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Original Article

Territoriality in *Drosophila*: indirect effects and covariance with body mass and metabolic rate

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Territoriality (i.e., defense of a resource) is the outcome of behavioral interactions that can result in selective advantages in many vertebrates and invertebrates. Since territoriality is expressed in a social context, an individuals' territoriality may change according to the phenotype of the opponents that they are confronted with (termed "indirect effects"). Defending a territory may also confer energetic costs to individuals, which could be reflected in their standard metabolic rate (SMR), a key component of an ectotherms' energy budget. Here, we measured territoriality using dyadic contests, body mass, and SMR using flow-through respirometry, twice in each of 192 adult male *Drosophila melanogaster*. Territoriality, body mass, and (whole-animal) SMR were all significantly repeatable. However, essentially all the among-individual variation in SMR was shared with body mass, as indicated by a very strong among-individual correlation (r_{ind}) between body mass and SMR. The among-individual correlation between territoriality and SMR also tended to be positive, suggesting the presence of underlying metabolic costs to territoriality. Although indirect effects on territoriality were present but weak (accounting for 8.4% of phenotypic variance), indirect effects on territoriality were negatively and significantly correlated with body mass. This indicates that larger individuals tended to suppress their opponents territoriality. Variation among individuals in their ability to suppress territoriality in others was not associated with their own territoriality or SMR.

Key words: **opponent effects, performance model, social behavior, standard metabolic rate, territory defense**

INTRODUCTION

Territoriality, or the defense of a resource (e.g., food and/or mates) from other individuals, is a behavior that is associated with increased reproductive success in some vertebrate (Clutton-Brock 1989; Davies 1991) and invertebrate (Thornhill 1979; Baker 1983) species. Territorial males can gain access to females directly by defending groups of them (i.e., female defence polygyny; Emlen and Oring 1977); for example, dominant male hamadryas baboons and elephant seals defend and mate with groups of multiple females (Le Boeuf 1974; Swedell et al. 2013). Territorial males can also gain access to females indirectly, by defending a resource that is essential to females (i.e., resource defence polygyny; Emlen and Oring 1977). For example, male arthropods such as hylaeine and wool-carder bees defend flowers that are a source of pollen for females (Alcock and Houston 1996; Starks and Reeve 1999), while burying beetles defend vertebrate carcasses on which females lay eggs (Scott 1998; Suzuki et al. 2006).

In the common fruit fly (*Drosophila melanogaster*), a polygamous insect, males will establish and defend small territories containing a food resource which is attractive to females for feeding and/or oviposition (Hoffmann 1987a; Hoffmann and Cacoyianni 1990). Unsurprisingly, studies in *D. melanogaster* and related species have shown that male flies exhibiting territorial behavior were more successful at mating compared to nonterritorial males (Dow and von Schilcher 1975; Hoffmann 1987a,1988; White and Rundle 2015). In such a "resource defense mating system" (Procter et al. 2012), acquiring and defending a territory involves male–male aggressive interactions which can include behaviors that range in intensity from wing threats to physical fights (e.g., foreleg fencing, lunging at one another, holding, and tussling together). Such interactions normally end when one male retreats or is chased away from the food by the other (Chen et al. 2002; White and Rundle 2015). Body size has been shown to be an important determinant of the outcome of dyadic encounters over a food source in *Drosophila* (Partridge and Farquhar 1983; Hoffmann 1987a,1987b; Partridge et al. 1987; White and Rundle 2015), although body mass did not change as a result of artificial selection for increased territoriality (Hoffmann 1988). Territoriality is often observed to be context dependent in *Drosophila*: Key factors that impact the expression of this behavior include male density and the size of the resource (Hoffmann and Cacoyianni 1990). The increased energetic requirement of defending a territory when male density is high, and/or when the territory is large, may make it sufficiently costly that the reproductive benefits are no longer a sufficient incentive (Baker 1983; Emlen and Oring 1977).

Defending a territory is likely to be energetically demanding given that it requires an individual to be active, and even

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aggressive, toward conspecifics. In ectotherms, a key component of an individual's energy expenditure is their standard metabolic rate (SMR), which represents the lowest metabolic rate of a postabsorptive, nonreproductive, and inactive adult measured at a specified ambient temperature (Hulbert and Else 2004). There is considerable variation in SMR among individuals (Speakman et al. 2004), some of which may relate to investment in potentially energy-demanding behaviors such as territoriality. Careau et al. (2008) formulated the "performance" and "allocation" models which predict contrasting relationships between SMR and metabolically demanding behaviors. The performance model predicts a positive relationship between SMR and territoriality, under the assumption that SMR reflects the size of the metabolic machinery needed to sustain the higher levels of energy expenditure in highly active individuals. Conversely, the allocation model predicts a negative relationship between SMR and territoriality, assuming that limitations on energy budgets create trade-offs between energy allocated to activity versus maintenance.

Given that both behavioral and metabolic traits typically have moderate repeatabilities (Nespolo and Franco 2007; Bell et al. 2009; White et al. 2013), their association at the phenotypic level will reflect covariation occurring both at the among- and within-individual levels. These arise from different mechanisms (Dingemanse and Dochtermann 2013). Therefore, it is important to partition the phenotypic correlation (r_p) into its component parts—the among-individual correlation (r_{ind}) and within-individual (i.e., residual) correlation (r_e) —to gain a more complete understanding of the relationship between behavioral and metabolic traits (e.g., Careau et al. 2019; Videlier et al. 2019; Cornwell et al. 2020). One particularly important reason to partition r_p in this way is to separate the genetic and permanent environmental underpinnings of the relationship (quantified by r_{ind}) from measurement error and micro-environmental sources of covariance (represented by r_e; Dingemanse and Dochtermann 2013; Careau and Wilson 2017).

Territoriality is expressed in a social context and is thus likely to depend on the phenotype of conspecifics (Wilson et al. 2009, 2011). Therefore, neglecting the effects of social partners on an individual's territorial behavior can pose inherent problems. A solution is to consider territoriality not as a trait belonging solely to the individual, but rather as the outcome of an interaction between multiple phenotypes (Moore et al. 2002). Whenever data are available on multiple individuals repeatedly acting both as focal and "opponent," it is possible to estimate the extent to which individuals differ in: 1) territoriality (i.e., direct effects), 2) how they influence the expression of territoriality in others (i.e., indirect effects), and 3) whether direct and indirect effects are correlated (Dingemanse and Araya-Ajoy 2015). The direct–indirect correlation is of particular interest because it indicates whether individuals that are more territorial than others on average also elicit (if positive) or suppress (if negative) territoriality in others with which they interact. For example, the positive direct–indirect correlation in Wilson et al. (2009) indicates that aggressive mice elicit greater aggressiveness in other mice during interactions. An additional possibility is that indirect effects are correlated with individual differences in other traits. For example, Santostefano et al. (2016) have shown that crickets that elicit more aggressiveness in conspecifics also tended to be more explorative and more active. To our knowledge, the possibility that metabolic rate may be a key component underlying indirect effects in behavior has not been examined.

Our goal is to estimate the repeatability of territoriality (defense of a food/egg laying resource), body mass, and SMR in a population of *D. melanogaster*, and to partition the phenotypic correlation (r_p) of these traits to estimate r_{ind} and r_e. We use a laboratory population that is adapted to a life cycle that includes a 4-day mating phase at low density each generation, which should increase the incidence of territorial behavior compared to most *Drosophila* lab populations (Hoffmann and Cacoyianni 1990). Furthermore, a previous study on this population has shown that, in males, SMR is positively correlated with locomotor activity recorded in isolation (Videlier et al. 2019); therefore, assuming territoriality is energetically expensive, we expected a positive r_{ind} between SMR and territoriality. Territoriality was assayed between randomly paired males and each male was paired with a different partner in their two tests. As a result, individual males in our study were repeatedly observed both as a focal individual and as an "opponent." This also allows us to estimate whether indirect effects occurred during the staged dyadic encounters, and if so, whether they were associated with individual differences in body mass and SMR.

MATERIALS AND METHODS

Study animals

In 2016, a stock population of *D. melanogaster* was established from a previously described laboratory-adapted population (MacLellan et al. 2012). This new stock was maintained with a life cycle that included a 4-day mating phase. This mating phase took place in an environment (8 oz. culture bottles) that featured increased structural complexity (i.e., dividers inserted into the food and two coiled piper cleaners inside the bottle) and reduced adult density (10 males and 10 females per bottle) compared to standard maintenance techniques (Videlier et al. 2019). The stock was maintained on a standard cornmeal-based food vials (90 g/L cornmeal, 100 g/L turbinado sugar, 40 g/L yeast, and 12 g/L agar) and subsequent assays used the same food.

Study design

To partition the phenotypic correlation into r_{ind} and r_e, individual virgin males had their territoriality, SMR, and (wet) body mass measured twice each, with a 48-h holding period separating the two sets of paired measurements. Measurements were performed in three blocks spanning three generations of the stock population, with each block consisting of 64 males. At the beginning of each block, virgin adults were collected within 8 h of eclosion and separated by sex using light CO_2 anesthesia. Females were discarded and males were marked with red or green fluorescent powder (Brilliant Group Inc., Richmond, CA) and stored in holding vials at a density of 10 flies per vial (70 per color treatment) with food and with live yeast added. Beginning 24 h following eclosion/marking, 64 (32 per color treatment) randomly selected males (1 day post-eclosion) were randomly paired and added to separate behavioral "arenas" in which territoriality was measured for 80 min. Following the territoriality assay, flies were transferred to individual holding vials (with a small amount of food) until the late evening when they were subsequently measured for metabolic rate overnight, which corresponds to the period of lowest locomotor activity in this population (Videlier et al. 2019). The body mass of each individual was measured the morning after respirometry (2 days post-eclosion) using an MX5 microbalance (Mettler Toledo, Columbus, OH) to a precision of 0.001 mg. Flies were returned to individual holding vials (with food) for 24 h before receiving a second application of fluorescent powder and held for 24 h. The day after re-marking

(4 days post-eclosion), flies were paired with a different individual and measured once more for territoriality, metabolic rate, and body mass at the same time intervals as described above.

The first block consisted of a factorial design in which half of the males received a different color for the second territoriality assay than the first. However, due to issues with distinguishing residual powder from first treatment from new powder applied during the second treatment, this design was abandoned for subsequent blocks and each fly received the same color treatment for both measures. Flies that could not be distinguished prior to the second assay were discarded (n = 8).

Territoriality measurements

To quantify territoriality, two males were aspirated without anesthesia into a cylindrical arena, similar to Hoffmann (1987a) and White et al. (2015), that contained a single, small food resource in the centre. The arena consisted of two petri dish bases (100 mm diameter), both in standard orientation (i.e., neither inverted), with one acting as the lid such that it nested slightly within the one below it. The bottom petri dish was lined with moist filter paper to maintain a humid environment. A food cup (37 mm diameter, 6 mm height), containing 5 mL of standard cornmeal-based food medium (see above) and with a small amount of yeast paste in the middle, was placed on the filter paper, in the centre of the dish. A small hole was cut in the top petri dish to allow flies to be introduced. The hole was sealed with tape for the duration of a trial. The assays were conducted in a brightly lit room and arenas were placed on a white background with white cardboard partitions separating individual replicates to minimize visual disturbance.

Males were allowed to acclimate for 1 h after introduction to their arena, during which they had full access to the food. Observations began between 10:00 and 12:00 and lasted 80 min. Over this period, spot checks of each arena were done every 4 min, recording the presence/absence of each male on the surface of the food. Territoriality scores were calculated for both individuals in each arena. A fly would receive a score of one if it was on the food or zero if it was off the food during a given check. Paired flies (i.e., dyads) were scored independently, allowing each fly to attain a maximum score of 20 for the territoriality assay, regardless of their opponent's score. Although our behavioral measure quantified presence/absence on the food resource, past studies have shown that *Drosophila* males find such a resource attractive and will occupy and defend it during dyadic encounters, sometimes aggressively such that they exclude the other male (Hoffmann and Cacoyianni 1990; Lim et al. 2014; White and Rundle 2015). After the behavioral assay had been completed, flies were anesthetized using CO_2 and transferred to individual holding vials with food.

Metabolic rate measurement

Metabolic rate was measured as CO_2 production using flow-through respirometry in an incubator at a constant temperature of 25 °C. The setup was composed of four separate units, each containing a differential CO_2 analyzer (Li-Cor7000; Li-Cor Biosciences, Lincoln, NE) and a 16-channel system composed of a flow-distribution manifold, a main board, and an activity board (MAVEn; Sable Systems International, North Las Vegas, NV). Li-Cor analyzers were set in differential mode in the 0–200 ppm range and calibrated simultaneously before each block with (1) pure nitrogen (zero CO_2) and (2) certified 14 ppm CO_2 gas. Although the Li-Cor analyzers have a nominal 1% accuracy, with the current setup and proper calibration it is possible to take measurements with a ±0.1 ppm accuracy. The system receives an influx of dry, CO_2-free air from a PG14L purge gas generator (Peak Scientific, Glasgow, Scotland), distributed to the reference cell (A) of each separate Li-Cor analyzer. Airstreams are then re-humidified by passing through Nafion tubing submerged in distilled water. The humidified air is pushed through the flow-distribution manifold, separating it into 17 streams (16 chamber channels + 1 baseline channel), of which only the baseline was actively regulated, at a flow rate of 20 mL min^{-1}. The non-baseline channels maintained roughly equivalent flow rates (range: 15–25 mL min^{-1}) and were measured using a second mass flow meter on the main board of the MAVEn before entering the Li-cor's measurement cell (B).

Flies were aspirated without anesthesia into individual clear plastic tubes (40 mm high × 6 mm diameter). CO_2 production was measured for 10 h overnight, from 21:00 to 7:00, corresponding to the period of lowest activity in this population (Videlier et al. 2019). Each MAVEn sequentially measured all 16 chambers for 120 s (dwell time) with baseline measurements (120 s) taken every 16 chambers (interleave ratio = 16). Therefore, individual flies were measured for 2 min every 34 min, totaling 17 measures per fly overnight. Chambers were placed directly above the MAVEn's activity board, consisting of three infrared sensors per chamber, which recorded instantaneous movements throughout the measurements.

ExpeData (Sable Systems International) was used for data transformations and extraction. Raw activity data from each channel (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 16 chambers in a given MAVEn unit) was corrected for drift using multiple baseline correction measures. CO_2 production was calculated by multiplying flow rate by the fractional concentration of CO_2. The first 40 s of each measurement was discarded to allow the system to fully equilibrate after changing between chambers. The nadir function was used to extract the lowest 20 s continuous period of CO_2 production from the remaining 80 s of each measurement. Additionally, the average flow rate, water pressure, temperature, light intensity, and activity (ACT_{20}) were extracted during this 20 s interval. Previous studies have shown that activity expressed in the 20 s prior to the metabolic measurements (ACT_{20p}) also affects metabolic rate (Videlier et al. 2019, 2021), so this was also extracted. For each fly, the lowest of the 17 measures of CO_2 production was selected to be representative of SMR. During one of the respirometry runs in the third block, the baseline channel displayed abnormal drift before self-correcting mid-trial. This effect was present in all four MAVEn units and lasted about the same duration. To correct for this, data with improper baseline readings were not used in the calculation of SMR. In this run, only the last 7 measures of CO_2 production were taken for each fly. Unpredictable factors such as death, escape, and indistinguishable treatment contributed to missing values, yielding a total of 192 individuals that were measured for territoriality (n = 348 observations) and SMR (n = 347 observations).

Statistical analyses

All of the parameters of interest were estimated with a single multivariate mixed model fit using ASReml-R (Butler et al. 2018), with the behavioral score, body mass, and SMR as the dependent variables. Test sequence (first vs. second) was fitted

as a fixed effect to all traits. The model also included time of day (range 10:00–12:00) and color treatment (green vs. red) fitted to the behavioral score only. Fixed effects fitted to SMR only included temperature, and activity during (ACT_{20}) and immediately prior (ACT_{20p}) to the 20 s that coincided with the SMR measurement. Other nuisance variables like flow rate, water vapor, and light intensity were also initially fitted to SMR, but were not significant and had small effect sizes so they were dropped from the model. The significance of fixed effects was determined using conditional Wald *F*-statistic with the denominator degrees of freedom determined following Kenward and Roger (1997). All continuous variables were standardized to a mean of 0 and a variance of 1 prior to analysis. Visual inspection of the residuals and random effects suggested slight deviations from normality, especially for the territoriality variable, but these were well below the level at which mixed models tend to produce biased parameter estimates (Schielzeth et al. 2020).

The model included random effects of focal male identity and opponent identity fitted to the behavioral score. The variance components associated with these random effects provide estimates respectively of the direct effect (V_{foc}; focal identity) and indirect (V_{opp}; opponent identity) effect. Since behavior was recorded on both individuals in each dyad, a certain degree of nonindependence exists in the data (i.e., we obtained two behavioral observations per dyad). To account for this, we also included dyad as a random effect fitted to the behavioral score. The variance component associated with the dyad random effect (V_{dyad}) was unconstrained during estimation, which is important as observations within dyads may positively or negatively covary (Bijma 2014). For body mass, the only random effect included was focal male identity to account for the repeated measures on each individual. For SMR, we included as random effects the focal male identity and a "dummy" variable to account for the nonindependence of the 16 measurements within a given MAVEn unit on a given day (i.e., separate levels for each day × unit combination; V_{maven}). Note that indirect and/or dyad effects are not likely to occur in body mass and SMR because these traits were measured individually (e.g., one male per chamber); for such an effect to occur, strong opponent and/or dyad effects from the territorial assay preceding respirometry would have to carry-over to SMR and mass measurements.

A 3 × 3 correlation matrix ("corgh" structure in ASReml-R) was fitted at the residual level to estimate the within-individual variances ($V_{e\text{-terr}}$, $V_{e\text{-mass}}$, and $V_{e\text{-SMR}}$) and correlations among territoriality, body mass, and SMR (r_e). At the among-individual level, a 4 × 4 correlation matrix (Supplementary Table S1) was fitted to estimate all six among-individual correlations (r_{ind}) between the V_{foc} and V_{opp} components consisting of: 1) the correlations between focal individual identity effects on territoriality, body mass, and SMR (i.e., $r_{foc\text{-terr,foc-mass}}$, $r_{foc\text{-terr,foc-SMR}}$, $r_{foc\text{-mass,foc-SMR}}$), 2) the correlation between focal and opponent identity effects on territoriality (i.e., $r_{foc\text{-terr,opp-terr}}$), and 3) the correlations between opponent identity effects on territoriality and focal identity effects on both body mass (i.e., $r_{foc\text{-mass,opp-terr}}$) and SMR (i.e., $r_{foc\text{-SMR,opp-terr}}$). Significance of correlations was determined using likelihood ratio tests with 1 *df* that compared an unconstrained model with one in which the correlation of interest was fixed to zero. Adjusted individual repeatability for body mass and SMR was calculated as $R_{foc\text{-trait}} = V_{foc\text{-trait}} / (V_{foc\text{-trait}} + V_{e\text{-trait}})$, where "trait" is either body mass or SMR. As a nuisance variable, V_{maven} was excluded in calculating $R_{foc\text{-SMR}}$ (Wilson 2018). For territoriality, individual repeatability was calculated as $R_{foc\text{-terr}} = V_{foc\text{-terr}}/(V_{foc\text{-terr}} + V_{opp\text{-terr}} + V_{dyad} + V_{e\text{-terr}})$ and the "opponent repeatability" as $R_{opp\text{-terr}} = V_{opp\text{-terr}}/(V_{foc\text{-terr}} + V_{opp\text{-terr}} + V_{dyad} + V_{e\text{-terr}})$. The approximate standard errors (SE) for the repeatability estimates were determined using the delta method. Best linear unbiased predictors (BLUPs) and residuals were extracted from this model to obtain graphic representations of the correlations.

RESULTS

Fixed effects

While color treatment did not affect territoriality, test sequence and time of day both had significant negative effects (Table 1A), indicating that males less frequently occupied the food in the second test and in tests conducted later in the morning. As expected, SMR was positively correlated with activity and temperature (Table 1B). Although temperature and activity represent unwanted variance in SMR measurements, including these as covariates statistically controlled for these effects. Test sequence also had a positive and significant effect on SMR (Table 1B).

Among- and within-individual (co)variances

After accounting for the above-mentioned fixed effects, the multivariate mixed model yielded estimates of the among-individual (i.e., direct) variances of the focal individual (V_{foc}) for territoriality, body mass, and SMR that were all substantial relative to their standard errors (Table 2A). The individual repeatability estimate was high for body mass ($R_{foc\text{-mass}} = 0.821 \pm 0.028$) and moderate for territoriality ($R_{foc\text{-terr}} = 0.299 \pm 0.071$) and SMR ($R_{foc\text{-SMR}} = 0.324 \pm 0.072$). The among-individual correlation between body mass and SMR was very strong and highly significant ($r_{foc\text{-mass,foc-SMR}} = 0.985 \pm 0.094$; Table 2B), while that between territoriality and SMR was positive but nonsignificant, although it approached so ($r_{foc\text{-terr,foc-SMR}} = 0.345 \pm 0.188$; $P = 0.066$; Figure 1; Table 2B). The among-individual correlation between territoriality and body mass was weaker and nonsignificant ($r_{foc\text{-terr,foc-SMR}} = 0.163 \pm 0.121$; $P = 0.187$; Table 2B). All of the residual correlations were weak and nonsignificant (Table 2C).

Table 1

Fixed effects from a multivariate mixed model for (A) territoriality, (B) wet body mass, and (C) SMR in 192 male *D. melanogaster*. Shown are the estimates (± se), denominator degrees of freedom (df_{den}), conditional Wald *F*-statistic, and *P*-values associated with the effects of test sequence and other effects fitted to territoriality (color treatment) and SMR (time of day, activity during (ACT_{20}) and activity prior (ACT_{20p}) to measurement, and temperature)

Source	Estimate ± SE	df_{den}	*F*	*P*
(A) territoriality				
Intercept	5.996 ± 1.631			
$Color_{[red]}$	−0.134 ± 0.101	96.6	1.8	0.1862
Test sequence	−0.251 ± 0.098	59.1	6.6	0.0130
Time of day	−0.008 ± 0.002	132.9	11.1	0.0011
(B) Body mass				
Intercept	−0.110 ± 0.101			
Test sequence	0.060 ± 0.054	129.2	1.3	0.2633
(C) SMR				
Intercept	−0.362 ± 0.137			
Test sequence	0.253 ± 0.087	20.5	8.3	0.0089
ACT_{20}	0.412 ± 0.039	329.5	110.1	<0.0001
ACT_{20p}	0.382 ± 0.040	338.0	90.9	<0.0001
Temperature	0.147 ± 0.037	63.2	15.9	0.0002

Indirect effects on territoriality

The variance estimate associated with the identity of the opponent ($V_{opp\text{-}terr}$; indirect effects) during the territoriality assays was considerably lower than that of the direct effect variance (Table 2) and the "opponent repeatability" for territoriality was correspondingly low ($R_{opp\text{-}terr}$ = 0.084 ± 0.070). This indicates that weak indirect effects occurred in our territoriality assay, although covariances with direct effects are still possible as V_{opp} was not zero. Indeed, the correlation between opponent identity effects on territoriality and focal identity effects on body mass was significant and negative ($r_{foc\text{-}mass,opp\text{-}terr}$ = −0.421 ± 0.242; P = 0.036; Figure 2; Table 2B). Finally, the correlations between direct and indirect effects were nonsignificant for both territoriality and SMR (Table 2B).

Table 2

Random effects from a multivariate mixed model of territoriality (terr), wet body mass (mass) and SMR in 192 male *D. melanogaster*. (A) Variance components included direct among-individual variances (V_{foc}; focal identity) fitted to all three traits, and indirect among-individual variance (V_{opp}; opponent identity). Other random effects included dyad effects (V_{dyad}) fitted to territoriality only and respirometry unit (V_{maven}) fitted to SMR only. Also included are the (B) among-individual correlations between direct and indirect phenotypic variance ($r_{foc,\ opp}$), and (C) residual correlations among in territoriality and SMR. Bold denotes significant correlations via a likelihood ratio test with 1 *df* ($P < 0.05$).

	Estimate ± SE	χ^2	P
(A) Variance components			
$V_{foc\text{-}terr}$	0.273 ± 0.074		
$V_{foc\text{-}mass}$	0.831 ± 0.099		
$V_{foc\text{-}SMR}$	0.123 ± 0.032		
$V_{opp\text{-}terr}$	0.077 ± 0.065		
$V_{dyad\text{-}terr}$	0.154 ± 0.089		
$V_{maven\text{-}SMR}$	0.025 ± 0.014		
$V_{e\text{-}terr}$	0.411 ± 0.091		
$V_{e\text{-}mass}$	0.181 ± 0.024		
$V_{e\text{-}SMR}$	0.256 ± 0.029		
(B) Among-individual correlations			
$r_{foc\text{-}terr,foc\text{-}mass}$	0.163 ± 0.122	1.74	0.1869
$r_{foc\text{-}terr,foc\text{-}SMR}$	0.345 ± 0.188	3.38	0.0661
$r_{foc\text{-}mass,foc\text{-}SMR}$	**0.985 ± 0.094**	**78.9**	**<0.0001**
$r_{foc\text{-}terr,opp\text{-}terr}$	0.168 ± 0.318	0.26	0.6115
$r_{foc\text{-}mass,\ opp\text{-}terr}$	**−0.421 ± 0.242**	**4.38**	**0.0363**
$r_{foc\text{-}SMR,opp\text{-}terr}$	−0.150 ± 0.279	0.28	0.5938
(C) residual correlations			
$r_{e\ terr\text{-}mass}$	−0.070 ± 0.103	0.47	0.4926
$r_{e\ terr\text{-}SMR}$	−0.035 ± 0.096	0.13	0.7134
$r_{e\ mass\text{-}SMR}$	0.060 ± 0.093	0.40	0.5264

DISCUSSION

We measured territoriality, body mass, and SMR twice in a large sample of 192 individual male *D. melanogaster* to estimate: 1) the among-individual (i.e., direct effect) variances and repeatabilities of territoriality, body mass, and SMR; and 2) their among- (r_{ind}) and within-individual (r_e) correlations. Moreover, we tested 3) whether individuals differed in the extent to which they influenced territoriality in others (i.e., indirect effects); and 4) whether these indirect effects were correlated with direct effects on territoriality, body mass, and SMR. We detected direct effect variances for all traits and found that that the repeatability of body mass was high while that of territoriality and SMR were moderate. The among-individual correlation between territoriality and SMR (i.e., $r_{foc\text{-}terr,foc\text{-}SMR}$) was positive although marginally nonsignificant (Figure 1). In contrast to the direct effect on territoriality, the indirect effect was weak. Therefore, males consistently differed in their territoriality and varied little in how they influenced the expression of territoriality in an opponent during a dyadic trial. Finally, the direct–indirect correlation between an individual's body mass and their effect on an opponent's territoriality (i.e., $r_{foc\text{-}mass,opp\text{-}terr}$) was negative and significant, indicating that the weak indirect effects we detected in territoriality were associated with individual differences in body mass. Therefore, within the context of dyadic encounters over a relatively large patch of food (see below), larger individuals suppress territoriality of their opponents by more often excluding those they interact with from the food resource.

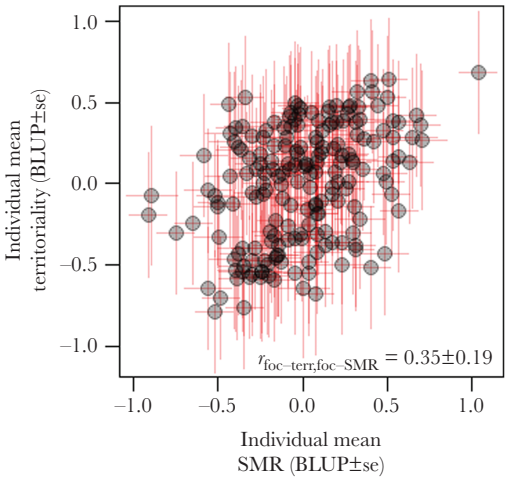

Figure 1
Representation of the marginally nonsignificant (P = 0.066) positive among-individual correlation between territoriality and SMR in 192 male *D. melanogaster*, displayed using the best linear unbiased predictors (i.e., BLUPs ± se) extracted from a multivariate mixed model (Tables 1 and 2).

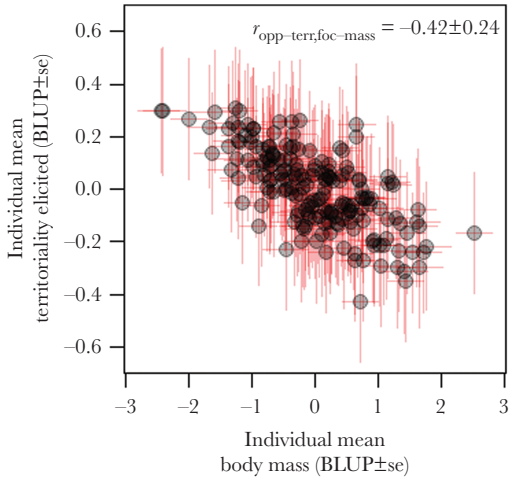

Figure 2
Representation of the significant negative correlation between indirect effects in territoriality and direct effects in wet body mass in 192 male *D. melanogaster*, displayed using the best linear unbiased predictors (i.e., BLUPs ± SE) extracted from a multivariate mixed model (Tables 1 and 2).

Repeatability

The repeatability of body mass was, unsurprisingly, very high, whereas estimates for territoriality and SMR were more moderate but still fairly precise. Our estimate for territoriality ($R_{foc\text{-}terr}$ = 0.298 ± 0.072) is only slightly less than the average of 0.37 for behavioral traits in general (Bell et al. 2009). The estimate for SMR was similar ($R_{foc\text{-}SMR}$ = 0.334 ± 0.054) and matches a previous estimate for this population (R = 0.33 ± 0.06; Videlier et al. 2019). However, these estimates are not directly comparable because Videlier et al. (2019) conditioned SMR on body mass (by including mass as a fixed effect), whereas we included body mass as a response variable in a multivariate model. Our approach has the advantage of allowing estimation of the among- and within-individual variance in body mass and its covariance with SMR. In our case, the among-individual body mass-SMR correlation was extremely strong ($r_{foc\text{-}mass,foc\text{-}SMR}$ = 0.985 ± 0.094), indicating that essentially all of the among-individual variance in SMR was shared with body mass, and conditioning SMR on body mass yields a repeatability of zero.

Relationship between territoriality and SMR

A recent meta-analysis showed that behaviors with known consequences for energy gain or expenditure tended to be positively correlated with maintenance metabolism (Mathot et al. 2019). A territory provides access to food, and hence a source energy, but is likely to have energetic costs arising from having to aggressively defend it (e.g., wing threats, foreleg fencing, lunging, holding, and tussling). The overall energy costs of territoriality are unknown and determining this would require comparing the overall energy expended on territoriality against total daily energy expenditure. Nevertheless, the positive (although nonsignificant P = 0.066) $r_{foc\text{-}terr,foc\text{-}SMR}$ suggests that increased SMR is a cost associated with the metabolic machinery required for territorial behavior, lending some support to the performance model (Careau et al. 2008). Fitness in male *D. melanogaster* increases with the number of mates (Bateman 1948) and, if territory defense provides access to females and hence mating opportunities (Dow and von Schilcher 1975; Hoffmann 1987a,1988; White and Rundle 2015), selection may favor energy investment in territoriality even if it generates a physiological maintenance cost in terms of a higher SMR. Relative to standard *Drosophila* laboratory populations, territoriality may be a more important component of male fitness in our population given the low-density mating environment with added structural complexity that they experience during their 4-day mating phase every generation. Previous studies have shown that such complex mating environments alter sexual interactions, reducing mating rates and male-induced harm of females in this species (Yun et al. 2017, 2019). Recognizing that additional work is needed to confirm this suggested link between territoriality and SMR, it would be interesting test whether the strength of this correlation depends on mating environment.

Indirect effects

Indirect effects on territoriality were present but weak (8.4% of phenotypic variance). In our assays, the observed frequency of both males occupying the food (40% of observations) was similar to levels expected under independence (39.7%, calculated as the product of the marginal occupancy probabilities), suggesting that one male was not excluding the other from the resource. While this could indicate the absence of territorial behavior in this population, a more likely explanation involves the division of the food resource into "sub-territories." Studies in insects, including *D. melanogaster*, show that territorial behavior changes, and monopolization becomes less common, as the size of the resource increases (Baker 1983; Hoffmann and Cacoyianni 1990; Lim et al. 2014). Hoffmann and Cacoyianni (1990) used a very similar assay to ours and found that males more aggressively defended a food resource of 20 mm in diameter compared to a smaller (8 mm) or larger (40 mm) resource. Our resource was 37 mm in diameter and approached the size at which Lim et al. (2014) found no effect of one male on how frequently the second male occupied the food patch (although occupancy of proximal areas to the food were affected). Thus, it is possible in our case that males were unwilling or unable to monopolize the resource and they effectively subdivided it, with each male holding its own (sub)territory. In such a situation, more nuanced measures of territoriality may be useful beyond simply occupancy (e.g., Lim et al. 2014). Other factors also influence territoriality in *D. melanogaster* including social environment (e.g., the presence of females and the density of competitor males) and the amount of food itself (Hoffmann and Cacoyianni 1990; Lim et al. 2014). As the nature of the behavioral interactions change in response to such factors, the magnitude of the indirect variance may change, and further insight could be gained by estimating the magnitude of indirect effects under varying conditions.

Under exploitative competition for a shared resource, a negative direct–indirect correlation is expected to arise if highly competitive individuals control the resource to the exclusion of less competitive individuals. In our case, the direct–indirect correlation ($r_{foc\text{-}terr,opp\text{-}terr}$) was low and nonsignificant, indicating that the weak indirect effects we observed were not driven by individual differences in territoriality per se. However, the significant and negative $r_{foc\text{-}mass,opp\text{-}terr}$ indicated that larger individuals tended to suppress territoriality in those they interacted with. This effect may arise from competitive ability: Large individuals may simply be better at excluding others from the resource than small individuals, as observed in eastern chipmunks (Couchoux et al. 2021). A nonmutually exclusive explanation is that, because of their higher energetic requirements, larger flies may need to defend a greater portion of the resource for their own compared to smaller flies. However, this possibility is not supported by the fact that that $r_{foc\text{-}SMR,opp\text{-}terr}$ was not significant, suggesting that the mass influence on the opponent territoriality was independent of its shared variation with SMR. As with the indirect effect itself, the direct–indirect correlations are likely to be sensitive to the biotic and abiotic conditions of the assay. An interesting topic for future study will be to examine whether and how the direct–indirect correlations change in response to variation in factors like size of the food resource, female presence/absence, and density of competitors.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at *Behavioral Ecology* online.

Table S1. Structure of the symmetric among-individual correlation matrix for focal and opponent territoriality, focal body mass, and focal standard metabolic rate (SMR). Direct (V_{foc}) and indirect (V_{opp}) variances are given along the diagonal and among-individual correlations (r_{ind}) are on the off diagonals.

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AUTHOR CONTRIBUTIONS

M.T., V.C., and H.D.R. conceived the project and study design, M.T. and M.V. collected the data, M.T. and V.C. analyzed the data, and all authors wrote the manuscript.

Data Availability: Analyses reported in this article can be reproduced using the r-script and data provided by Tremblay et al. (2021).

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