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Sex-specific genetic (co)variances of standard metabolic rate, body mass and locomotor activity in *Drosophila melanogaster*

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Abstract

A longstanding focus in evolutionary physiology concerns the causes and consequences of variation in maintenance metabolism. Insight into this can be gained by estimating the sex-specific genetic architecture of maintenance metabolism alongside other, potentially correlated traits on which selection may also act, such as body mass and locomotor activity. This may reveal potential genetic constraints affecting the evolution of maintenance metabolism. Here, we used a half-sibling breeding design to quantify the sex-specific patterns of genetic (co)variance in standard metabolic rate (SMR), body mass and daily locomotor activity in *Drosophila melanogaster*. There was detectable additive genetic variance for all traits in both sexes. As expected, SMR and body mass were strongly and positively correlated, with genetic allometry exponents ($b_A \pm SE$) that were close to 2/3 in females (0.66 ± 0.16) and males (0.58 ± 0.32). There was a significant and positive genetic correlation between SMR and locomotor activity in males, suggesting that alleles that increase locomotion have pleiotropic effects on SMR. Sexual differences in the genetic architecture were largely driven by a difference in genetic variance in locomotor activity between the sexes. Overall, genetic variation was mostly shared between males and females, setting the stage for a potential intralocus sexual conflict in the face of sexually antagonistic selection.

KEYWORDS

basal metabolic rate, Diptera, energetics, **G**-matrix, sexual dimorphism

1 | 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 studies (Brown et al., 2004; Réale et al., 2010; Ricklefs & Wikelski, 2002). Maintenance metabolism reflects the energy invested in body maintenance (e.g. cellular turnover), and this represents a large proportion of an individual's total energy budget. Maintenance metabolism determines the standard or basal metabolic rate (SMR/BMR) in ectotherms and endotherms, respectively, and is quantified as the minimal energy expenditure of a resting, post-absorptive, nonreproductive, adult at a given temperature during the inactive part of the diurnal cycle. Thus, maintenance metabolism can be thought of as the 'idling cost' of an individual's metabolic machinery. SMR and BMR are quite variable within (Videlier et al., 2019; White et al., 2013) and among species, and understanding the causes and consequences of this variation is a major focus in evolutionary physiology (Burton et al., 2011; Pettersen et al., 2018).

Numerous comparative studies have shown that metabolic rate (SMR, BMR and related traits) correlates with ecological factors such as ambient temperature (Clarke & Fraser, 2004; Schulte, 2015). Differences in maintenance metabolism that correlate with biotic and abiotic factors suggest adaptive divergence, a prerequisite of which is 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. As expected for such a labile trait that is challenging to measure (Lighton & Halsey, 2011), h^2 estimates are generally moderate to low (Pettersen et al., 2018; White & Kearney, 2013), but do indicate the presence of V_A.

Selection, however, rarely acts on single traits in isolation (Lande & Arnold, 1983; Walsh & Blows, 2009), and a comprehensive understanding of the genetic basis of SMR therefore goes beyond the simple existence of V_A. Additional insight can be gained by estimating the genetic basis of SMR alongside other, potentially correlated traits on which selection may also act (i.e. the additive genetic (co) variance matrix, **G**). 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, and it is a likely target of selection itself. To account for its strong correlation with mass, past studies generally consider mass-specific metabolic rates by dividing SMR by mass, or condition metabolic rate on mass by including it as a covariate. However, by treating mass as a separate trait (Hagmayer et al., 2020; Tieleman et al., 2009), V_A can be estimated in both SMR and body mass, as well as their additive genetic correlation (r_A). Selection can also be quantified for each trait on its own, correlational selection between the traits can be considered (Videlier et al., 2021), and the response to selection can be partitioned into direct and correlated effects (Lande & Arnold, 1983) although collinearity arising from a strong correlation between these traits may require substantial sample sizes. Modelling (co)variances between mass and SMR also allows the allometry of metabolic traits to be quantified at the additive genetic level, which may be useful in linking micro- and macro-evolutionary patterns in metabolic allometry (Beaman et al., 2020; Glazier, 2005).

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

$$\mathbf{G}_{fm} = \begin{bmatrix} \mathbf{G}_f & \mathbf{B} \\ \mathbf{B}^T & \mathbf{G}_m \end{bmatrix}. \quad (1)$$

Here, $\mathbf{G}_f$ and $\mathbf{G}_m$ are submatrices quantifying the genetic (co)variances for the traits in females and males, respectively, and **B** is a submatrix of cross-sex (or 'between-sex') genetic covariances both within and between traits ('within-trait' cross-sex covariances on the diagonal, 'between-trait' cross-sex covariances on the off-diagonal of **B**). $\mathbf{B}^T$ is the transpose of **B**. **B** 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 close or equal to one, and a response to selection in one sex will produce a correlated response in the other sex in the same direction. A positive r_{fm} constrains the response to sexually antagonistic selection on a trait, generating intralocus conflict that hampers the evolution of sexual dimorphism and favours mechanisms that decrease r_{fm} (Bonduriansky & Chenoweth, 2009). Consistent with this, r_{fm} is negatively correlated with the magnitude of sexual dimorphism across traits (Poissant et al., 2010). Although estimates of r_{fm} exist for various morphological (Poissant et al., 2008; Sztepanacz & Houle, 2019; Tarka et al., 2014; Turk et al., 2018) and behavioural (Han & Dingemanse, 2017; Kralj-Fišer et al., 2019; Long & Rice, 2007) traits, there are few examples for metabolic rate (Jumbo-Lucioni et al., 2010; but see, Boratyński et al., 2013). The other elements of **B**, consisting of the cross-sex between-trait covariances, quantify the genetic relationship between one trait in males and another trait in females. Overall, **B** shapes correlated responses to selection between the sexes, affecting trait evolution and the evolution of sexual dimorphism (Cox et al., 2017; Gosden et al., 2012; Ingleby et al., 2014; Rapkin et al., 2017).

Additional insight into the genetic architecture of SMR may be gained by considering its covariance with other energetically expensive traits that are likely to be under selection and hence may constrain or otherwise alter its evolution. Locomotor activity is one example. If the metabolic machinery that supports locomotor activity creates an 'idling cost' in terms of increased SMR (a scenario referred to as the 'performance model' of energy management, Careau et al., 2008), then locomotor activity should correlate positively with SMR. For example, sustaining high levels of energy expenditure may require larger digestive organs (to process food faster and/or extract more energy) that have high mass-specific metabolic rates, and this would be reflected by a higher energy turnover at rest (i.e. higher SMR). By contrast, the allocation model posits that, because SMR constitutes a relatively large portion of an individual's energy budget, investment in it may come at the cost of reduced energy available for other energetically expensive traits, generating negative correlations between them (Careau et al., 2008). Considerable attention has been given to testing the contrasting expectations of these models over the past decade (Mathot & Dingemanse, 2015; Mathot et al., 2019), with mixed results at the phenotypic level. For example, a recent study found that, while SMR and locomotor activity were positively correlated among male *D. melanogaster*, the correlation was negative in females (Videlier et al., 2019). The joint consideration of SMR, body mass and locomotor activity in a sex-specific genetic analysis provides insight into the shared genetic

basis of these key traits that affect energy budgets, and doing so is an important step in understanding SMR evolution and divergence.

We quantify SMR, body mass and daily locomotor activity via a half-sibling quantitative genetic breeding design in *D. melanogaster*. It is easy to obtain a large pedigreed set of *Drosophila* and to control environmental conditions while phenotyping, but their small size and hence low metabolic rate make it challenging to precisely measure SMR on large numbers of single individuals as needed for multivariate quantitative genetics studies. While a few broad-sense heritability estimates exist for *Drosophila* metabolic rate (Alton et al., 2017; Jumbo-Lucioni et al., 2010; Khazaeli et al., 2005; Matoo et al., 2019; Montooth et al., 2003), very little attention has been given to estimates of V_A and we are aware of only a single study that quantified h^2 of resting metabolic rate (i.e. metabolic rate when not all of the conditions for SMR were met; Castañeda & Nespolo 2013). Overall, our estimates reveal V_A for all traits (SMR, body mass and locomotor activity) in both sexes, some differences in genetic architecture driven in large part by a difference in V_A in locomotor activity between the sexes, and substantial shared genetic variation between males and females that reveal the potential for ongoing intralocus sexual conflict.

2 | METHODS

2.1 | Study 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 a.m./p.m.) 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 stock has a specific life cycle in which egg-to-adult development occurs 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 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* laboratory populations. Males were discarded after the mating phase and surviving females were allowed to lay eggs for 24 hr in vials to create the next generation, after which they were also discarded.

2.2 | Breeding design

A paternal half-sibling breeding design was performed on a laboratory-adapted *D. melanogaster* population in which 125 virgin males (sires) were each mated to either four (N = 108) or two (N = 17) virgin females (dams) for 48 hr. These females subsequently oviposited for 24 hr in separate vials. Offspring were collected from an average of 2.86 dams/sire (range = 2–3). For logistic reasons, the breeding design was performed over seven blocks of 16–20 unique 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 locomotor activity (hereafter 'activity') following Videlier et al. (2019). SMR was measured on individual flies via a 64-chamber flow-through respirometry system over a 12-hr period overnight (from ~19:00 to ~07:00), when locomotor activity is lowest (for details, see supplemental materials). Locomotor activity was measured on individual flies over a 22-hr period as the number of movements across a detector beam in a narrow chamber (cleaned polycarbonate tubes; 65 mm long, 5 mm diameter) in which the fly could only walk (for details, see supplemental materials). Although the use of a single beam to detect activity unavoidable underestimate activity, activity scores produced by the DAM2 activity monitors (Trikinetics, Waltham, MA, USA) are usually strongly correlated with total distance travelled (Gilestro, 2012). The metabolic and locomotor activity assays were conducted in separate incubators with a regulated temperature of 25 ± 1°C (matching those of the stock) that was undisturbed for the duration of the recording . After metabolic or activity measurements, flies were dried at 50°C overnight and dry body mass was subsequently measured using a MX5 microbalance (Mettler Toledo, Columbus, OH). Due to occasional deaths or escapes, 182 individuals did not have a mass measurement paired with their SMR or activity measurement, while equipment malfunction resulted in 193 individuals not having a SMR or activity measurement (but had their mass measured). These individuals were nevertheless kept in the analysis, for a total of 2,664 individuals (1,334 females and 1,330 males) included in our final data set (available on Dryad at https://doi.org/10.5061/dryad.j3tx95xd0).

2.3 | Statistical analyses

Activity was $\log_{10}$-transformed, and then, all continuous variables were standardized to a mean = 0 and variance = 1. ASReml-R (Butler et al., 2018) was used to fit multivariate animal models with SMR, activity and body mass as response variables. Preliminary analysis showed that partitioning the additive genetic matrices separately by sex greatly improved the fit over a model that pooled the sexes (likelihood ratio test, LRT: $\chi^2_6 = 956.9$, $p < .0001$), so all subsequent analyses fit 6 × 6 models in which SMR, mass and activity were treated as separate traits in males and females, thereby estimating the full $\mathbf{G}_{fm}$ (Equation 1).

All models included block and age as categorical, fixed effects fitted to all traits. We also fitted other potential nuisance variables specific to a subset of the traits including 'monitor identity' for 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.

For SMR, we included fixed effects of temperature, flow rate, water vapour, light intensity, and average activity during and immediately prior to the metabolic measurement (ACT_{20} and ACT_{20p}; log_{10}-transformed and standardized). Nonsignificant nuisance variables including fly density in the holding vials, light intensity and flow rate were excluded from the final models. Significance of fixed effects was evaluated using conditional Wald *F*-statistics (Table S1) employing Kenward and Roger (1997) denominator degrees of freedom (*df*).

All multivariate animal models included individual identity associated with the pedigree, allowing the phenotypic variance to be partitioned into additive genetic (V_A) and residual (V_R) variances (Wilson et al., 2010). Dam identity was 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 apparatus on a given day (i.e. separate levels for each day × unit combination; termed 'MAVEn_run'; Videlier et al. (2019)).

We estimated additive genetic and residual covariance matrices by specifying an unstructured general matrix ('us') at the additive genetic and residual levels. SMR and activity were measured in different individuals so their residual covariances (within and between sexes) cannot be estimated and were fixed to zero. Because most V_{CE} estimated were at the lower boundary of the parameter space (i.e. 0), we did not estimate covariances at that level (i.e. a 'diag' structure was used for dam identity; Table S2). To facilitate hypothesis testing and biological interpretation, we ran a second, identical model in which correlation matrices were estimated directly, thereby scaling each covariance relative to its associated trait variances. This was done by specifying an unstructured general correlation matrix ('corgh') at the additive genetic and residual levels, which yielded the same likelihood estimate as the covariance model.

Narrow-sense heritability was estimated for each sex-specific trait as $h^2 = V_A/V_P$, where V_P is the total phenotypic variance, calculated as the sum of V_A, V_{CE} and V_R. To assess the significance of genetic and residual correlations, we used separate LRTs with 1 *df* to compare an unconstrained 'corgh' model to one in which the correlation was fixed to 0. Similar LRTs were performed comparing the unconstrained 'corgh' model to a reduced model in which the correlation was fixed to 0.99999 with 0.5 *df* (to provide a one-tailed test given the correlation boundary of one, Dominicus et al., 2006). To visualize r_A, breeding values were extracted from the model as the best linear unbiased predictors (Houslay & Wilson, 2017). The overall importance of the cross-sex genetic covariances (i.e. **B**) was tested by comparing the fit of an unconstrained model to one in which all covariances in **B** were fixed to 0 using an LRT with 9 *df* Potential asymmetry in the upper versus lower 'triangles' of **B** was tested by comparing the unconstrained model to one in which the reciprocal trait correlations were forced to take on a single, shared value in an LRT with 3 *df*

Finally, 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 to estimate the proportional relationship between SMR and body mass, thus providing insight into metabolic allometry. The genetic (b_A) and residual (b_e) slopes between SMR and mass were calculated by dividing the genetic or residual covariance between SMR and mass by its associated variance in mass.

3 | RESULTS

3.1 | Within-sex genetic (co)variances (G_f and G_m)

A model estimating separate $\mathbf{G}_f$ and $\mathbf{G}_m$ matrices had a significantly better fit over a single, pooled **G** (see Methods); therefore, all of the following results come from a fully specified 6 × 6 model in which SMR, mass and activity were treated as separate traits in males and females. In males, there was substantial V_A for activity with a corresponding narrow-sense heritability ($h^2 \pm SE$) that was relatively high (0.620 ± 0.108), whereas in females V_A and heritability (0.188 ± 0.098) were noticeably lower (Figure 1a and S2). By contrast, moderate V_A was detected in both SMR and body mass and these differed much less between males and females, contributing to smaller differences in sex-specific h^2 estimates (Figures 1a and 2).

There was a strong, positive r_A (±*SE*) between SMR and body mass in females (0.963 ± 0.194), whereas this was somewhat weaker in males (0.681 ± 0.155; Figure 3a,b). By contrast, there was little evidence of a r_A between activity and body mass in either sex, although the point estimate in females was slightly negative (−0.283 ± 0.222; Figure 3c,d). The r_A between SMR and activity was moderate and positive in both sexes, although the point estimate in females was somewhat smaller, but also less precise (0.240 ± 0.296), than that in males (0.355 ± 0.156; Figure 3e,f).

3.2 | Cross-sex genetic covariances (B)

Allowing non-zero estimates of the within and between-trait cross-sex additive genetic covariances (i.e. **B**) significantly improved model fit (LRT: $\chi^2_9 = 71.25$, $p < .0001$), indicating shared V_A between males and females for one or more of these traits. All three r_{fm} were positive and significantly greater than zero (Table S3, Figure 1e-g). The r_{fm} (±*SE*) point estimate for activity was particularly high (0.983 ± 0.281) and did not differ significantly from one. Estimates for SMR and body mass were lower and differ significantly from 1, but both were still significantly positive (i.e. >0; Table S3).

The between-trait cross-sex r_As were generally positive although more variable and one point estimate were negative (between female activity and male mass), but not significantly so. Two estimates differed significantly from zero and several from one (Table S3). There was some indication of asymmetry between the lower and upper 'triangles' of **B**, but this was not significant (LRT: $\chi^2_3 = 1.90$, $p = .59$).

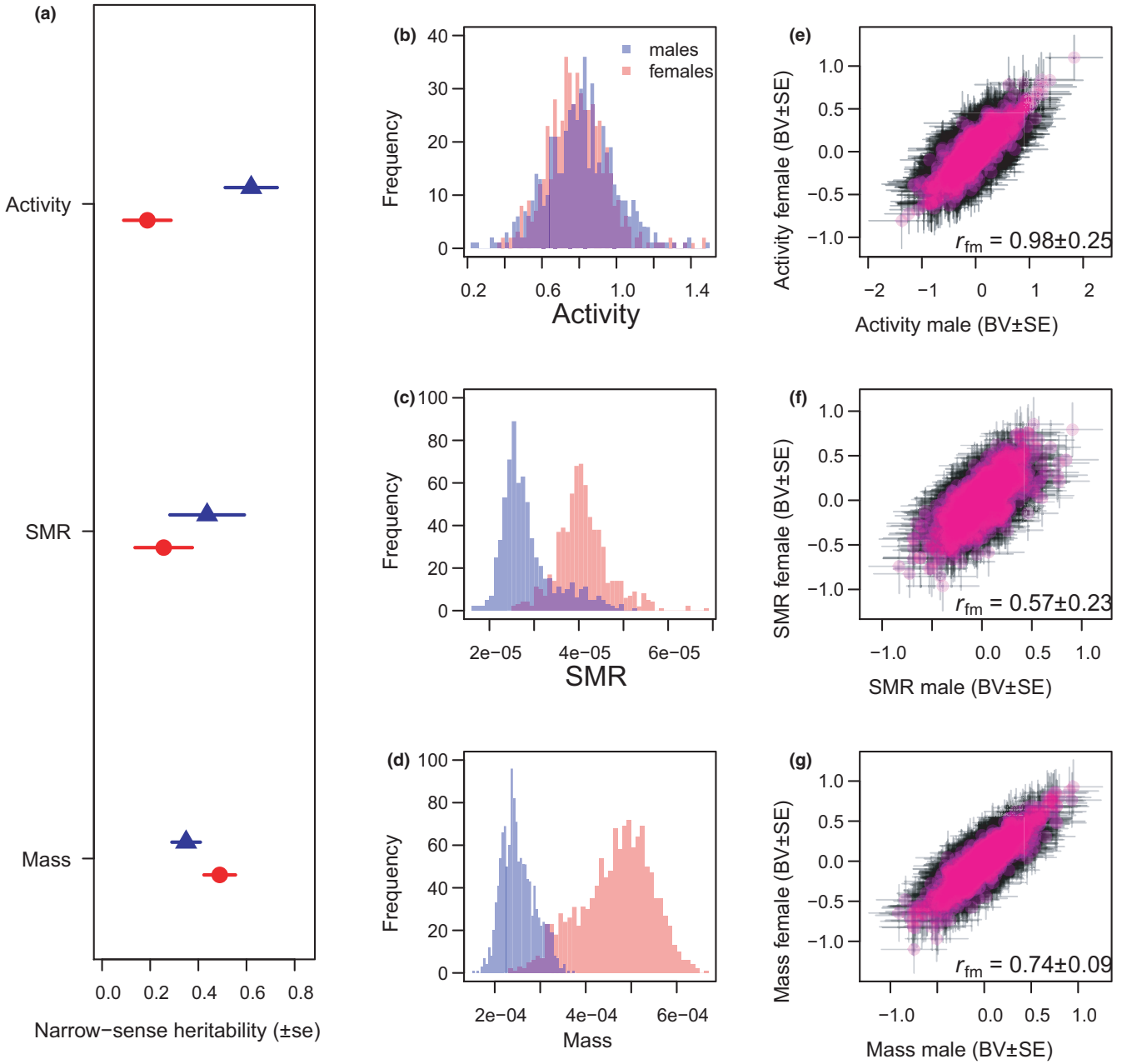

FIGURE 1 (a) Narrow-sense heritability for body mass, standard metabolic rate (SMR) and locomotor activity in female (red circles) and male (blue triangles) *Drosophila melanogaster*. Frequency distribution of (b) locomotor activity, (c) SMR and (d) body mass of females (red), males (blue) and overlap (purple). Sexual dimorphism was significant for each trait although varied in magnitude, being greatest for body mass (female mean 1.84× male mean; $F = 13{,}380$, $df_{den} = 2{,}246.6$, $p < .001$), intermediate for SMR (female mean 1.43× male mean; $F = 3{,}335$, $df_{den} = 1{,}116.9$, $p < .001$), and weakest, and in the opposite direction for activity (male mean 1.09× female mean; $F = 6.0$, $df_{den} = 871.9$, $p = .014$). Plots of the additive genetic breeding values (BV) extracted from the 6 × 6 multivariate animal model for (e) locomotor activity, (f) SMR and (g) body mass, showing their respective cross-sex genetic correlations ($r_{fm} \pm SE$)

3.3 | Allometric slopes

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

4 | DISCUSSION

A quantitative genetic study of a suite of traits provides insight into shared genetic variation and can reveal potential genetic constraints that can alter responses to selection. Further partitioning genetic variation between the sexes allows the construction of the sex-specific additive genetic (co)variance matrix ($\mathbf{G}_{fm}$) that includes

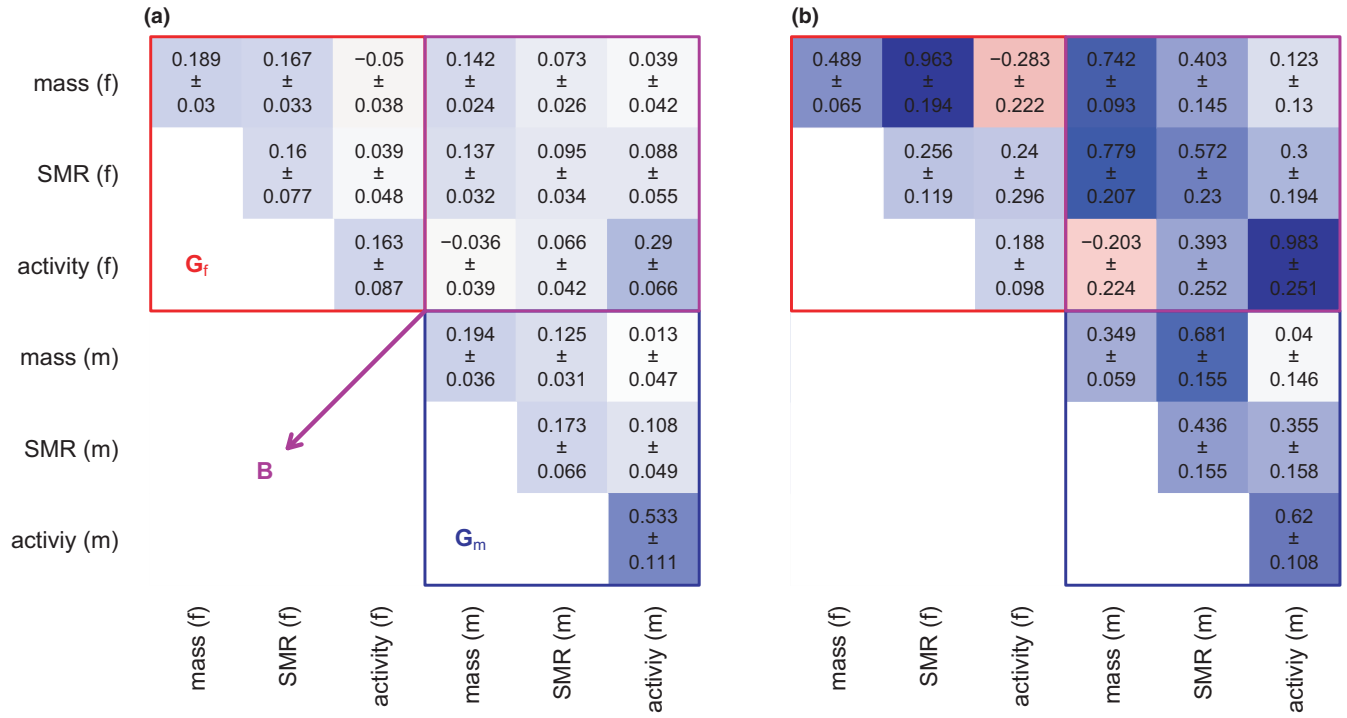

FIGURE 2 (a) Additive genetic (co)variance matrix from a sex-specific 6 × 6 multivariate animal model featuring body mass, standard metabolic rate (SMR) and locomotor activity in male (m) and female (f) *Drosophila melanogaster*. Additive genetic variances are on the diagonal, and additive genetic covariances (±*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 on the off-diagonals (±*SE*). Colour gradient reflects the relative magnitudes of the estimates (red for negative, blue for positive and white for near zero)

B, providing insight into shared genetic variation between males and females. Here, we examined the genetic (co)variances of SMR, 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 are likely to affect, and potentially constrain, evolutionary responses. Furthermore, females and males differed significantly in their genetic architecture, mainly driven by differences in V_A in activity.

4.1 | Within-sex genetic (co)variances: G_f and G_m

Little is known about the genetic basis of maintenance metabolism in ectotherms despite its fundamental importance in ecology and life-history studies (Réale et al., 2010; Ricklefs & Wikelski, 2002). The few V_A and h^2 estimates that exist are low to moderate and often nonsignificant (Pettersen et al., 2018). Moreover, these estimates are generally mass-specific (i.e. SMR divided by body mass) or mass-conditioned (i.e. by including body mass as a covariate) and thus confound the genetic bases of SMR and body mass. A multivariate approach allows estimates of the V_A for both traits, as well as their r_A with each other and with other ecologically (and hence evolutionary) relevant traits (Walsh & Blows, 2009). Here, r_As among SMR, body mass and activity suggest a great deal of shared V_A due to pleiotropy and/or linkage disequilibrium. Such shared V_A can generate severe genetic constraints because selection on one trait will result in indirect (i.e. correlated) response in the other trait.

Unsurprisingly, the r_A between SMR and body mass was positive and strong in both sexes, consistent with the few positive genetic association found between body mass and SMR (White et al., 2019). A shared genetic basis to variation in these traits will contribute to their correlated evolution and likely reflects, in part, the fundamental fact that it requires more energy to maintain a larger body. Although a strong r_A between SMR and body mass suggests strong pleiotropy (or linkage) and limited scope for independent evolution of the two traits, it is not directly informative with respect to allometric scaling of metabolic rate, a topic that has been intensively studied among species for a long time (Glazier, 2005). For maintenance metabolism, the interspecific scaling exponent usually ranges between a 2/3 to 3/4 power function, indicating that metabolic rate does not scale linearly with body mass (i.e. larger animals have a reduced maintenance cost per unit mass). Surprisingly, we are aware of no estimate of the allometric slope at the genetic level (b_A) within a population. Estimating b_A may help bridge micro- and macro-evolutionary approaches to studying allometry because, in a bivariate plane representing genetic variation in these two traits, b_A represents the genetic 'line of least resistance' along which populations/species are most likely to diverge (Schluter, 1996). Here, the genetic allometric slope in females (0.658 ± 0.159) and males (0.581 ± 0.324) more closely correspond to a 2/3 than a 3/4 scaling exponent. Interspecific allometry exponents in other flying insect taxa match

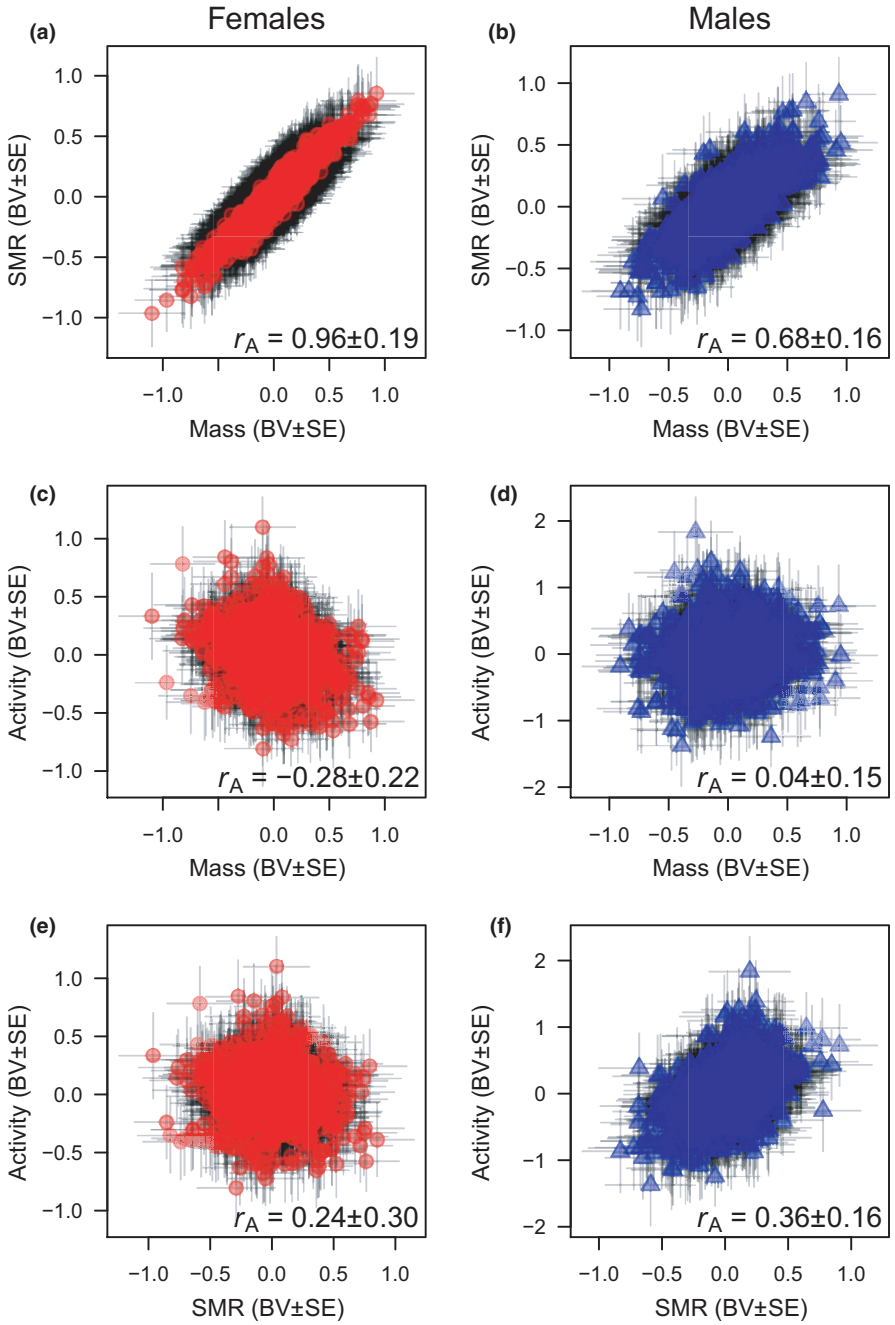

FIGURE 3 Bi-variate plots of the additive genetic breeding values (BV) for body mass, standard metabolic rate (SMR) and locomotor activity in female (left panels) and male (right panels) *Drosophila melanogaster*, depicting the additive genetic correlations ($r_A \pm SE$) among these traits

this specific scaling exponent (Niven & Scharlemann, 2005), suggesting that species divergence has occurred along the genetic line of least resistance.

Returning to genetic correlations, in males activity shared some V_A with SMR ($r_A = 0.355 \pm 0.158$), but not with body mass (0.040 ± 0.146). This is surprising given the extensive V_A shared between body mass and SMR, and it implies that it is the mass-independent component of variation in SMR that is shared with activity. A similar partitioning was not possible in females because essentially none of the V_A in SMR was independent of mass. The genetic independence of activity and body mass in males implies that selection on activity will not produce a correlated response in body mass (and vice versa), while selection on activity would alter SMR in the same direction. Metabolic rate and flight (another aspect of locomotor activity) were mapped to the same region on the third chromosome among males in another *D. melanogaster* population (Montooth et al., 2003), also suggesting the possibility of a shared genetic basis of SMR and activity. If locomotor activity is a reasonable proxy for overall activity, then the positive correlation we observed with SMR supports the performance model in males in that the metabolic machinery necessary to express energetically expensive traits such as locomotion generates 'idling costs' that increase SMR. Similar results were detected at the phenotypic level in this population (Videlier et al., 2019) and a recent meta-analysis of phenotypic studies also supported the performance model (Mathot et al., 2019). The evolutionary implications of such idling costs are

unclear, but the genetic association detected here indicates shared genetic variation that may alter, and potentially constrain, responses to selection on either trait.

In females, aside from the mass-SMR correlation discussed above, the interpretation of the other r_As is more speculative given their lower precision. Nevertheless, point estimates indicate that some genetic variation is shared between locomotor activity and both SMR and body mass. While the point estimate between locomotor activity and body mass is negative, it was not significantly different from zero. The relatively small and nonsignificant r_A between locomotor activity and SMR supports neither the performance nor the allocation models of energy management. Instead, the results suggest the 'independent' model, whereby SMR and activity neither promote nor constrain each other within the total energy budget (Careau & Garland, 2012). This could arise, for instance, if metabolic machinery necessary for locomotion can be largely shut down when not in use such that idling costs are absent.

4.2 | Between-sex genetic covariances: B matrix

B provides insight into the extent to which V_A is shared between females and males, both within and between traits. Surprisingly, few studies have estimated **B**, most notably when including a metabolic trait. A possible reason is that quantifying **B** requires measurements on pedigreed males and females for multiple traits, along with substantial sample sizes to fit a complex multivariate model. Doing so is important, however, because selection is often sex-specific and shared V_A between sexes will thus have consequences for evolutionary responses (Wyman et al., 2013). For example, **B** can constrain (Gosden et al., 2012; Ingleby et al., 2014; Lewis et al., 2011) or permit (Cox et al., 2017; Rapkin et al., 2017; Sztepanacz & Houle, 2019) the evolution of sexual dimorphism. 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 lie along the diagonal of **B**. In our case, the associated r_{fm} for all three of the traits, representing scaled covariances, was significantly greater than zero, indicating a shared genetic basis between males and females for each trait. This suggests the possibility of ongoing intralocus sexual conflict if selection is sexually antagonistic on any of these traits. The shared V_A for locomotor activity is perhaps not surprising given the weak sexual dimorphism observed in this trait in our population (Figure 1b). A previous study of the same population, using the same beam-cross method, also found no sexual dimorphism in daily locomotor activity (Videlier et al., 2019). By contrast, Long and Rice (2007) found a strong sexual dimorphism in locomotor activity in another *D. melanogaster* population, but quantified locomotor activity in a very different context and using a different method. Long and Rice (2007) also demonstrated sexually antagonistic selection on locomotor activity alongside a strong r_{fm} in their population, suggesting ongoing intralocus sexual conflict. The weak sexual dimorphism and a strong r_{fm} are in line with the general trend of negative association between sexual dimorphism and r_{fm} (Bonduriansky & Locke, 2005; Cox et al., 2017; Poissant et al., 2010).

Our r_{fm} estimates for body mass and SMR were lower and significantly less than one, demonstrating the existence of some independent V_A in these traits between males and females. A reduced r_{fm} suggests a history of sexually antagonistic selection as its presence will favour mechanisms to lower r_{fm}, allowing males and females to evolve to their sex-specific trait optima and hence resolving the intralocus conflict (Bonduriansky & Chenoweth, 2009). The existence of widespread sexual dimorphism in body size across taxa, including in *D. melanogaster*, further suggests a history of sexually antagonistic selection for this trait. Sexual dimorphism in SMR has received less attention, and its interpretation is more challenging given confounding dimorphism in mass. Videlier et al. (2021) estimated phenotypic selection on mass and SMR in the current population, but conclusions concerning sexual antagonism are unclear. Selection differentials were positive and similar for both traits in males and females, but gradients were nonsignificant (due to the strong mass-SMR covariances) and, while nonlinear selection was detected in females only, it was present over a range of trait values that were absent in males.

Finally, between-trait cross-sex correlations were variable but, in general, not strong. Of note are the reciprocal correlations between mass in one sex and SMR in the other, both of which were significantly greater than zero. Strong correlations in these cases are perhaps not surprising given the strong within-sex correlations between SMR and mass in both males and females. Still, the between-trait cross-sex correlations could have been low if within-sex correlations were mediated by different sets of polymorphisms. The fact that the between-trait cross-sex correlations were nearly as high as within-sex correlations suggest high cross-sex correlations in pleiotropic effects of polymorphisms on body size and SMR. Finally, **B** can be asymmetrical between its lower and upper components, reflecting a situation in which covariances between traits differ depending on which sex is expressing which trait (Gosden & 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 and power was likely reduced given the relatively large uncertainties in these estimates.

5 | CONCLUSION

Insight into the quantitative genetics of SMR, body mass and locomotor activity was gained by estimating the sex-specific genetic covariance matrix ($\mathbf{G}_{fm}$) for these traits. Our results revealed some difference between females and males in their genetic (co)variances, driven in large part by a difference in V_A in locomotor activity between the sexes. While there was detectable V_A in SMR, it was largely shared with that of mass, and almost entirely so in females. In addition, the **B** matrix revealed substantial shared genetic variation between the sexes for all three traits, generating positive and

strong r_{fm}s that highlight the potential for intralocus sexual conflict. While phenotypic selection has been previously quantified (Videlier et al., 2021), results were inconclusive and such estimates may be biased by environmentally-induced covariance between the fitness and the traits. 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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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

VC and HDR conceived of the project, all authors contributed to study design, and MV collected the data. MV and VC analysed the data with input from HDR, and all authors wrote the manuscript.

DATA AVAILABILITY STATEMENT

All data and analysis code used in this paper can be found on Dryad at https://doi.org/10.5061/dryad.j3tx95xd0.

PEER REVIEW

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OPEN RESEARCH BADGES

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data are available at https://doi.org/10.5061/dryad.j3tx95xd0.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Supplementary methods and tables for the article:

"Sex-specific genetic (co)variances of standard metabolic rate, body mass and locomotor activity in *Drosophila melanogaster*"

Metabolic measurements

The flow-through respirometry 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.

Flies were gently placed, without anaesthesia, separately into chambers made of clear plastic tubes (40 mm high by 6 mm diameter). Each metabolic chamber was placed over 3 infra-red activity detectors. The raw outputs from the activity detectors 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. Metabolic rate (MR; CO_2 production) 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 MR using the "nadir" function in ExpeData. For each extracted bout of MR, we recorded 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 MR measurement. For each respirometry run, the lowest of the 21 extracted MR values was selected per individual as their standard metabolic rate (SMR).

Locomotor activity assay

Locomotor activity outside of respirometry was measured using three DAM2 activity monitors (Trikinetics, Waltham, MA, USA), each capable of measuring 32 separate flies. For each set of measurements, 96 flies were anesthetised with CO_2 and then individually and randomly placed in separate, cleaned 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 around 11:00 and lasted for 24 hours. The first two hours of data were discarded to allow acclimation to the experimental conditions. Locomotor activity for each fly was calculated as the average number of movements during the remaining 22 hours. Although the use of a single beam to detect activity unavoidable underestimate activity, activity scores produced by the DAM monitors are usually strongly correlated with total distance travelled (Gilestro, 2012).

Table S1. Sources of variation of body mass (MASS), standard metabolic rate (SMR), and locomotor activity (ACT) in female (F) and male (M) *D. 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	MASS	Fem	-2.129	0.102	131.2	4.6	0.000
	SMR	Fem	-0.492	0.145			
	ACT	Fem	-0.568	0.661			
	MASS	Mal	0.344	0.117			
	SMR	Mal	-0.269	0.150			
	ACT	Mal	-0.733	0.632			
Block	MASS	Fem	…	…	184.1	20.5	0.000
Block	SMR	Fem	…	…	109.8	1.8	0.114
Block	ACT	Fem	…	…	118.0	9.7	0.000
Block	MASS	Mal	…	…	177.6	28.0	0.000
Block	SMR	Mal	…	…	107.3	1.2	0.287
Block	ACT	Mal	…	…	139.4	9.8	0.000
Age	MASS	Fem	…	…	931.1	237.1	0.000
Age	SMR	Fem	…	…	110.3	17.7	0.000
Age	ACT	Fem	…	…	219.5	6.5	0.000
Age	MASS	Mal	…	…	722.3	19.2	0.000
Age	SMR	Mal	…	…	104.5	10.6	0.000
Age	ACT	Mal	…	…	221.9	1.0	0.408
Mass category (ACT)	MASS	Fem	-0.007	0.035	1136.4	0.0	0.840
Mass category (ACT)	MASS	Mal	-1.122	0.043	1174.1	692.4	0.000
Monitor (1)	ACT	Fem	…	…	539.6	0.0	0.986
Monitor (1)	ACT	Mal	…	…	479.7	2.7	0.071
Water Vapor	SMR	Fem	0.142	0.042	87.4	11.5	0.001
Water Vapor	SMR	Mal	0.001	0.045	100.5	0.0	0.988
Temperature	SMR	Fem	0.184	0.044	88.2	17.3	0.000
Temperature	SMR	Mal	0.142	0.046	110.3	9.5	0.003
Activity during measure	SMR	Fem	0.315	0.038	677.5	68.0	0.000
Activity during measure	SMR	Mal	0.325	0.030	605.9	117.5	0.000
Activity before measure	SMR	Fem	0.319	0.037	658.8	73.1	0.000
Activity before measure	SMR	Mal	0.415	0.031	648.0	173.4	0.000

Table S2. Complete list of random effects estimates from the 6×6 multivariate animal model with standardized (N(0,1)) 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 covariances (Cov_A) were fitted between all six traits, whereas residual covariances (Cov_e) were only estimable between mass and SMR and mass and activity within each sex.

component	estimate	SE	z.ratio	bound
V_{MAVEn} - female SMR	0.024	0.015	1.581	P
V_{MAVEn} - male SMR	0.041	0.015	2.639	P
V_{CE} - female mass	0.000	NA	NA	B
V_{CE} - female SMR	0.037	0.040	0.913	P
V_{CE} - female activity	0.000	NA	NA	B
V_{CE} - male mass	0.000	NA	NA	B
V_{CE} - male SMR	0.008	0.030	0.268	P
V_{CE} - male activity	0.000	NA	NA	B
V_A - female mass	0.358	0.043	8.315	U
Cov_A - female SMR - female mass	0.245	0.043	5.712	U
V_A - female SMR	0.246	0.089	2.763	U
CovA - female activity - female mass	-0.002	0.046	-0.039	U
CovA - female activity - female SMR	0.048	0.055	0.873	U
V_A - female activity	0.298	0.095	3.122	U
Cov_A - male mass - female mass	0.195	0.034	5.659	U
Cov_A - male mass - female SMR	0.153	0.042	3.673	U
Cov_A - male mass - female activity	0.109	0.049	2.220	U
V_A - male mass	0.377	0.051	7.435	U
Cov_A - male SMR - female mass	0.096	0.032	3.047	U
Cov_A - male SMR - female SMR	0.102	0.037	2.784	U
Cov_A - male SMR - female activity	0.099	0.046	2.163	U
Cov_A - male SMR - male mass	0.149	0.037	4.019	U
V_A - male SMR	0.182	0.068	2.692	U
Cov_A - male activity - female mass	0.099	0.051	1.959	U
Cov_A - male activity - female SMR	0.110	0.062	1.774	U
Cov_A - male activity - female activity	0.428	0.075	5.677	U
Cov_A - male activity - male mass	0.136	0.058	2.348	U
Cov_A - male activity - male SMR	0.137	0.052	2.645	U
V_A - male activity	0.670	0.119	5.629	U
V_e - female mass	0.143	0.028	5.055	P
Cov_e - female SMR - female mass	0.079	0.033	2.427	P
V_e - female SMR	0.389	0.060	6.472	P
Cove - female activity - female mass	0.031	0.039	0.811	P
V_e - female activity	0.622	0.091	6.857	P
V_e - male mass	0.288	0.036	7.889	P
Cov_e - male SMR - male mass	0.040	0.030	1.338	P
V_e - male SMR	0.213	0.041	5.157	P
Cov_e - male activity - male mass	-0.066	0.046	-1.436	P
V_e - male activity	0.255	0.089	2.868	P

Table S3 Additive genetic (r_A) and residual (r_e) correlations between body mass, locomotor activity, and standard metabolic rate (SMR) in *D. melanogaster*. Likelihood ratio test (LRT) 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_1	P	$\chi^2_{0.5}$	P
r_A - females	*mass*	*SMR*	0.963	0.194	**37.46**	**<0.001**	0.03	0.606
r_A - females	*mass*	*activity*	-0.283	0.222	1.55	0.213	**25.36**	**<0.001**
r_A - females	*SMR*	*activity*	0.240	0.296	0.64	0.423	**5.38**	**0.007**
r_A - males	*mass*	*SMR*	0.681	0.155	**19.34**	**<0.001**	**2.43**	**0.049**
r_A - males	*mass*	*activity*	0.040	0.146	0.07	0.787	**43.98**	**<0.001**
r_A - males	*SMR*	*activity*	0.355	0.158	**5.13**	**0.024**	**8.05**	**0.001**
r_{fm}	*mass*	*mass*	0.742	0.093	**42.01**	**<0.001**	**9.08**	**0.001**
r_{fm}	*SMR*	*SMR*	0.572	0.230	**8.01**	**0.005**	**2.59**	**0.044**
r_{fm}	*activity*	*activity*	0.983	0.251	**22.35**	**<0.001**	0.00	0.757
r_A - between-sex cross-trait	*female SMR*	*male mass*	0.779	0.207	**20.92**	**<0.001**	1.47	0.103
r_A - between-sex cross-trait	*female activity*	*male mass*	-0.203	0.224	0.84	0.359	**21.63**	**<0.001**
r_A - between-sex cross-trait	*female mass*	*male SMR*	0.403	0.145	**8.18**	**0.004**	**7.84**	**0.002**
r_A - between-sex cross-trait	*female activity*	*male SMR*	0.393	0.252	2.52	0.113	**4.94**	**0.010**
r_A - between-sex cross-trait	*female mass*	*male activity*	0.123	0.130	0.85	0.357	**46.23**	**<0.001**
r_A - between-sex cross-trait	*female SMR*	*male activity*	0.300	0.194	2.58	0.108	**10.17**	**<0.001**
r_e - females	*mass*	*SMR*	0.307	0.078	**9.39**	**0.002**	NA	NA
r_e - females	*mass*	*activity*	0.123	0.096	1.55	0.212	**37.54**	**<0.001**
r_e - males	*mass*	*SMR*	0.154	0.094	2.09	0.148	NA	NA
r_e - males	*mass*	*activity*	0.000	0.122	0.00	0.996	NA	NA