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Sex-Specific Among-Individual Covariation in Locomotor Activity and Resting Metabolic Rate in *Drosophila melanogaster*

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ABSTRACT: A key endeavor in evolutionary physiology is to identify sources of among- and within-individual variation in resting metabolic rate (RMR). Although males and females often differ in whole-organism RMR due to sexual size dimorphism, sex differences in RMR sometimes persist after conditioning on body mass, suggesting phenotypic differences between males and females in energy-expensive activities contributing to RMR. One potential difference is locomotor activity, yet its relationship with RMR is unclear and different energy budget models predict different associations. We quantified locomotor activity (walking) over 24 h and RMR (overnight) in 232 male and 245 female *Drosophila melanogaster* that were either mated or maintained as virgins between two sets of measurements. Accounting for body mass, sex, and reproductive status, RMR and activity were significantly and moderately repeatable (RMR: $R = 0.33 \pm 0.06$; activity: $R = 0.58 \pm 0.03$). RMR and activity were positively correlated among ($r_{ind} = 0.26 \pm 0.09$) but not within ($r_e = 0.05 \pm 0.06$) individuals. Moreover, activity varied throughout the day and between the sexes. Partitioning our analysis by sex and activity by time of day revealed that all among-individual correlations were positive and significant in males but nonsignificant or even significantly negative in females. Such differences in the RMR-activity covariance suggest fundamental differences in how the sexes manage their energy budget.

Keywords: basal metabolic rate, energy expenditure, insects, personality, sexual dimorphism, standard metabolic rate.

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 that affect MR is therefore a key endeavor 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, postabsorptive, nonreproductive, 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), mollusks (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 intraspecific 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 or 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 nonresting 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 the high levels of energy expenditure associated with activity and reproduction (Careau et al. 2008). By contrast, in the independent model of energy management, increased nonresting expenditure is dissociated from maintenance costs and hence SMR, owing 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 nonresting energy expenditure (Careau 2017). Empirical support exists for all three models, as various relationships have been observed between SMR (or basal MR for endotherms)

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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 behaviors 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 elevate MR by only 5%–10%, in species that walk a lot this may represent a nontrivial component of the nonresting 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 behavior 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 proved 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 minimize 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 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 focusing 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 that 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 in 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 (nonreproductive) was intentionally violated in half of our subjects in their second measurement, and we therefore refer to our measurements as resting metabolic rate (RMR). In doing so, our experimental protocol allowed us to evaluate how MR and activity were affected not only by body mass and sex but also by 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.

Material and Methods

Study Animals and Husbandry

A stock population was established in February 2016 from a large sample of a laboratory-adapted population of

Drosophila melanogaster that had originally been collected in Dundas, Ontario, Canada, in 2006 (MacLellan et al. 2012). This new stock was maintained with discrete nonoverlapping generations in 64 vials at 25°C, at 50% relative humidity, and with a 12L : 12D photoperiod (lights turn on at 7:00 and off at 19:00) 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 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 with 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, PA) filled with 75 mL of food (fig. A1; figs. A1–A3 are available online). 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 provided individuals with places to hide (e.g., to avoid unwanted sexual attention) and may have resulted in the expression of a richer repertoire of sexual and other behaviors (e.g., search effort and territory defense in males, escape behaviors 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 640 individuals of each sex were then randomly chosen and evenly distributed among 64 bottles (i.e., 10 females and 10 males per bottle). After 4 days in these bottles, males were discarded and surviving females were evenly transferred among 64 standard glass culture vials (28.5 mm × 95 mm, containing 10 mL of 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 mating protocol described above 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 h after flies were collected as virgins, locomotor activity was recorded for 24 h, after which individuals were directly transferred into a chamber in which MR was measured for 9 h overnight. After respirometry, each fly was weighed to the nearest 0.001 mg on an MX5 microbalance (Mettler Toledo, Columbus, OH) and then transferred to a standard food vial either individually or together with an opposite-sex individual from the same block. After 36 h 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), each capable of measuring 32 separate flies. For each set of measurements, 64 flies were anesthetized 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), 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 h. The first 2 h 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 h (ACT_{avg}). As is typical for *D. melanogaster* (Helfrich-Forster 2000; Ferguson et al. 2015), locomotor activity followed a bimodal pattern with peaks occurring immediately after changes in lighting (fig. 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. 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 were switched off, which corresponds to the evening peak (ACT_{ep}); (3) between the two peaks when the lights were on (ACT_{day}); and (4) between the two peaks when the lights were off

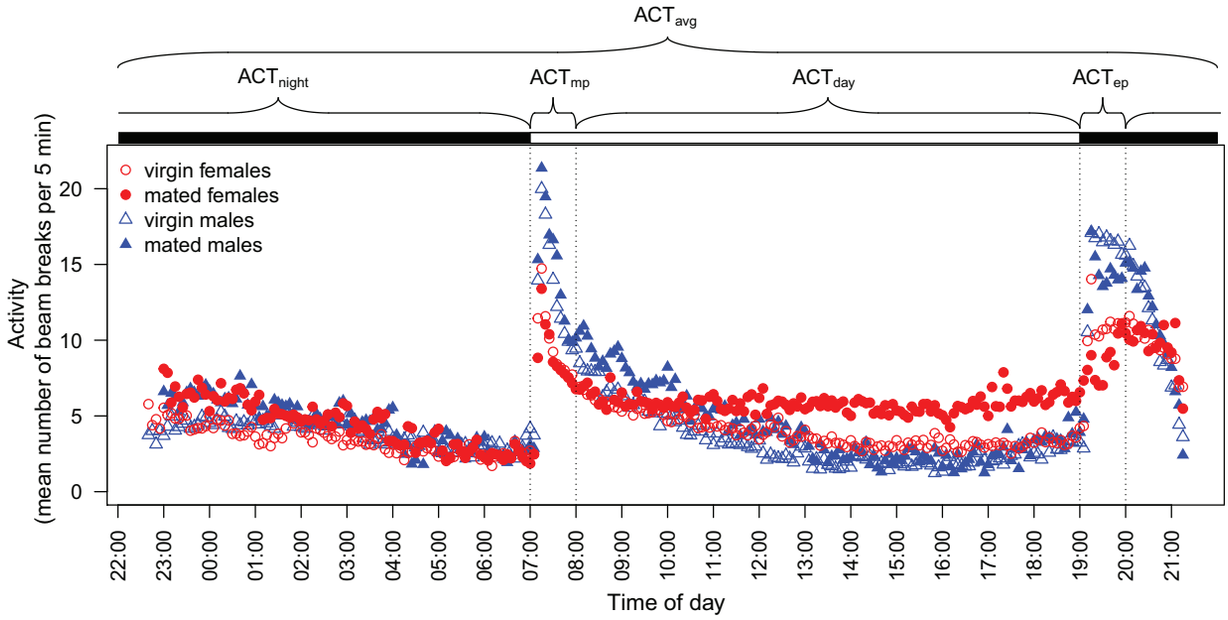

Figure 1: Daily variation in locomotor activity ($n = 496$) in male (triangles) and female (circles) *Drosophila melanogaster* that were either virgin (open) or mated (filled). Black and white boxes show when incubator lights were on or off. There are two major peaks of activity occurring immediately after switching 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}), nonpeak day (ACT_{day}), evening peak (ACT_{ep}), and nonpeak night (ACT_{night}).

(ACT_{night}). Overall, we collected $n = 956$ activity measurements from $n = 496$ individuals.

Metabolic Rate Measurement

CO_2 production ($\dot{V}CO_2$) 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 consisting of a differential CO_2 analyzer (Li-Cor7000; Li-Cor Biosciences, Lincoln, NE) and a 16-channel flow management, data acquisition, and signal processing system (Maven; Sable Systems, North Las Vegas, NV). 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 analyzers 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 (A) of each CO_2 analyzer. Afterward, each airstream was humidified by flowing through Nafion tubing (DuPont, Wilmington, DE) submerged in distilled water. The rehumidified airstream 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 min^{-1}. The approximately equivalent flow rates in the nonbaseline channels (range: 15–25 mL min^{-1}) were maintained by means of matched flow resistances based on microorifice flow restrictors. A second mass flow meter on the Maven's main board measured the actual flow rate of each selected airstream before it was automatically directed through the measurement cell (B) of the CO_2 analyzer.

Flies were gently placed without anesthesia into individual chambers made of clear plastic tubes (40 mm high × 6 mm diameter). Measurements were performed for 9 h overnight, between 21:00 and 7:00, which correspond to the period of lowest activity in this population (fig. 1). Each Maven was set to measure each chamber for 120 s (dwell time) with baseline measurements (120 s) 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 versus 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-s lag with the activity channels. $\dot{V}CO_2$ 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 $\dot{V}CO_2$ measurements (Lighton 2008). 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 s of each measurement was discarded to allow the system to fully equilibrate after changing between chambers. From the remaining 80 s of each measurement period, we extracted the lowest 20-s continuous bout of $\dot{V}CO_2$ using the nadir function in ExpeData. In addition to the average of the lowest 20 s, 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 s immediately prior to the $\dot{V}CO_2$ measurement (ACT_{20p}). For each respirometry run, the lowest of the 16 extracted $\dot{V}CO_2$ values was selected per individual (fig. A2). Because one of the criteria for measuring SMR (nonreproductive) 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 midway 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 standardized to a mean of 0 and a variance of 1. ACT_{20} and ACT_{20p} were log transformed before standardization. Sex was treated as a binary variable (female = 0, male = 1), as were 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 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 included since no individuals were mated in the first measurement. We also tested for possible three-way interactions; none were significant ($P > .07$ 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 vapor, 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 nonindependence 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 described 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 the nadiv package (Wolak 2012) in R version 3.4.4 (R Core Team 2018) to calculate ~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 described above using 95% CIs.

Results

RMR: Effect of Activity during the Metabolic Measurements

For a given individual, RMR was selected as the lowest $\dot{V}CO_2$ value from among the 16 separate measurements collected over the 9-h 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. A2*A*). 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 $\dot{V}CO_2$ value, although some flies were moderately but regularly active throughout the entire respirometry run such that some activity occurred even during their lowest $\dot{V}CO_2$ measurement (fig. A2*B*). As expected, this remaining activity was positively correlated with MR (table A2*A*, fig. A2*C*; tables A1–A3 are available online). 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 analyzing 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. A3).

RMR: Mass, Sex, and Reproductive Effects

As expected, RMR increased with body mass (fig. 2*A*) and was also significantly higher in mated compared with virgin individuals (fig. 2*B*). The effect of reproductive status did not vary by sex or with body mass, as indicated by the nonsignificant two-way interactions (table 1, pt. A). Although RMR did not differ between males and females when accounting for body size (table 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 in females (table 1, pt. A, fig. 2*A*). After accounting for all of these sources of variation, the repeatability ($R \pm$ SE) of RMR was 0.342 ± 0.055 (see table A1 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 1, pt. B). Males and females did not differ significantly in average activity (table 1, pt. B), and none of the two-way interactions 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 nonpeak night (fig. 1; table 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 terms 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 A1).

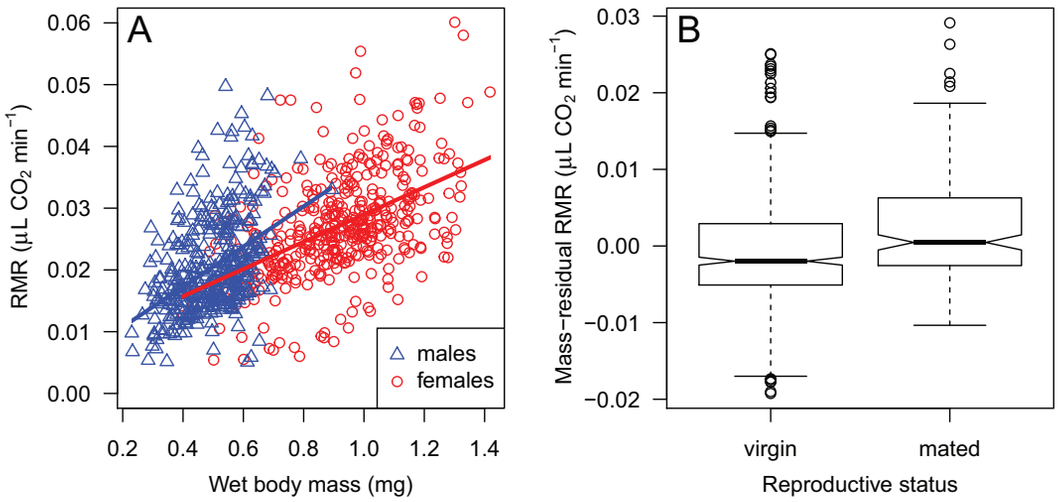

Figure 2: Resting metabolic rate (RMR) as a function of wet body mass (*A*) and reproductive status (*B*; virgin vs. mated) in *Drosophila melanogaster*. The body mass–RMR relationship is shown separately for males (triangles) and females (circles) with corresponding linear regressions. RMR is conditioned on wet mass when testing the effect of reproductive status.

Table 1: Sources of variation in resting metabolic rate (RMR; pt. A) and locomotor activity (ACT_{avg}; pt. B) in a laboratory-adapted population of *Drosophila melanogaster*

Trait, source	Estimate	SE	df_{den}	*F*	*P*
A. RMR:					
Intercept	.07	.09	8.8	.62	.452
Sex	.20	.07	488.1	1.11	.293
Body mass	**.69**	**.04**	**518.2**	**440.60**	**<.001**
Reproductive status	**.17**	**.04**	**654.3**	**20.78**	**<.001**
Sex × body mass	**.25**	**.06**	**522.5**	**15.18**	**<.001**
Sex × reproductive status	.07	.13	656.1	.27	.602
Body mass × reproductive status	.02	.06	670.3	.12	.734
B. ACT_{avg}:					
Intercept	−.03	.09	21.0	.13	.722
Sex	−.12	.15	521.4	.91	.341
Body mass	**−.15**	**.08**	**434.6**	**4.22**	**.040**
Reproductive status	**.23**	**.07**	**700.6**	**10.34**	**.001**
Sex × body mass	−.07	.15	648.5	.23	.633
Sex × reproductive status	−.36	.26	689.3	1.92	.167
Body mass × reproductive status	.12	.13	684.0	.92	.338

Note: 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. Boldfacing indicates significance ($P < .05$). Shown are estimates of each fixed effect with their standard error (SE), denominator degrees of freedom (df_{den}; numerator df = 1 in all cases), conditional Wald *F* statistic, and *P* value. ACT_{avg} = average activity.

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 to 0.10]; table 3). This correlation was stronger and again significant at the among-individual level ($r_{ind} = 0.26$ [0.13 to 0.39]; table 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 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 to 0.13]; table 3). Again, correlations were stronger at the among-individual level and ranged from 0.20 to 0.26, except for activity during the evening peak when it was weaker and nonsignificant (0.08). At the within-individual level, all correlations between RMR and activity were nonsignificant, except for a positive association with activity during night (table 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 A3, fig. 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 to −0.20]). Sex-specific correlations are significantly different (i.e., 95% CIs were nonoverlapping; table A3, fig. 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 nonsignificant among-individual correlation at the population level (see above; fig. 3; table 3). Within-individual correlations were nonsignificant in most cases (table A3).

Discussion

We measured locomotor activity and RMR twice in a large number (>400) of male and female *Drosophila 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 nonsignificant 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

Table 2: Sources of variation in locomotor activity during morning peak (ACT_{mp}; pt. A), nonpeak day (ACT_{day}; pt. B), evening peak (ACT_{ep}; pt. C), and nonpeak night (ACT_{night}; pt. D; see table 1 for details)

Trait, source	Estimate	SE	df_{den}	*F*	*P*
A. ACT_{mp}:					
Intercept	−.14	.09	15.6	2.27	.152
Sex	**.40**	**.14**	**532.6**	**20.44**	**<.001**
Body mass	**−.23**	**.07**	**494.2**	**4.32**	**.038**
Reproductive status	.02	.08	826.5	.05	.828
Sex × body mass	**−.34**	**.14**	**586.7**	**6.11**	**.014**
Sex × reproductive status	−.14	.28	819.5	.25	.615
Body mass × reproductive status	−.06	.13	815.9	.18	.670
B. ACT_{day}:					
Intercept	−.01	.08	27.6	.01	.946
Sex	**−.74**	**.14**	**417.5**	**37.83**	**<.001**
Body mass	**−.23**	**.08**	**294.3**	**13.94**	**<.001**
Reproductive status	**.37**	**.07**	**733**	**25.87**	**<.001**
Sex × body mass	−.01	.14	610.8	<.01	.951
Sex × reproductive status	**−.72**	**.26**	**723.6**	**7.81**	**.005**
Body mass × reproductive status	.02	.12	719.1	.04	.849
C. ACT_{ep}:					
Intercept	−.08	.08	25.9	.99	.329
Sex	**.87**	**.14**	**425.5**	**63.28**	**<.001**
Body mass	.05	.07	319.1	2.78	.097
Reproductive status	**−.17**	**.08**	**790.0**	**4.67**	**.031**
Sex × body mass	−.17	.14	580.7	1.6	.206
Sex × reproductive status	.46	.27	782.5	2.95	.086
Body mass × reproductive status	.08	.13	778.8	.33	.565
D. ACT_{night}:					
Intercept	.05	.12	12.5	.19	.669
Sex	.32	.15	572.8	2.79	.095
Body mass	.07	.08	577.1	.1	.753
Reproductive	−.05	.08	816	.26	.611
Sex × body mass	.13	.15	596	.81	.368
Sex × reproductive status	.01	.29	808.1	<.01	.99
Body mass × reproductive status	.13	.14	804.2	.88	.349

Note: Boldfacing indicates significance ($P < .05$).

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). When conditioned on body mass, however, RMR did not differ significantly between the sexes (table 1, pt. A). Interestingly, the RMR–body mass relationship did differ between males and females, with males showing a steeper slope than females (fig. 2*A*). A steeper slope could arise if, compared with females, males disproportionately 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. 1998; 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 1, pt. A). 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 effect 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 h after the period during

Table 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 laboratory-adapted population of *Drosophila melanogaster*

Model	r_p (95% CI)	r_{ind} (95% CI)	r_e (95% CI)
RMR × ACT_{avg}	**.08 (.06 to .10)**	**.26 (.13 to .39)**	.05 (−.03 to .13)
RMR × ACT_{mp}	**.02 (.01 to .03)**	**.21 (.06 to .37)**	−.06 (−.14 to .02)
RMR × ACT_{day}	**.05 (.04 to .06)**	**.20 (.07 to .33)**	−.01 (−.08 to .07)
RMR × ACT_{ep}	**.01 (.007 to .013)**	.08 (−.07 to .22)	−.02 (−.10 to .06)
RMR × ACT_{night}	**.11 (.08 to .13)**	**.26 (.11 to .41)**	**.15 (.07 to .23)**

Note: Correlations are presented with 95% confidence intervals (CIs), and boldfacing indicates significance (i.e., CIs that do not include zero). ACT_{avg} = average activity; ACT_{day} = activity during day; ACT_{ep} = activity during evening peak; ACT_{mp} = activity during morning peak; ACT_{night} = activity during night.

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 5 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 behavior (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-Forster 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). 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 nonpeak 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 behavioral 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, ACT_{avg} = 0.581 ± 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 multichannel flow-through respirometry system to measure RMR twice in more than 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. The pooled estimates across sexes and activity components (i.e., peak vs. nonpeak periods) also obscured substantial heterogeneity in this RMR-activity relationship. Further partitioning by sex and 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 nonsignificant in three activity periods and significant but negative in the fourth (fig. 3). To date, rare examples of a sex-specific relationship between RMR and behavior have been observed in rodents (Lantová et al. 2011; Šíchová et al. 2014) with opposite correlations between proactive behaviors and RMR in males and

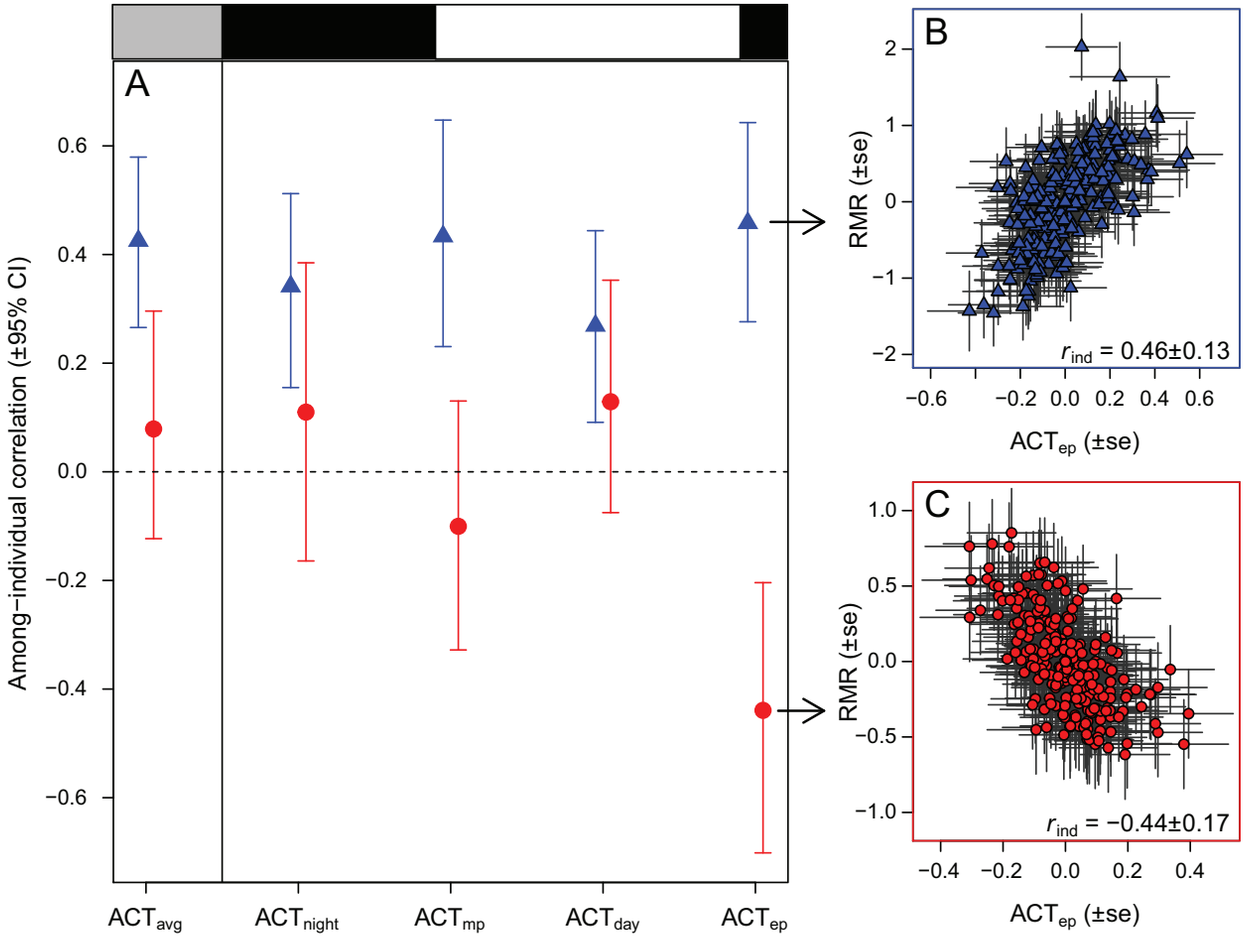

Figure 3: *A*, Among-individual correlations (r_{ind}) with 95% confidence intervals (CIs) between resting metabolic rate (RMR) and the different activity variables (ACT$_{avg}$, ACT$_{mp}$, ACT$_{day}$, ACT$_{ep}$, and ACT$_{night}$; see fig. 1). Triangles denote males, and circles denote females. Black and white boxes show the lighting status (white vs. black = lights on vs. off; gray = average across both periods) 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 males (*B*) and females (*C*).

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 behaviors 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 behaviors may be more common in our population compared with other *Drosophila* laboratory populations because its complex mating environment was specifically designed to allow the expression of a richer repertoire of sexual and other behaviors (see fig. A1). Recent experiments have shown that such environmental complexity alters sexual selection and sexual conflict (MacLellan et al. 2009; 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 nonresting 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 with

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 behaviors 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. A1) may also allow females to avoid male harassment by hiding instead of via more energy-expensive resistance and escape behaviors.

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 nonresting 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 with 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 occurred only 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. Behavioral studies that track diurnal patterns of investment in various energy-demanding activities (locomotion, foraging, reproduction, etc.) would be useful to 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 address 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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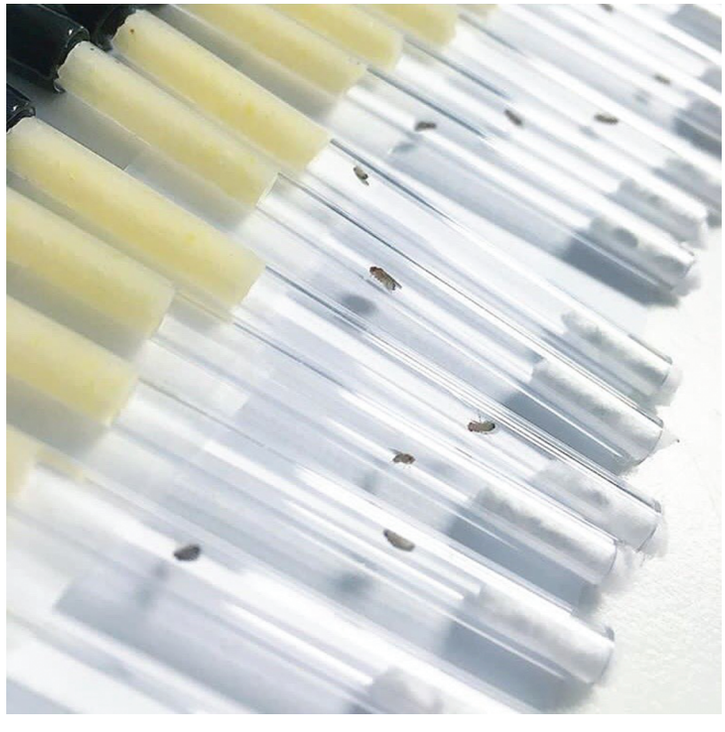

Drosophila melanogaster in activity chambers. Photo credit: Vincent Careau.