**0.279**	**0.030**	**0.534**
D) females day 8:									
w	0.310	-0.015	0.031						
SMR	-0.015	0.739	0.579	-0.015	-0.082	0.052	-0.144	-0.325	0.022
Mass	0.031	0.579	0.725	0.031	-0.034	0.097	0.158	-0.006	0.331
E) females day 9:									
w	0.251	0.012	-0.020						
SMR	0.012	0.792	0.647	0.012	-0.059	0.082	0.139	-0.056	0.357
Mass	-0.020	0.647	0.728	-0.020	-0.088	0.048	-0.151	-0.374	0.048
F) females day 10:									
w	0.323	0.128	0.149						
SMR	0.128	0.877	0.733	**0.128**	**0.042**	**0.203**	-0.024	-0.206	0.161
Mass	0.149	0.733	0.821	**0.149**	**0.061**	**0.223**	**0.203**	**0.004**	**0.396**

Table S3.3. (Co)variance matrix between relative fitness (*w*), standard metabolic rate (SMR), body mass, and the three variables from second-orders of SMR and body mass for each day of emergence (Day 8, 9, or 10) in males and females. See Table 3.2 for the calculation of selection non-linear selection gradients (γ).

		(co)variance matrix						selection differentials			selection gradients		
		w	SMR	Mass	SMR^2	Mass^2	SMR x Mass	*C*	CI low	CI up	γ	CI low	CI up
A) males day 8:	*w*	0.648	0.067	0.042	0.062	0.068	0.083						
	SMR	0.067	0.743	0.457	0.244	-0.118	-0.009						
	Mass	0.042	0.457	0.772	-0.033	-0.136	-0.128						
	SMR^2	0.062	0.244	-0.033	2.023	0.310	0.717	0.062	-0.108	0.248	-0.028	-0.230	0.269
	Mass^2	0.068	-0.118	-0.136	0.310	1.189	0.678	0.068	-0.054	0.192	0.045	-0.241	0.371
	SMR x Mass	0.083	-0.009	-0.128	0.717	0.678	0.926	0.083	-0.031	0.196	0.087	-0.168	0.282
B) males day 9:	*w*	0.852	0.140	0.154	-0.010	0.051	-0.013						
	SMR	0.140	0.786	0.574	0.198	0.283	0.152						
	Mass	0.154	0.574	0.791	0.178	0.560	0.315						
	SMR^2	-0.010	0.198	0.178	1.686	0.922	1.115	-0.010	-0.199	0.162	0.038	-0.586	0.515
	Mass^2	0.051	0.283	0.560	0.922	1.918	1.307	0.051	-0.146	0.234	0.053	-0.431	0.630
	SMR x Mass	-0.013	0.152	0.315	1.115	1.307	1.343	-0.013	-0.156	0.137	-0.097	-0.594	0.368
C) males day 10:	*w*	0.850	0.188	0.231	0.040	0.178	0.107						
	SMR	0.188	0.793	0.600	0.432	0.450	0.459						
	Mass	0.231	0.600	0.777	0.498	0.515	0.511						
	SMR^2	0.040	0.432	0.498	1.830	1.397	1.573	0.040	-0.145	0.226	-0.180	-0.649	0.332
	Mass^2	0.178	0.450	0.515	1.397	2.240	1.802	0.178	-0.021	0.365	0.271	-0.313	0.915
	SMR x Mass	0.107	0.459	0.511	1.573	1.802	1.743	0.107	-0.075	0.280	-0.095	-0.600	0.362
D) females day 8:	*w*	0.857	0.186	0.236	0.041	0.182	0.110						
	SMR	0.186	0.793	0.600	0.432	0.449	0.458						
	Mass	0.236	0.600	0.777	0.498	0.515	0.511						
	SMR^2	0.041	0.432	0.498	1.830	1.397	1.574	0.002	-0.121	0.122	-0.068	-0.413	0.299
	Mass^2	0.182	0.449	0.515	1.397	2.240	1.802	-0.055	-0.145	0.034	-0.254	-0.553	0.090
	SMR x Mass	0.110	0.458	0.511	1.574	1.802	1.743	-0.008	-0.085	0.065	0.133	-0.189	0.442
E) females day 9:	*w*	0.251	0.014	-0.020	-0.108	-0.152	-0.129						
	SMR	0.014	0.795	0.648	0.431	0.289	0.351						
	Mass	-0.020	0.648	0.728	0.299	0.286	0.294						
	SMR^2	-0.108	0.431	0.299	1.756	1.323	1.508	-0.108	-0.226	0.016	-0.074	-0.540	0.321
	Mass^2	-0.152	0.289	0.286	1.323	1.719	1.528	**-0.152**	**-0.251**	**-0.043**	-0.194	-0.756	0.316
	SMR:Mass	-0.129	0.351	0.294	1.508	1.528	1.574	**-0.129**	**-0.229**	**-0.021**	0.036	-0.352	0.491
F) females day 10:	*w*	0.322	0.125	0.147	0.037	0.048	0.038						
	SMR	0.125	0.876	0.733	0.651	0.569	0.596						
	Mass	0.147	0.733	0.821	0.631	0.620	0.588						
	SMR^2	0.037	0.651	0.631	3.019	2.207	2.545	0.037	-0.146	0.180	-0.061	-0.519	0.369
	Mass^2	0.048	0.569	0.620	2.207	2.435	2.276	0.048	-0.164	0.215	-0.058	-0.657	0.466
	SMR x Mass	0.038	0.596	0.588	2.545	2.276	2.415	0.038	-0.151	0.185	0.026	-0.424	0.485

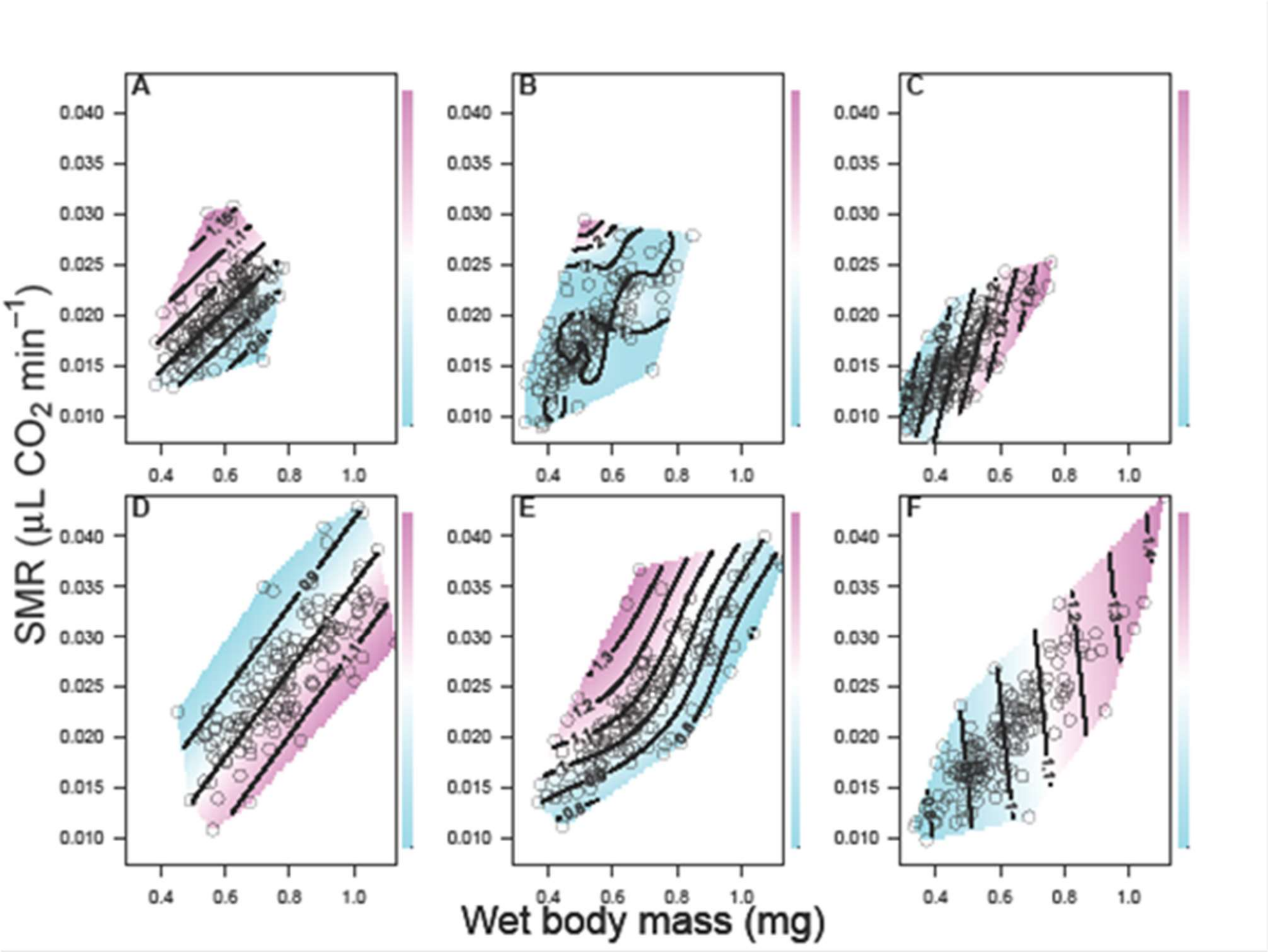

Figure S3.2. Standard metabolic rate (SMR) as function of wet body mass in A) male for day 8 of emergence, B) male for day 9 of emergence, C) male for day 10 of emergence, D) female for day 8 of emergence, E) female for day 9 of emergence and F) female for day 10 of emergence. The contour map (thin-plate spline) shows how predicted relative fitness varies as function of SMR and body mass. Points represent individual.

CHAPTER 4: Standard metabolic rate in *Drosophila melanogaster*: sex-specific genetic architecture and co-variance with body mass and locomotor activity.

This chapter has been writing for publication in collaboration with Vincent Careau (VC) and Howard D. Rundle (HDR). I designed the experiment with the help of VC and HDR. I collected the data and analyzed it with input from VC and HDR. All authors wrote the manuscript.

Introduction

Metabolic rate – the "fire of life" – represents the energy turnover generated by ongoing biological processes within an organism (Kleiber 1961) and, as such, is a fundamental aspect of animal physiology, ecology, and life-history evolution (Ricklefs and Wikelski 2002; Brown et al. 2004; Réale et al. 2010). Maintenance metabolism reflects the energy invested into the body maintenance (e.g., cellular turnover, basic body function) which represent a large proportion of the total energy budget of an individual. In ectotherms and endotherms, this is referred to as the standard metabolic rate (SMR) and basal metabolic rate (BMR) respectively, and are quantified as the minimal level of energy expenditure in a post-absorptive, nonreproductive, and inactive adult (at a given temperature for SMR) (Rolfe and Brown 1997; Careau et al. 2014). Maintenance metabolism can be thought of as the ‘‘idling cost" of an individual's metabolic machinery. SMR and BMR are quite variable within (Nespolo and Franco 2007; White et al. 2013; Videlier et al. 2019) and among species (Konarzewski and Ksiazek 2013; White and Kearney 2013), and understanding the causes and consequences of this variation in maintenance metabolism is a major focus of evolutionary physiology (Mcnab and Morrison 1963; Burton et al. 2011; Pettersen et al. 2018).

Numerous comparative studies have shown that SMR and other metabolic traits correlate with ecological factors such as temperature (Nespolo et al. 2003; Clarke and Fraser 2004; DeVries and Appel 2013; DeVries et al. 2013; Schulte 2015; Auer et al. 2018), altitude (Chown and Gaston 1999; Rourke 2000), and diet (Van Leeuwen et al. 2012; Auer et al. 2015, 2016*b*; Piper 2017; Ayayee et al. 2018). Differences in maintenance metabolism that correlate with biotic and abiotic factors suggest adaptive divergence, a pre-requisite of which is the presence of additive genetic variance (V_A) in metabolic rate (Falconer 1962). Accordingly, substantial effort has been devoted to estimating V_A and its associated narrow-sense heritability (h^2) for maintenance metabolism and other related metabolic traits, primarily in mammals and birds but also in insects as well. However, quantifying individual metabolic rate, and more specifically SMR, is challenging because of the highly standardized condition under which measurements must be performed. Doing so in a small ectotherm, like *Drosophila melanogaster*, has additional challenges due to their extremely low individual metabolic rate. Few estimates of V_A for SMR have been attempted in small ectotherms (Carter et al. 2012; Bruning et al. 2013; Nespolo et al. 2014) although there are more estimates for metabolic rate under less standardized conditions (e.g., resting metabolic rate or routine metabolic rate). As expected for such a labile trait, h^2 estimates are generally moderate to low for metabolic rate (Bushuev et al. 2011; White and

Kearney 2013; Pettersen et al. 2018), but do indicate at least some V_A by which adaptation could occur.

Selection, however, rarely acts on single traits in isolation (Lande and Arnold 1983; Walsh and Blows 2009) and a comprehensive understanding of adaptive variation in SMR therefore goes beyond the simple existence of V_A. Additional insight can be gained by estimating the genetic architecture of SMR alongside other, potentially genetically correlated, traits (i.e., the additive-genetic variance-covariance matrix (**G**) of a set of traits). Body mass is of primary interest in this respect as it is consistently found to correlate strongly with SMR (Burton et al. 2011; White 2011) because larger animals consume more energy due to their greater tissue volume. To account for this, past studies generally correct SMR for body mass to consider mass-specific metabolic rates. However, additional insight can be gained by treating body mass as a separate trait. By doing so, one can estimate V_A in both SMR and body mass, as well as their genetic correlation (r_A). Selection can also (in theory) be quantified for each trait on its own, correlational selection between the traits can be considered (see chapter 2), and the response to selection can be partitioned into direct and correlated effects for each (Lande and Arnold 1983). Another advantage of this approach is that it provides an estimate of the allometry of metabolic traits at the additive genetic level, which may help link micro- and macro-evolutionary patterns of metabolic allometry (Glazier 2005; Beaman et al. 2020).

In insects, body mass is often highly sexually dimorphic with females usually being larger than males (Teder 2014). Metabolic rate is also sexually dimorphic in at least some taxa (Schimpf et al. 2012; Tomlinson and Phillips 2015; Arnqvist et al. 2017), and the allometric scaling of SMR or BMR has been shown to be sex specific in some cases (Ryan and Hopkins 2000; Mathot et al. 2013; Videlier et al. 2019). Such sex-specific effects can be incorporated into quantitative genetic approaches by treating a given set of traits as separate traits in males and in females. In doing so, the **G** matrix is partitioned into a larger matrix quantifying the sex-specific genetic architecture $\mathbf{G}_{\mathrm{fm}}$ (Lande 1980; Cheverud et al. 1985) for the set of traits:

$$\mathbf{G}_{\mathrm{fm}} = \begin{bmatrix} \mathbf{G}_{\mathrm{f}} & \mathbf{B} \\ \mathbf{B}^{\mathrm{T}} & \mathbf{G}_{m} \end{bmatrix},$$

where $\mathbf{G}_{\mathrm{f}}$ and $\mathbf{G}_{\mathrm{m}}$ represent the genetic (co)variance matrices for the traits separately in females and males respectively, and **B** is a matrix of cross-sex (also termed between-sex) genetic (co)variances both within and between traits (‘within-trait’ cross-sex covariances are on the diagonal, ‘between-trait’ cross-sex covariances are on the off-diagonal); $\mathbf{B}^{\mathrm{T}}$ is the transpose of **B**. The **B** matrix provides insight into the shared genetic basis of traits in males and females. For example, if the sexes share most of their V_{A} for a given trait, then the cross-sex genetic correlation (r_{fm}, a standardized version of a cross-sex genetic covariance) will be strong and positive (i.e., close or equal to one), and a response to selection in one sex will produce a correlated response in the other sex. A positive r_{fm} will constrain the response to sexually-antagonistic selection on the trait, generating intralocus conflict that hampers the evolution of sexual dimorphism and favours mechanisms that decrease r_{fm} (Bonduriansky and Chenoweth 2009; Van Doorn 2009). Consistent with this, r_{fm} is negatively correlated with the magnitude of sexual dimorphism across traits (Poissant et al. 2010). Although r_{fm} have been estimated for various morphological (Poissant et al. 2008; Potti and Canal 2011; Tarka et al. 2014; Turk et al. 2018; Sztepanacz and Houle 2019) and behavioural traits (Long and Rice 2007; Han and Dingemanse 2017; Han et al. 2019; Kralj-Fišer et al. 2019), estimates for metabolic rate are rare but do exist for bank voles (Boratyński et al. 2013) and *D. melanogaster* (Jumbo-Lucioni et al. 2010).

Considering the covariance of body mass and SMR is not sufficient on its own to capture the full extent of potential genetic constraints for these traits. The other elements of the **B** matrix, consisting of the cross-sex between-traits covariances, quantify the genetic relationship between one trait in males and another trait in females. Asymmetry can be exhibited within **B** if the lower and upper triangles of **B** differ. This asymmetry occurs when the covariances between traits differ in a manner dependent upon which sex is expressing which trait (Gosden and Chenoweth 2014), implying genetic divergence between sexes and thus shaping correlated response to selection in a sex-specific way. All together, **B** shapes correlated responses to selection between the sexes, affecting trait evolution and the evolution of sexual dimorphism (Gosden et al. 2012; Ingleby et al. 2014; Cox et al. 2017; Rapkin et al. 2017).

Additional insight into the genetic architecture of SMR may be gained by considering genetic correlations with other energetically expensive traits such as the daily locomotor activity. When seen as the "idling cost" of an individual's metabolic machinery, as the performance model of energy management suggests (Careau et al. 2014), SMR is predicted to correlate positively with activity. In contrast, the allocation model posits that, because SMR constitutes a relatively large portion of an individual's energy budget (Careau et al. 2008), investment in it may come at the cost of reduced energy available for allocation to other energetically expensive traits, thereby generating negative correlations between them (Careau et al. 2014). Considerable attention has been given to testing the contrasting expectations of the performance, independent and allocation models over the past decade (Portugal et al. 2016; Careau 2017), with mixed results at the phenotypic level. For example, a recent study found that while SMR and locomotor activity were positively correlated at the among-individual level in male *D. melanogaster*, the correlation was negative in females (Videlier et al. 2019). The joint consideration of SMR, body mass, and daily locomotor activity in a fully sex-specific genetic analysis, may therefore provide additional insight into the genetic architecture of metabolism and its association with the energy budget.

Here, we quantified SMR, body mass, and daily locomotor activity by performing a half-sibling quantitative genetic breeding design. *D. melanogaster* is a good model species for this sort of investigation because they have a short generation time and a large pedigreed set of individuals can be obtained and phenotyped under relatively constant conditions (Roff and Mousseau 1987). Although a few broad-sense heritability estimates exist for metabolic rate in *Drosophila* (Montooth et al. 2003; Khazaeli et al. 2005; Jumbo-Lucioni et al. 2010; Alton et al. 2017; Matoo et al. 2019), we are aware of only a single published h^2 estimate for RMR (resting metabolic rate, similar to SMR but with some conditions not controlled) (Castañeda and Nespolo 2013) and none for SMR. Overall, our estimate of the sex-specific genetic architecture (i.e. $\mathbf{G}_{\mathrm{fm}}$) for this set of traits reveals some differences between males and females that demonstrate the importance of considering this suite of traits separately in males and females, and suggest the presence of important shared genetic variation between traits and also between sexes.

Methods

Population and breeding design

A stock population was established in February of 2016 from a large sample of a laboratory-adapted population of *D. melanogaster* that was originally collected in Dundas, ON in 2006 (MacLellan et al. 2012). Since then, this stock has been maintained with discrete, non-overlapping generations at 25°C, 50% relative humidity, and with a 12L:12D photoperiod (lights switch at 7 am/pm) on a standard cornmeal-based food (90 g/L cornmeal, 100 g/L turbinado sugar, 40 g/L yeast and 12 g/L agar). The population had a specific life cycle in which egg-to-adult development occurred over nine days in standard *Drosophila* glass culture vials (28.5 mm × 95 mm). Following this, emerged adults experienced a 4-day ‘mating phase’ that took place in an environment (8 oz. culture bottles) with reduced density (10 males and 10 females/bottle) and increased spatial complexity (i.e., dividers inserted into the food and two coiled piper cleaners inside the bottle) compared to standard *Drosophila* laboratory populations. Males were discarded after the mating phase and surviving females were allowed to lay eggs for 24 h in vials to create the next generation, after which they were also discarded.

Using this stock, we conducted a paternal half-sibling breeding design in which 125 virgin males (sires) were each mated to either four (*N*=108) or two (*N*=17) virgin females (dams) for 48 hours. These females were subsequently allowed to oviposit for 24 hours in separate laying vials. Offspring were collected from an average of 2.86 dams/sire (range = 2-3) depending on female survival and productivity. To make the experiment manageable, the breeding design was performed over seven blocks of 16-20 sires each, except for one block in which there were 11 sires, generating a total of 349 families.

From each family, four male and four female offspring were phenotyped, two of each sex for SMR and two different individuals of each sex for general locomotor activity (thereafter, activity). SMR and activity were measured using previously described protocols (Videlier et al. 2019). After metabolic or activity measurements, flies were placed at 50°C overnight in a drying oven and dry body mass was subsequently measured using a MX5 microbalance (Mettler Toledo, Columbus, OH). Dry body mass was therefore measured for all phenotyped individuals. After accounting for rare handling errors and occasional death, a total of 2,482 individuals (1,252 females and 1,230 males) were phenotyped.

Statistical analyses

All continuous variables were standardised to a mean of zero and a variance of one. Activity variables, including general locomotor activity and two nuisance variables recorded during the metabolic measurements (average activity during and immediately prior to the metabolic measurement; ACT_{20} and ACT_{20p}), were log-transformed before standardization. Analyses were conducted in ASReml-R (Butler et al. 2009). First, a 3×3 multivariate animal model was fit using SMR, activity, and body mass as response variables, pooling the sexes. A sex-specific 3×3 multivariate animal model was then fit that estimated separated **G** and residual matrices for each sex, providing estimates of $\mathbf{G}_f$ and $\mathbf{G}_m$. Partitioning the additive genetic and residual matrices this way greatly improved the fit of the model (Likelihood Ratio Test (LRT): $\chi^2 = 983.6$, $df=11$, $P < 0.0001$), so subsequent analyses treated SMR, activity and body mass as separate traits in males and females. This was done by fitting 6×6 multivariate animal models to estimate the full $\mathbf{G}_{\mathrm{fm}}$ matrix, consisting of $\mathbf{G}_{\mathrm{f}}$, $\mathbf{G}_{\mathrm{m}}$ and **B**.

All models included block (categorical variable with seven levels) and age (categorical variable with five levels ranging to 1 to 5 days post emergence) as fixed effects fitted to all traits. We also fitted other potential nuisance variables specific to a subset of traits. These included 'monitor identity' for locomotor activity and, for body mass, a variable indicating whether the individual went through the respirometry or activity assay to control for differences in the environment immediately prior to mass measurement. Finally, for SMR we included as fixed effects the temperature, flow rate, water vapour, light intensity, and average activity during and immediately prior to the metabolic measurement (ACT_{20} and ACT_{20p}). We also fitted fly density in the holding vial as covariate to all traits, but it was never significant ($P > 0.19$ in all cases) and was therefore excluded from the final models presented here. Other nonsignificant nuisance variables, including light intensity and flow rate, were excluded as well. Significance of fixed effects was evaluated using a conditional Wald *F*-statistic (Table S4.2) and the denominator degrees of freedom were determined following Kenward and Roger (1997).

All multivariate animal models included individual identity associated with the pedigree information, which allowed to partition the phenotypic variance into additive genetic variance (V_A) and residual variance (V_R) (Kruuk 2004; Wilson et al. 2010). Dam identity was also included as a random effect to capture common environment variance (V_{CE}), with potential contributions from dominance and maternal genetic variance as well. For SMR, we also included a random effect to account for the non-independence of the 16 measurements with a given 'unit' (i.e. MAVEn measurement apparatus; Videlier et al. 2019) on a given day (i.e., separate levels for each day × unit combination; named MAVEn_run). We first estimated additive genetic and residual covariance matrices, but to facilitate hypothesis testing and biological interpretation, we subsequently directly estimated correlation matrices instead, which scale covariances relative to both trait variances. This was done by specifying an unstructured general correlation matrix ('corgh') at the additive genetic and residual level. SMR and activity were measured in different individuals so residual correlations between these traits (within and between sexes) cannot be estimated and were fixed to zero in all cases. Because most V_{CE} estimated were at the lower boundary of the parameter space (i.e., 0), we did not attempt to estimate correlations at that level (i.e., a "diag" structure was used for dam identity) (Table S4.1).

Narrow sense heritability (h^2) was estimated for each sex-specific trait as: $h^2 = V_A / V_P$ (Kruuk 2004; Wilson et al. 2010), where V_P is the total phenotypic variance, calculated as the sum of V_A, V_{CE}, and V_R (Wilson 2018). To assess the significance of genetic and residual correlations, we used separate LRTs with one degree of freedom to compare an unconstrained model to one in which the correlation was fixed to 0 or to 0.99999. To visualize genetic correlations, best linear unbiased predictors (BLUP) were extracted (Hadfield et al. 2010; Houslay and Wilson 2017). The overall importance of the cross-sex genetic covariances (i.e. **B**) matrix was tested by comparing the fit of an unconstrained model to one in which all covariances in **B** were fixed to zero using a LRT with 9 degrees of freedom. Potential asymmetry of in the upper vs. lower triangles of **B** was tested by comparing the unconstrained model to one in which the two sections were forced to be the same as the average of the two triangles.

Finally, an additional and final model was performed to estimate the genetic allometry of SMR. A 4×4 multivariate animal model was fitted with sex-specific SMR and body mass as response variables (i.e. excluding activity), employing the corresponding fixed and random effects from above. SMR and body mass were not standardized but were $\log_{10}$ transformed. Such transformation provided information on the proportional relationship between SMR and body mass and thus insight into metabolic allometry. Genetic and residual slopes (*b*) between SMR and MASS were calculated as: $b = \mathrm{Cov}_{\mathrm{SMR\text{-}MASS}} / V_{\mathrm{MASS}}$.

Results

Genetic (co)variances: $\mathbf{G}_f$ *and* $\mathbf{G}_m$

In males, there was substantial V_A for locomotor activity with a corresponding narrow-sense heritability that was relatively high (h^2 ± se: 0.620 ± 0.108), whereas in females V_A and heritability (h^2 ± se: 0.188 ± 0.098) were noticeably lower (Figs. 4.1 and 4.2). In contrast, moderate genetic variation was detected in both SMR and body mass and these differed much less between males and females, contributing to smaller differences in sex-specific heritabilities (Figs. 4.1 and 4.2).

There was a strong, positive genetic correlation between SMR and body mass in females ($r_A = 0.963 \pm 0.194$), whereas this was somewhat weaker in males ($r_A = 0.681 \pm 0.155$; Fig. 4.3B). In contrast, there was little evidence of a genetic correlation between locomotor activity and body mass in either sex, although the point estimate in females was slightly negative ($r_A = -0.283 \pm 0.222$; Fig. 4.3C-D). The genetic correlation between SMR and locomotor activity was moderate and positive in both sexes, although the point estimate in females was somewhat smaller and less precise ($r_A = 0.240 \pm 0.296$) than that in males ($r_A = 0.355 \pm 0.156$; Fig. 4.2E,F).

Cross-sex genetic covariances: **B** *matrix*

Inclusion of non-zero within and between-trait cross-sex additive genetic covariances (i.e. **B**) significantly improved the fit of the model (LRT $\chi^2 = 71.25$, $df = 9$, $P < 0.0001$), indicating shared V_A between males and females for at least some combinations of these traits. All three within-trait cross-sex genetic correlations (i.e. r_{fm}) were positive and significantly different from zero (Table 4.1, Fig. 4.2E-G). The r_{fm} point estimate for activity was particularly high (0.983 ± 0.281); only that for body mass was significantly less than one, although the point estimate for SMR was lower but also less precise (Table 4.1).

The between-trait cross-sex genetic correlations were generally positive although more variable and one-point estimate was negative (between female activity and male mass), but not significantly so. Two estimates differed significantly from zero and several from one (Table 4.1). There was some indication of asymmetry between the lower and upper ‘triangles’ of **B**, but this was not significant (LRT $\chi^2 = 1.993$, $df = 3$, $P = 0.574$).

Allometric slopes

Analysis of $\log_{10}$-transformed SMR and body mass estimated the genetic allometric slope (b_A) for females as $b_A = 0.658 \pm 0.159$, with a similar value of $b_A = 0.581 \pm 0.324$ in males. The residual allometric slopes (b_e) were somewhat shallower, with values of $b_e = 0.399 \pm 0.167$ in females and $b_e = 0.342 \pm 0.165$ in males.

Discussion

Estimating the genetic architecture between traits provides insight into shared genetic variation and can reveal potential genetic constraints that may alter their response to selection. Further partitioning genetic variation between the sexes allows the construction of the full sex-specific additive genetic (co)variance matrix ($\mathbf{G}_{\mathrm{fm}}$) that includes **B** providing insight into shared genetic variation between males and females. Here, we examined the genetic architecture of SMR, general locomotor activity and body mass in female and male *D. melanogaster*. Point estimates suggest V_A in all three traits, but also shared genetic variation between sexes and traits that will affect, and potentially constrain evolutionary responses. Furthermore, females and males differed in their genetic architecture, mainly driven by differences in genetic variance in locomotor activity.

Within-sex genetic architecture: $\boldsymbol{G}_f$ *and* $\boldsymbol{G}_m$

Little is known about the genetic basis of the maintenance metabolism in ectotherms despite its fundamental importance in ecology, and life-history evolution (Ricklefs and Wikelski 2002; Réale et al. 2010). The few V_A and h^2 estimates that exist are low to moderate and often non-significant (Pettersen et al. 2018). Moreover, these V_A and h^2 are generally corrected for the body mass, and thus do not distinguish the genetic basis of SMR and body mass. A multivariate approach allows estimates of the genetic variance for both traits, as well as their genetic correlation with each other and with other ecologically relevant traits (Walsh and Blows 2009). Here, the genetic correlations between SMR, body mass, and locomotor activity suggest a great deal of shared genetic variance due to pleiotropy and linkage disequilibrium. A shared genetic variance may indicate the presence of genetic constraint because selection on one trait will result in indirect (i.e., correlated) response in the other trait.

Unsurprisingly, the genetic correlation r_A between SMR and body mass was strong in both males and females with both being significantly greater than zero and not significantly different from one. This result is consistent with the positive genetic association found between body mass and metabolic traits (White et al. 2019). Additional insight into the genetic relationship between SMR and body mass, and their correlated evolution, can be gained through estimating the genetic allometric slope (b_A). Doing so is a required step if we want to bridge micro- and macro-evolutionary parameters because, in a bivariate plane representing genetic variation in these two traits, b_A represents the genetic “line of least resistance” along which species should most likely diverge (Schluter 1996). The allometric scaling of metabolic rate has been intensively studied at the inter-specific levels for a long time (Kleiber 1961; Glazier 2005; Beaman et al. 2020). For maintenance metabolism, the interspecific allometric scaling exponent usually ranges between a 2/3 and 3/4 power function, which means that metabolic rate does not scale linearly with body mass. The mechanism underlying the exact scaling relationship is still debated, and multiple mechanisms have been proposed (White and Kearney 2013). Here, the genetic allometric slope in females ($b_A = 0.658 \pm 0.159$) and males ($b_A = 0.581 \pm 0.324$) most closely correspond to the 2/3 scaling exponent. Interspecific allometry exponents in other insect taxa matched this specific scaling exponent (Niven and Scharlemann 2005), suggesting that species divergence may have occurred along the genetic line of least resistance.

Returning to genetic correlations, in males, locomotor activity shared genetic variance with SMR but not with body mass, indicated by the positive and significant r_A between locomotor activity and SMR and the weak and not significant r_A between locomotor activity and body mass. This result appear surprising given the large genetic variance shared between body mass and SMR, suggesting that the weakening of the genetic constraint between SMR and body mass was enough for the mass-independent component of variation in SMR to be shared with activity. A similar partitioning was not possible in females as essentially most of genetic variation in SMR was common to mass. The genetic independence between locomotor activity and body mass implies that selection on locomotor activity will not produce a correlated response in body mass (and vice versa), while selection on activity will affect SMR. This result is consistent with a genetic mapping study conducted in males of another *D. melanogaster* population, in which metabolic rate and another locomotor activity (flying) were mapped in the same region on the third chromosome (Montooth et al. 2003). All together, these results support the performance model in males where SMR reflects the “idling cost” of the metabolic machinery required to express energetically expensive traits such as locomotor activity. Similar results were detected at the phenotypic level in this population (Videlier et al. 2019). Male *Drosophila* improve their reproductive success by increasing their mating rate (Bateman 1948; Hall 1994) and therefore should be selected for an increase in locomotor activity. In addition to the energetic cost of activity itself, an increase in SMR suggests additional energy costs related to body maintenance.

In females, the interpretation of the other genetic correlations (aside from mass-SMR) is somewhat more challenging due to their lower precision. Nevertheless, point estimates indicate that some genetic variation is shared between locomotor activity and both SMR and body mass. The point estimate for r_A between locomotor activity and body mass is negative, suggesting that the shared genetic variance has opposite effects on these traits, but this correlation was not significantly different from zero. The relatively small and nonsignificant r_A between locomotor activity and SMR supports the independent model of energy management whereby SMR and activity neither promote nor constrain each other within the total energy budget. Similar results were detected at the phenotypic level in this population (Videlier et al. 2019).

Between-sex genetic architecture: ***B*** *matrix*

Along with the estimate of $\mathbf{G}_f$ and $\mathbf{G}_m$, the full $\mathbf{G}_{fm}$ matrix provides additional insight into the extent to which genetic variance is shared between females and males both within and between traits (i.e., **B**). Surprisingly, few studies have estimated **B**, especially when including a physiological trait. A possible reason of this is that quantifying **B** requires measurements on both pedigreed males and females for multiple traits, along with substantial sample sizes to fit such a complex multivariate model. Shared genetic variance between sexes has been shown to have consequences for the predicted evolutionary response to selection (Wyman et al. 2013). **B** can contribute to the evolution of sexual dimorphism by constraining (Lewis et al. 2011; Gosden et al. 2012; Ingleby et al. 2014) or promoting it (Cox et al. 2017; Rapkin et al. 2017; Sztepanacz and Houle 2019). In our case, including **B** significantly improved the model fit, demonstrating that one or more of these correlations matter to the genetic architecture of SMR, body mass and locomotor activity.

The within-trait cross-sex genetic covariances are found along the diagonal of **B**. In our cases, all three of these associated correlations (r_{fm}), representing scaled covariances, were significantly greater than zero, indicating shared genetic basis of these traits between females and males. This indicates the possibility of ongoing intralocus sexual conflict if selection is sexually antagonistic on any of these traits (Bonduriansky and Chenoweth 2009; Van Doorn 2009). The shared genetic variance for locomotor activity was surprisingly strong, especially given known sex-specific patterns of how activity varies through the day (Ferguson et al. 2015; Videlier et al. 2019) and the sex difference in variances of locomotion activity. A similar pattern was found in another *Drosophila* population in which a very strong r_{fm} was estimated despite pronounced sexual dimorphism (Long and Rice 2007). Long and Rice (2007) also found that selection on activity was sexually antagonist implying the presence of intralocus sexual conflict over this trait.

Cross-sex genetic correlations for body mass and SMR were lower, but the only r_{fm} significantly lower than one was for body mass, suggesting the presence of some independent genetic variance in this trait between males and females. This suggests a past history of sexually antagonistic selection, which is not surprising given widespread sexual dimorphism in body size across taxa. Indeed, the resolution of intralocus sexual conflict requires a lowering of r_{fm} to facilitate the evolution of sexual dimorphism (Bonduriansky and Chenoweth 2009). For SMR, the point estimate of r_{fm} was even lower but, was also less precise and hence was marginally non-significant when tested against a value of one ($P = 0.107$). This suggests some independence of the genetic variance in SMR between the sexes and hence a potential past history of sexually antagonist selection on this trait. Our previous estimates of phenotypic selection also suggested that the possibility of sex-specific selection for SMR and/or body mass in this population, although not for trait values that overlapped in males and females (Videlier et al. 2020).

The existence of some sexual dimorphism despite a strong r_{fm} may be somewhat surprising and not in accord with the general trend of negative correlation between sexual dimorphism and r_{fm} (Bonduriansky and Locke 2005; Poissant et al. 2010). But similar results have been found (Charmantier et al. 2017) and suggest that possible other factors such as the environment or the ontogeny could affect the observed phenotypic sexual dimorphism. It is also possible that sex-specific loci were fixed over time and therefore did not contributed to the genetic variance, which dissociate the observed sexual dimorphism and the shared genetic variance between sexes.

Finally, the between-trait cross-sex correlations were variable but, in general, not strong. Of note, however, are the reciprocal correlations between mass in one sex and SMR in the other, both of which were significantly greater than zero. This isn’t surprising given the strong within-sex correlations between SMR and mass. **B** can be asymmetrical between its lower and upper triangles, reflecting a situation in which covariances between traits differ in a manner dependent upon which sex is expressing which trait (Gosden and Chenoweth 2014). Asymmetry of **B** can also alter the predicted evolutionary responses. In our case, the point estimates suggest some asymmetry, but it was not significant.

Conclusion

Insight into the genetic architecture of SMR, body mass and locomotor activity was gained by estimating the fully sex-specific genetic matrix ($\mathbf{G}_{\mathrm{fm}}$). We revealed some difference between females and males in their genetic architecture, driven in large part by a difference in genetic variance in locomotor activity between the sexes. While there was detectable genetic variance in SMR, it was largely shared with that of mass, almost entirely so in females. In addition, the **B** matrix revealed that substantial genetic variation was shared between the sexes for all three traits, generating positive and strong cross-sex genetic correlations which indicate the potential for intralocus sexual conflict if selection is sexually antagonistic in this population. While phenotypic selection has been previously quantified (Videlier 2020), such estimates may be biased by environmentally-induced covariance between the fitness and the traits, so an important goal for future studies will be to estimate selection at the genetic level by including fitness within the context of a breeding design like that used here.

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Chapter 4

Wyman, M. J., J. R. Stinchcombe, and L. Rowe. 2013. A multivariate view of the evolution of sexual dimorphism. J. Evol. Biol. 26:2070–2080.

Table 4.1 Additive genetic (r_A) and residual (r_e) correlations between body mass, locomotor activity, and standard metabolic rate (SMR) *D. melanogaster*. Likelihood ratio test (LRT, 1df) were used to compare an unconstrained model to one in which each correlation was fixed to either zero (LRT-0) or one (LRT-1). Bold values denote significance ($P < 0.05$).

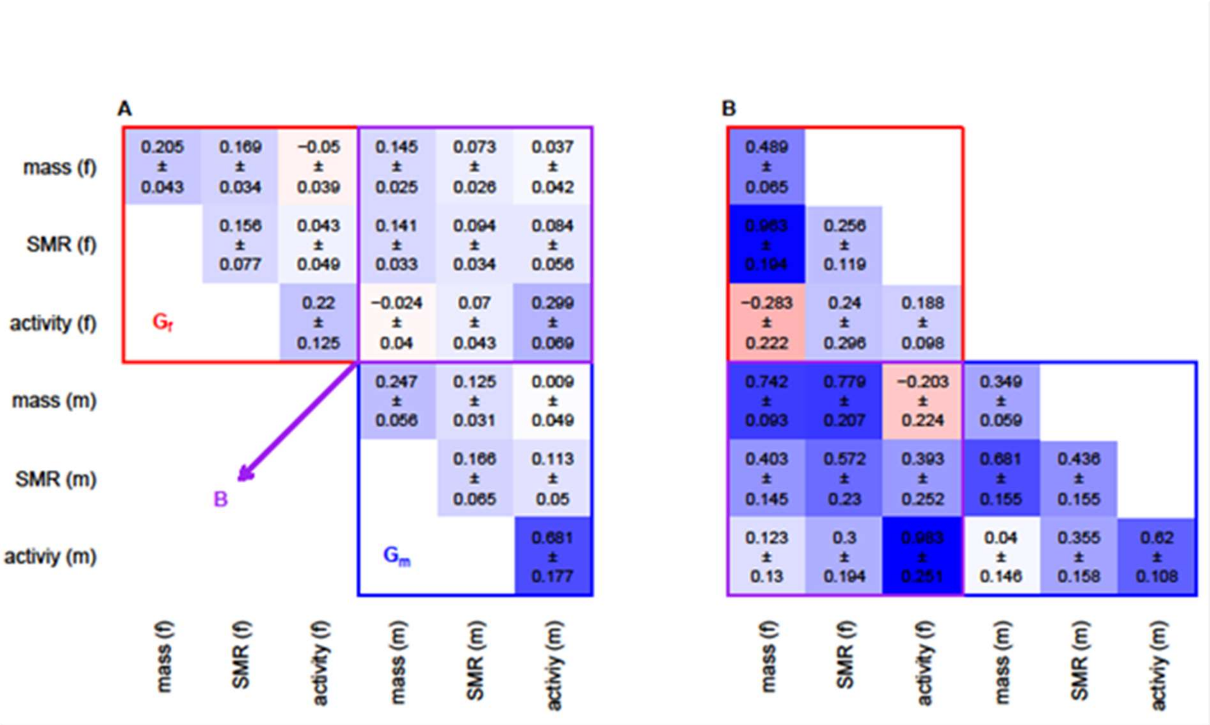
Correlation	Trait 1	Trait 2	Estimate	SE	LRT-0		LRT-1	
					χ^2	P	χ^2	P
additive genetic (r_A) - females	*Female mass*	*Female SMR*	0.963	0.194	**37.464**	**<0.001**	0.033	0.856
additive genetic (r_A) - females	*Female mass*	*Female activity*	-0.283	0.222	1.549	0.213	**25.355**	**<0.001**
additive genetic (r_A) - females	*Female SMR*	*Female activity*	0.24	0.296	0.643	0.423	**5.384**	**0.02**
additive genetic (r_A) - males	*Male mass*	*Male SMR*	0.681	0.155	**19.339**	**<0.001**	2.435	0.119
additive genetic (r_A) - males	*Male mass*	*Male activity*	0.04	0.146	0.073	0.787	**43.98**	**<0.001**
additive genetic (r_A) - males	*Male SMR*	*Male activity*	0.355	0.158	**5.128**	**0.024**	**8.05**	**0.005**
cross-sex (r_A) - r_{fm}	*Female mass*	*Male mass*	0.742	0.093	**42.008**	**<0.001**	**9.085**	**0.003**
cross-sex (r_A) - r_{fm}	*Female SMR*	*Male SMR*	0.572	0.23	**8.011**	**0.005**	2.595	0.107
cross-sex (r_A) - r_{fm}	*Female activity*	*Male activity*	0.983	0.251	**22.354**	**<0.001**	0.005	0.945
cross-sex cross-trait r_A	*Female SMR*	*Male mass*	0.779	0.207	**20.924**	**<0.001**	1.466	0.226
cross-sex cross-trait r_A	*Female activity*	*Male mass*	-0.203	0.224	0.843	0.359	**21.628**	**<0.001**
cross-sex cross-trait r_A	*Female mass*	*Male SMR*	0.403	0.145	**8.184**	**0.004**	**7.845**	**0.005**
cross-sex cross-trait r_A	*Female activity*	*Male SMR*	0.393	0.252	2.515	0.113	**4.94**	**0.026**
cross-sex cross-trait r_A	*Female mass*	*Male activity*	0.123	0.13	0.848	0.357	**46.23**	**<0.001**
cross-sex cross-trait r_A	*Female SMR*	*Male activity*	0.3	0.194	2.582	0.108	**10.171**	**0.001**
residual (r_e) - females	*Female mass*	*Female SMR*	0.307	0.078	**9.385**	**0.002**	**45.132**	**<0.001**
residual (r_e) - females	*Female mass*	*Female activity*	0.123	0.096	1.555	0.212	**37.539**	**<0.001**
residual (r_e) - males	*Male mass*	*Male SMR*	0.154	0.094	2.09	0.148	NA	NA
residual (r_e) - males	*Male mass*	*Male activity*	0	0.122	0	0.996	NA	NA

Figure legends

Figure 4.1. (A) Additive genetic (co)variance matrix from a sex-specific 6×6 multivariate animal model featuring body mass, SMR, and general locomotor activity in male (m) and female (f) *D. melanogaster*. Additive genetic variances (V_A ± SE) are on the diagonal and additive genetic covariances (± SE) are on the off-diagonals. (B) Additive genetic correlation matrix from the same 6×6 multivariate model, with narrow-sense heritabilities on the diagonal and genetic correlations (r_A) on the off diagonals (± SE). Color gradient reflects the magnitudes of the estimates (red for strongly negative, blue for strongly positive, white for near zero).

Figure 4.2. (A) Narrow-sense heritabilities (h^2) for body mass, standard metabolic rate (SMR), and general locomotor activity in female (red dots) and male (blue triangles) *D. melanogaster*. Inset are the frequencies distributions of trait values showing phenotypic sexual dimorphism in (B) general locomotor activity, (C) SMR, and (D) body mass of females (red) and males (blue). Each trait was sexual dimorphic (activity: F = 6.90, *df* = 1, P= 0.014; SMR:F = 3356, *df* = 1, P<0.001; and for body mass : F = 13400, *df* = 1, P<0.001). Plots of the additive genetic breeding values (BV) extracted from the 6×6 multivariate animal model, for (E) general locomotor activity, (F) SMR, and (G) body mass, showing their respective cross-sex genetic correlations (r_{fm} ± se).

Figure 4.3. Bi-variate plots of the additive genetic breeding values (BV) for body mass, standard metabolic rate (SMR), and locomotor activity in female (left column) and male (right column) *D. melanogaster,* depicting the additive genetic correlations (r_A ± SE) among these traits.



A

	mass (f)	SMR (f)	activity (f)	mass (m)	SMR (m)	activiy (m)
mass (f)	0.205 ± 0.043	0.169 ± 0.034	−0.05 ± 0.039	0.145 ± 0.025	0.073 ± 0.026	0.037 ± 0.042
SMR (f)		0.156 ± 0.077	0.043 ± 0.049	0.141 ± 0.033	0.094 ± 0.034	0.084 ± 0.056
activity (f)			0.22 ± 0.125	−0.024 ± 0.04	0.07 ± 0.043	0.299 ± 0.069
mass (m)				0.247 ± 0.056	0.125 ± 0.031	0.009 ± 0.049
SMR (m)					0.166 ± 0.065	0.113 ± 0.05
activiy (m)						0.681 ± 0.177

B

	mass (f)	SMR (f)	activity (f)	mass (m)	SMR (m)	activiy (m)
	0.489 ± 0.065					
	0.963 ± 0.194	0.256 ± 0.119				
	−0.283 ± 0.222	0.24 ± 0.296	0.188 ± 0.098			
	0.742 ± 0.093	0.779 ± 0.207	−0.203 ± 0.224	0.349 ± 0.059		
	0.403 ± 0.145	0.572 ± 0.23	0.393 ± 0.252	0.681 ± 0.155	0.436 ± 0.155	
	0.123 ± 0.13	0.3 ± 0.194	0.983 ± 0.251	0.04 ± 0.146	0.355 ± 0.158	0.62 ± 0.108

Figure 4.1.

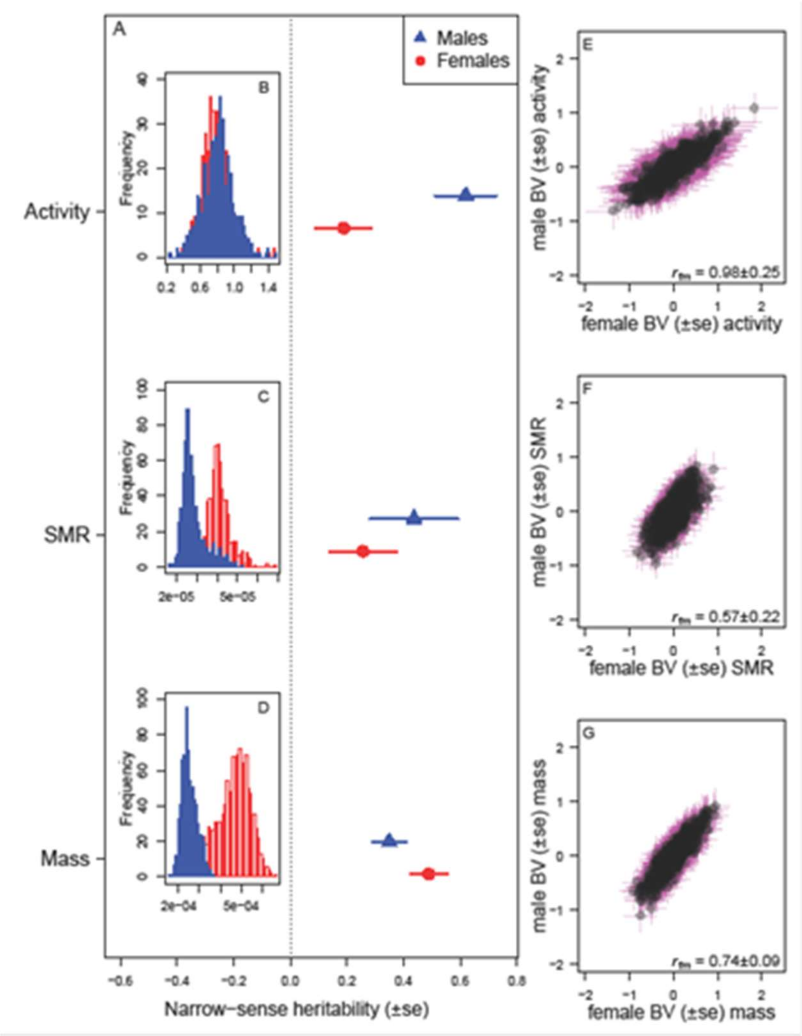

A
Males
Females
Activity
SMR
Mass
B
C
D
Frequency
0.2 0.6 1.0 1.4
2e−05 5e−05
2e−04 5e−04
−0.6 −0.4 −0.2 0.0 0.2 0.4 0.6 0.8
Narrow−sense heritability (±se)
E
F
G
male BV (±se) activity
female BV (±se) activity
r_{fm} = 0.98±0.25
male BV (±se) SMR
female BV (±se) SMR
r_{fm} = 0.57±0.22
male BV (±se) mass
female BV (±se) mass
r_{fm} = 0.74±0.09

Figure 4.2.

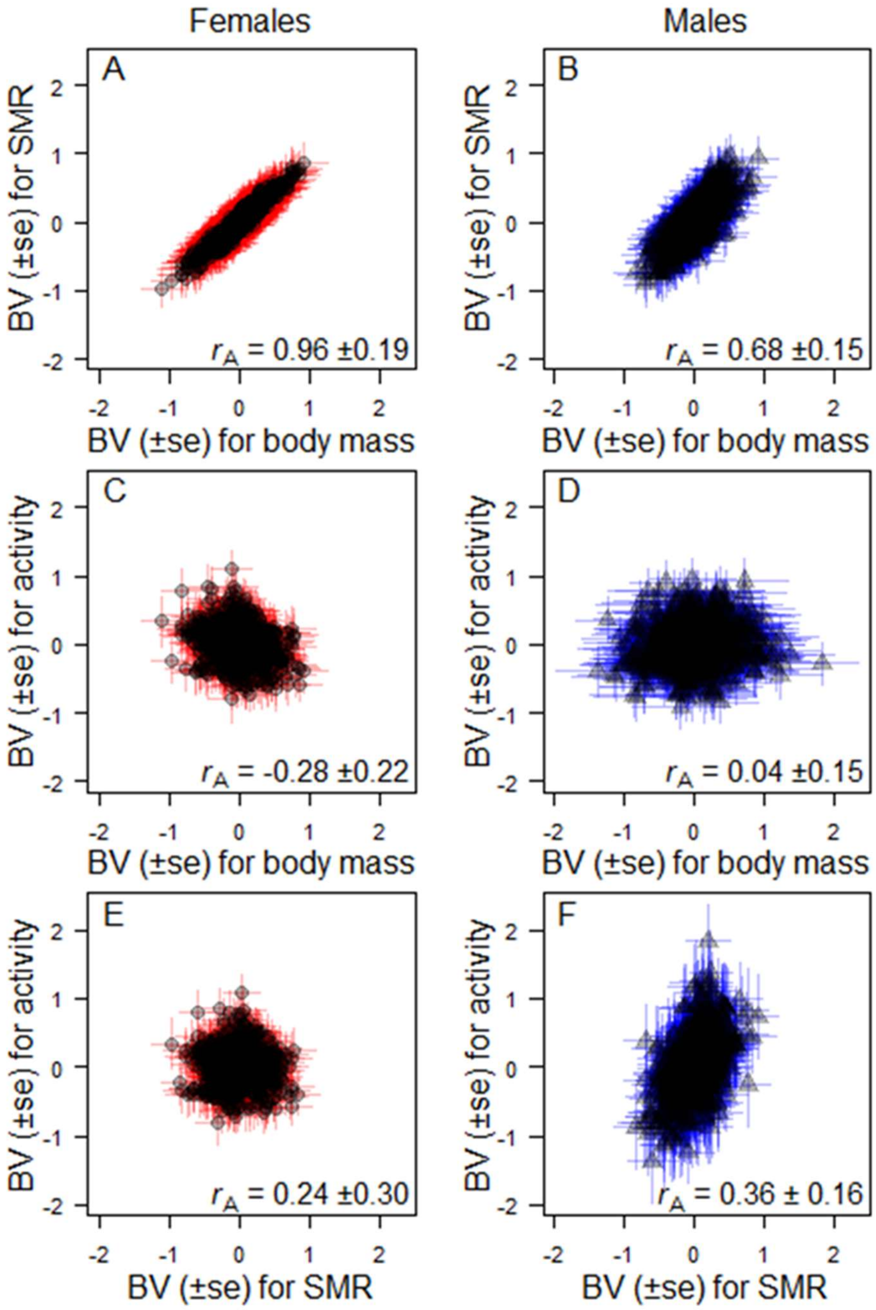

Females
Males
A
B
C
D
E
F
BV (±se) for SMR
BV (±se) for body mass
BV (±se) for activity
-2
-1
0
1
2
r_A = 0.96 ±0.19
r_A = 0.68 ±0.15
r_A = -0.28 ±0.22
r_A = 0.04 ±0.15
r_A = 0.24 ±0.30
r_A = 0.36 ± 0.16

Figure 4.3.

Chapter 4: Supplementary information

Table S4.1. Complete list of random effects estimates from the 6×6 multivariate animal model with standardized (mean = 0, variance = 1) body mass, standard metabolic rate (SMR), and locomotor activity fitted as response variables separately for females and males, including measurement unit (V_{MAVEn}; fitted to SMR only), common environment variance (V_{CE}), additive genetic variance (V_{A}), and residual variance (V_e). Additive genetic correlations (r_{A}) were fitted between all six traits, whereas residual correlations (r_e) were only estimable between mass and SMR and mass and activity within each sex.

	component	SE	z.ratio	bound
V_{MAVEn} - female SMR	0.008	0.012	0.658	P
V_{MAVEn} - male SMR	0.043	0.016	2.680	P
V_{CE} - female mass	0.000	NA	NA	B
V_{CE} - female SMR	0.045	0.039	1.150	P
V_{CE} - female activity	0.000	NA	NA	B
V_{CE} - male mass	0.000	NA	NA	B
V_{CE} - male SMR	0.010	0.030	0.325	P
V_{CE} - male activity	0.000	NA	NA	B
V_{A} - female mass	0.189	0.030	6.293	P
V_{A} - female SMR	0.160	0.077	2.079	P
V_{A} - female activity	0.163	0.087	1.869	P
V_{A} - male mass	0.194	0.036	5.317	P
V_{A} - male SMR	0.173	0.066	2.633	P
V_{A} - male activity	0.533	0.111	4.782	P
r_{A} - female SMR - female mass	0.963	0.194	4.960	U
r_{A} - female activity - female mass	-0.283	0.222	-1.274	U
r_{A} - female activity - female SMR	0.240	0.296	0.811	U
r_{A} - male mass - female mass	0.742	0.093	7.963	U
r_{A} - male mass - female SMR	0.779	0.207	3.766	U
r_{A} - male mass - female activity	-0.203	0.224	-0.906	U
r_{A} - male SMR - female mass	0.403	0.145	2.786	U
r_{A} - male SMR - female SMR	0.572	0.230	2.491	U
r_{A} - male SMR - female activity	0.393	0.252	1.558	U
r_{A} - male SMR - male mass	0.681	0.155	4.391	U
r_{A} - male activity - female mass	0.123	0.130	0.946	U
r_{A} - male activity - female SMR	0.300	0.194	1.551	U
r_{A} - male activity - female activity	0.983	0.251	3.909	U
r_{A} - male activity - male mass	0.040	0.146	0.272	U
r_{A} - male activity - male SMR	0.355	0.158	2.246	U
V_e - female mass	0.198	0.023	8.680	P
V_e - female SMR	0.419	0.055	7.672	P
V_e - female activity	0.705	0.090	7.842	P
V_e - male mass	0.362	0.031	11.613	P
V_e - male SMR	0.214	0.041	5.269	P
V_e - male activity	0.327	0.087	3.763	P
r_e - female SMR - female mass	0.307	0.078	3.917	U
r_e - female activity - female mass	0.123	0.096	1.282	U
r_e - male SMR - male mass	0.154	0.094	1.639	U
r_e - male activity - male mass	0.000	0.122	0.000	U

Table S4.2. Sources of variation of body mass (MAS), standard metabolic rate (SMR), and locomotor activity (ACT) in female (F) and male (M) *Drosophila melanogaster*, as estimated in the 6×6 multivariate animal model. Shown are estimate of each fixed effect with their standards errors (SE), denominator degrees of freedom (df_{den}; numerator $df = 1$ in all cases, except for Block, Monitor and Age), conditional Wald-*F* statistic, and *P*-values. Estimates for fixed effects with more than one level are not shown (…). Bold values denote significance ($P < 0.05$).

Source	Trait	Sex	Estimate	SE	df_{den}	*F(con)*	*P*
Intercept	MAS	F	-2.129	0.102	**131.2**	**4.642**	**<0.001**
	SMR	F	-0.492	0.145			
	ACT	F	-0.568	0.661			
	MAS	M	0.344	0.117			
	SMR	M	-0.269	0.150			
	ACT	M	-0.733	0.632			
Block	MAS	F	…	…	184.1	20.530	**<0.001**
Block	SMR	F	…	…	109.8	1.759	0.114
Block	**ACT**	**F**	**…**	…	**118.0**	**9.672**	**<0.001**
Block	**MAS**	**M**	**…**	…	**177.6**	**27.970**	**<0.001**
Block	SMR	M	…	…	107.3	1.249	0.287
Block	**ACT**	**M**	**…**	…	**139.4**	**9.750**	**<0.001**
Age	**MAS**	**F**	**…**	…	**931.1**	**237.100**	**<0.001**
Age	**SMR**	**F**	**…**	…	**110.3**	**17.670**	**<0.001**
Age	**ACT**	**F**	**…**	…	**219.5**	**6.485**	**<0.001**
Age	**MAS**	**M**	**…**	…	**722.3**	**19.230**	**<0.001**
Age	**SMR**	**M**	**…**	…	**104.5**	**10.560**	**<0.001**
Age	ACT	M	…	…	221.9	0.970	0.408
Mass category (SMR)	MASS	F	-0.007	0.035	1136.4	0.041	0.840
Mass category (SMR)	**MAS**	**M**	**-1.122**	**0.043**	**1174.1**	**692.400**	**<0.001**
Monitor (1)	ACT	F	…	…	539.6	0.014	0.986
Monitor (1)	ACT	M	…	…	479.7	2.662	0.071
Water Vapor	**SMR**	**F**	**0.142**	**0.042**	**87.4**	**11.520**	**0.001**
Water Vapor	SMR	M	0.001	0.045	100.5	0.000	0.988
Temperature	**SMR**	**F**	**0.184**	**0.044**	**88.2**	**17.300**	**<0.001**
Temperature	**SMR**	**M**	**0.142**	**0.046**	**110.3**	**9.547**	**0.003**
Activity during measure	**SMR**	**F**	**0.315**	**0.038**	**677.5**	**68.010**	**<0.001**
Activity during measure	**SMR**	**M**	**0.325**	**0.030**	**605.9**	**117.500**	**<0.001**
Activity before measure	**SMR**	**F**	**0.319**	**0.037**	**658.8**	**73.130**	**<0.001**
Activity before measure	**SMR**	**M**	**0.415**	**0.031**	**648.0**	**173.400**	**<0.001**

CHAPTER 5: GENERAL DISCUSSION

The central aim of my thesis was to understand among-individual variation in SMR in *Drosophila melanogaster*, including the extent to which additive genetic variation contributes to this, how selection acts on this variance, whether and how it associates with variation in locomotor activity, and whether all of these differ between females and males. Moreover, a general theme emerged in my thesis featuring the use of a multivariate approach in an attempt to dissociate SMR and body mass to better understand how selection acts on these traits and their genetic architecture.

In the second chapter, by repeatedly measuring the metabolic rate of multiple females and males, I estimated a moderate repeatability for RMR (resting metabolic rate, which is closely related to, but not SMR because not all individuals were virgin) in both sexes. I demonstrated that body mass, sex and reproductive status contributed to the among-individual variation of RMR. In parallel, I also measured general locomotor activity and showed that the among-individual phenotypic correlation between RMR and locomotor activity varied throughout the day and between the sexes. Results from this chapter suggest fundamental differences in how males and females manage their energy budget.

The presence of among-individual variation is one of the requirements for a trait to respond to selection. Thus, in the third chapter, I examined the covariance between the relative fitness and SMR in both sexes. I took advantage of a *Drosophila* laboratory population, adapted to a specific life cycle within a complex environment, to measure individual lifetime reproductive success; this is the same population I used in my other chapters. A multivariate approach was employed in the attempt to dissociate phenotypic selection on SMR from that on body mass, and to estimate both linear and nonlinear (including correlation) selection. In addition, I used a different multivariate formulation of the Lande and Arnold (1983) statistical approach to quantify selection. My approach estimated the residual covariance between fitness, SMR and body mass after accounting for various nuisance variables that were unique to each trait thereby avoiding a stats-on-stats approach. In males, linear selection differentials on SMR and body mass were positive and significant, but selection gradients were not significant due to the presence of a strong collinearity between SMR and body mass despite my substantial sample sizes. In females, nonlinear selection was detected, but also non-significant, again likely due in large part to the effects of collinearity. This result suggests that fitness surfaces differed between sexes, but this difference is unclear because males and females did not share the same phenotypic space.

For a response to selection to occur, a trait also requires the presence of genetic variance. Thus, in the fourth chapter, I estimated the additive genetic variance of SMR using a paternal half-sibling breeding design. To better understand the multivariate genetic architecture of SMR, I also measured two other relevant traits, body mass and the general locomotor activity, which have been shown to covary with SMR in my previous chapters. This allowed me to adopt a multivariate approach that considered the genetic (co)variance of SMR, body mass, and activity. To provide insight into how the genetic architecture differed between sexes, I also estimated a fully sex-specific genetic (co)variance matrix, composed of the genetic (co)variance matrix among these traits with each sex, along with the between-sex genetic (co)variance matrix. My results suggest the presence of additive genetic variances for all traits and some difference between males and females in their genetic architecture. Importantly, my results reveal that the majority of the additive genetic variation in SMR is shared with mass in both sexes, again highlighting the challenge of differentiating these traits. In addition, substantial genetic variation was shared between the sexes for all three traits, generating positive and strong cross-sex genetic correlations which indicated the potential for intralocus sexual conflict in this population.

Overall, my results provide further support to the possibility of sex-specific energy management strategies that may contribute to intralocus sexual conflict in this population. Ultimately, to confirm the presence of sexual conflict, sex-specific selection should be estimated at the genetic level. Indeed, the pedigreed population from the fourth chapter was also phenotyped for female and male fitness, following a similar protocol to that in chapter three (two males and two females from each half-sib family were assayed for fitness). The inclusion of fitness complicates the statistical analyses and interpretation of the results, and time constraints prevented me from included this in my thesis. Nevertheless, doing so is an important next step and I provide some discussion, and highlight some potential issues, with doing so here.

When trait and fitness data are available for a pedigreed population , genetic variance in fitness, and selection at the genetic level, can be estimated. According to secondary theorem of selection (STS, Morrissey et al. 2010), the predicted evolutionary response to selection of the trait (z) can be estimated as the genetic covariance between relative fitness and the trait, and this represents the genetic selection differential:

Equation 1. $$\Delta\bar{z} = \sigma_{A_{(z,w)}} = s_g,$$

where $\Delta\bar{z}$ is the predicted change in the mean trait of z, $\sigma_{A_{(z,w)}}$ is the genetic covariance between relative fitness (w) and z, and s_g is the genetic selection differential on z. Equation 1 can also be further developed into a multivariate approach to dissociate direct and indirect selection (Stinchcombe et al. 2014).

Equation 2. $$\beta_g = \boldsymbol{G}^{-1} s_g$$

where β_g is the vector of the genetic selection gradients and $\boldsymbol{G}^{-1}$ is the inverse of the genetic (co)variance matrix of the traits excluding relative fitness. This approach is more accurate than the classic phenotypic selection gradients approach (Lande and Arnold 1983) because it eliminates any possible problems of environmentally-induced covariance between the fitness and the traits (Stinchcombe et al. 2014). By expanding this to include the relative fitness for males and females, and also treating the other traits as sex-specific (i.e. as separate traits in males and females as in my fourth chapter), one could estimate the predicted evolutionary response for SMR, body mass, and general locomotor activity in both males and females from selection in both sexes. However, three possible issues may arise by using this method: a possible lack of genetic variance for the relative fitness may prevent the estimation of covariances; the estimation of a larger and more complex genetic matrix requires a large sample size; and the predicted evolutionary responses are estimated without uncertainty. Preliminary analyses have revealed the presence of additive genetic variance for relative fitness in both sexes. To estimate the uncertainty around the estimate of selection, a Bayesian approach can be used (O'Hara et al. 2008). Alternatively, a bootstrap approach would also be possible, although this must take the hierarchical structure of the dataset into account. Finally, interpretation of the resulting sex-specific matrix and the predicted evolutionary responses of the traits may be challenging, as it features additional quantities that have not been considered to date in the literature (e.g., the covariance of traits in one sex with fitness in the other sex). However, such an approach should provide comprehensive insight into whether and how selection differs between the sexes and the extent of sexual conflict for these traits. Then, and only then, I will be able to confidently confirm the presence or absence of a sexual conflict in the *Drosophila* population.

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