

# Functional traits in red flour beetles: the dispersal phenotype is associated with leg length but not body size nor metabolic rate

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## Summary

1. Individuals vary in their ability to disperse. Much of this variation can be described by covarying phenotypic traits that are related to dispersal (constituting the ‘dispersal phenotype’ or ‘dispersal syndrome’), but the nature of the associations among these traits is not well understood. Unravelling the associations among traits that potentially constitute the dispersal phenotype provides a foundation for understanding evolutionary trade-offs due to variation in dispersal.

2. Here, we tested five predictions pertaining to the relationships among physiological, morphological and movement traits that are associated with dispersal, using a species with a long history as a laboratory model for studying ecological phenomena, red flour beetles (*Tribolium castaneum*).

3. We identified a dominant axis of movement ability that describes variation in dispersal-related movement traits. Individuals that scored positively on this axis moved at higher speed, travelled longer distances, had lower movement intermittency and dispersed quicker to a specified area.

4. Relative leg length, but not body size nor routine metabolic rate related positively with movement ability, indicating a likely mechanistic relationship between increased stride length and movement ability.

5. Our data suggest that the dispersal phenotype may be more strongly linked to morphological traits than physiological ones. We demonstrate that associations among many functional traits do not necessarily conform to *a priori* expectations, and predict that the substantial intraspecific variation in trait values may be important for selection. Movement is a complex behavioural trait, but it has a mechanistic basis in locomotor morphology that warrants further exploration.

**Key-words:** activity, dispersal syndrome, locomotion, movement, physiology, routine MR, speed, *Tribolium castaneum*

## Introduction

The dispersal phenotype, or dispersal syndrome, is the specific expression of multiple phenotypic (physiological, morphological and behavioural) traits that facilitate efficient movement and effective dispersal (Ronce & Clobert 2012). The genetic and demographic structure of a population is significantly affected by the patterns of covariation

among traits constituting the dispersal phenotype (Ronce & Clobert 2012), and by the variation in dispersal phenotypes among individuals (Bowler & Benton 2005, 2009; Shaw & Kokko 2014).

An animal's size is often related to a range of physiological and fitness-related traits, including metabolic rate (White & Kearney 2013), predation success (Blanckenhorn 2000), mate choice (Jennions & Petrie 1997), fecundity (Honěk 1993) and movement propensity (Stevens *et al.* 2014). Across a range of taxa, body size correlates

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positively with movement and dispersal ability such that larger individuals have a stronger propensity to move, or move a greater distance (e.g. Anholt 1990; Benard & McCauley 2008; Sekar 2012; Stevens *et al.* 2012; Whitmee & Orme 2013). Explanations for a positive correlation are often directed towards the larger size or enhanced development of locomotor appendages that facilitate movement (Phillips *et al.* 2006; Laparie *et al.* 2013; Stevens *et al.* 2014). However, there are also examples where body size correlates negatively with dispersal (e.g. Hanski, Peltonen & Kaski 1991), or intermediate-sized individuals move more (e.g. McDevitt *et al.* 2013) due to complex interactions between the phenotype, population structure and the environment. In invasive cane toads *Rhinella marina*, dispersive individuals at the edge of their range that had a stronger propensity to move had longer bodies and greater endurance (Llewelyn *et al.* 2010), and relatively longer legs (Phillips *et al.* 2006). Functional locomotor morphology, such as leg length, wing shape or supporting muscle architecture for limbs, tends to relate positively with different movement characteristics and also has consequences for energy expenditure (Roff & Fairbairn 1991; Choi, Shim & Ricklefs 2003; Ducatez *et al.* 2012; Lowe & McPeck 2012).

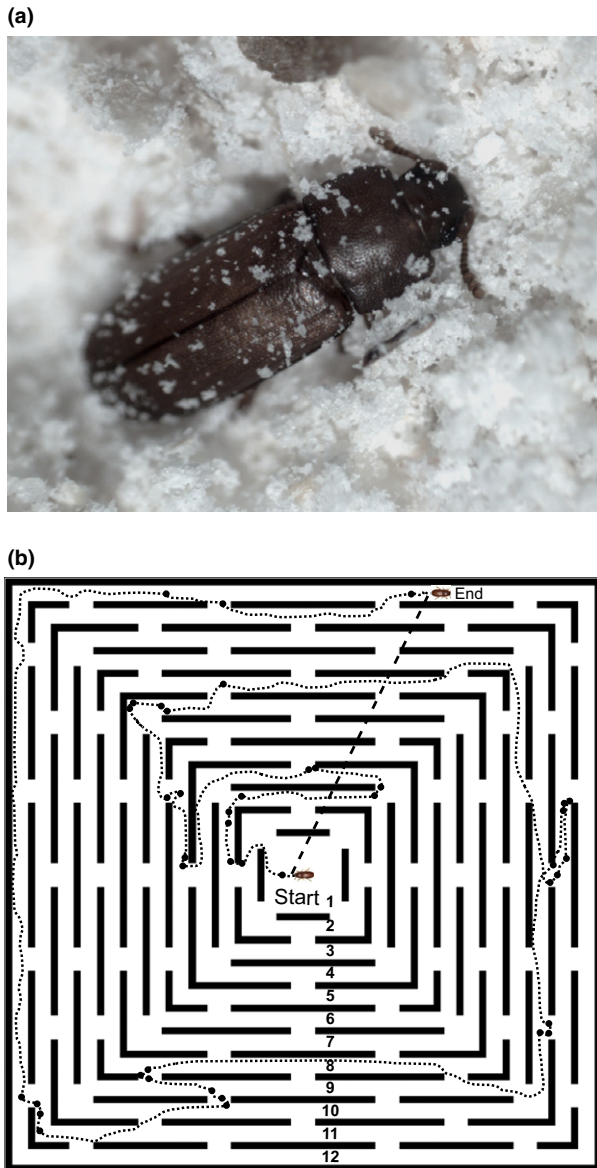
Rates of energy expenditure under different contexts are a particularly well-studied area of animal physiology with considerable attention devoted to the inter- and intraspecific variation in metabolic rate (Glazier 2005; Burton *et al.* 2011; White & Kearney 2013). Metabolic rate (MR), basally or at rest, is a proxy for maintenance energy expenditure and is strongly associated with temperature (Clarke & Fraser 2004), body size (White 2011) and physical activity (Speakman & Selman 2003). While there is certainly an association between resting MR and levels of voluntary activity, the mechanism and direction of this association is generally not well understood. These relationships can, however, be divided into two general models: performance and allocation (Careau *et al.* 2008). The performance model suggests that individuals with a higher MR at rest are able to attain and invest greater amounts of energy into activity [e.g. salmonids that have a higher standard MR are more aggressive (Cutts, Metcalfe & Taylor 1998)]. Alternatively, the allocation model suggests that the total energy available for an individual is partitioned between resting MR and activity, such that individuals with a lower resting MR have more energy left to allocate to activity (e.g. mice forced to run more to receive a food reward reduced their resting MR (Vaanholt *et al.* 2007)).

If the partitioning of energy into dispersal is a trade-off with energy available for other traits, then we expect that actively dispersing individuals would have a different phenotype to those that are less exploratory or active (Clobert *et al.* 2009). Even within a species, the morphology of locomotor structures can affect MR. For example, large wing morphs of sand field crickets *Gryllus firmus* have a higher MR than smaller wing morphs due to the higher

cost of maintaining the larger flight muscle tissues (Crnokrak & Roff 2002). MR appears to be related with dispersal, such that individuals from invasion front populations generally have higher MR than individuals that are less effective at moving (Haag *et al.* 2005; Niitepõld *et al.* 2009; Myles-Gonzalez *et al.* 2015).

Behaviours associated with dispersal are diverse and often challenging to measure. Proximate measurements that have been found to correlate with dispersal include distance travelled (Ducatez *et al.* 2012), movement speed (Phillips *et al.* 2006; Delgado *et al.* 2010), boldness and exploratory behaviours (Rehage & Sih 2004; Cote *et al.* 2010), and measures of locomotor activity (Socha & Zemek 2003). Intuitively, many movement traits will be fundamentally linked and therefore strongly associated (e.g. speed and distance travelled). As animal movement and ecology can be altered by behavioural patterns such as travelling along straight or tortuous paths (Brown, Phillips & Shine 2014), or moving intermittently (Bazazi *et al.* 2012), examining trait associations remains crucial to understanding drivers of movement. Simple measures of locomotor activity can, in some species, be broadly representative of complex movement behaviours. For example, spontaneous activity, the observable activity of an individual when not specifically externally stimulated (Ewing 1963), is used as a proxy for exploratory behaviour in *Drosophila* species (Martin 2003). The application of spontaneous activity as a simple movement metric in species other than *Drosophila* is rare. Hence, examining the associations between spontaneous activity and more complex measurements would be useful to identify whether a simple measurement may be a suitable alternative to more complex movement metrics.

In this study, we investigated dispersal phenotypes by examining the associations among body size, relative leg length, energy expenditure and movement behaviour in the red flour beetle *Tribolium castaneum* (Herbst 1797). This species and its close relative *Tribolium confusum* have long been used as models for laboratory-based microcosm and demographic studies of dispersal (e.g. Naylor 1961; Ziegler 1976; Zirkle, Dawson & Lavie 1988; Campbell & Hagstrum 2002; Łomnicki 2006; Melbourne & Hastings 2009); however, morphology, physiology and movement behaviour have not been studied concurrently with this study system. We aimed to test the following predictions. First, that the morphological and physiological traits (body size, leg length and metabolic rate) are all associated. Secondly, that body size is positively associated with movement, such that larger individuals move farther and faster. Thirdly, that leg length is positively associated with movement, such that individuals with longer legs relative to body size would have a greater capacity to move quickly and efficiently. Fourthly, that routine energy expenditure has an association with movement, supporting either the performance or the allocation model of energy expenditure. And finally, that spontaneous activity may be an appropriate proximal measurement for more complex movement traits.



**Fig. 1.** (a) Adult red flour beetle *Tribolium castaneum* (Herbst, 1797) moving through wheat flour. Photograph by Pieter Arnold. (b) Complex maze environment based on 12 evenly-spaced square radial passageways used to quantify aspects of movement. A typical movement path taken by an individual beetle is shown where the dotted line represents total path length and dashed line represents linear distance travelled from start to end of the trial. Points of no movement during the trial (behavioural intermittence) are shown as solid circles.

## Materials and methods

### STUDY SPECIES AND HOUSING

A laboratory population of *T. castaneum* (Fig. 1a) established from a wild-type line (QTC4) that was sourced from the Postharvest Grain Protection Team (Department of Agriculture, Fisheries and Forestry; Brisbane, QLD, Australia) was used throughout experiments. Stocks were maintained on 200 g of flour medium (95% wholemeal stoneground wheat flour; 5% torula yeast) in 1-L cylindrical containers under controlled conditions of

$29.5 \pm 1$  °C and cultured fortnightly to separate cohorts and refresh the medium. Pupae were collected from the stock containers and sexed by examining the external genitalia (Halstead 1963) under an Olympus SZ61 stereomicroscope (Olympus Australia Pty. Ltd., Notting Hill, VIC, Australia). Groups of five pupae were separated by the date of eclosion and by sex and then placed in 70-mL containers with 5 g of flour that was replaced fortnightly. Age in this study ranged from 5 to 105 days post-eclosion, where age was known within  $\pm 12$  h. At least 24 h prior to taking measurements, beetles were placed in empty 70-mL containers to fast before respirometry. A total of 290 individuals ( $n = 145$  males and  $n = 145$  females) were each measured once only for multiple traits in the following order: metabolic rate and spontaneous activity, movement behaviour, mass and morphometrics.

### TRAIT MEASUREMENT

All trait measurements were conducted as described in detail in Arnold, Cassey & White (2016a). Briefly, four beetles were measured at once, where each individual was placed into one of four 2-mL chambers in-line with a flow-through respirometry system utilizing two LI-7000 CO<sub>2</sub>/H<sub>2</sub>O analysers (Li-COR Inc., Lincoln, NE, USA) to measure CO<sub>2</sub> production through respiration over a 1-h period. CO<sub>2</sub> production was used as a proxy for metabolic rate (Lighton 2008), which, as measured in this study, was defined as routine metabolic rate (routine MR) due to the presence of spontaneous activity during measurement (Mathot & Dingemanse 2015). Spontaneous activity was measured synchronously with routine MR by using an infrared activity detector (LAM10H; TriKinetics Inc., Waltham, MA, USA). For each discrete interruption of the infrared beam, a value of 1 was recorded, and the total number of counts over the measurement period was converted to counts h<sup>-1</sup>.

Following spontaneous activity measurements, individuals were placed in a maze (Fig. 1b), which represents a spatially complex environment with barriers to movement and multiple junctions. In an open arena, *T. castaneum* will actively move towards and remain along the arena edge (P. A. Arnold, pers. obs.), whereas in a maze, movement is typically more varied among individuals and multiple movement attributes can be easily measured. Movement through the maze was video recorded (1280 × 720 px; 10 frames s<sup>-1</sup>) for 3 min, and then, individual movement paths were digitized and tracked in MATLAB software (The MathWorks, Inc., Natick, MA, USA) using an extended Kalman filter (Hedrick 2008). Minimum and maximum movement speed (calculated as the lower and upper 5th percentiles of speed during the trial), behavioural intermittence (as frequency of stops during the trial), path length (total length travelled during the trial), linear distance travelled (straight line distance between their positions at time 0 and time 180 s) and time to maze edge (reach the outer passageway) were calculated. Fresh mass (mg) was measured, and then, individuals were photographed dorsally and ventrally to measure elytron (modified hardened forewing; dorsal) length and width, pronotum (thorax; dorsal) length and width, and hind femur length using IMAGEJ software (v1.46r, National Institutes of Health, Bethesda, MD, USA).

### STATISTICAL ANALYSES

Data were tested for normality, homogeneity of variance and the presence of interactions. Spontaneous activity was centred and scaled around zero according to the Z-distribution for analyses. The morphometric measurements of elytron length and width, pronotum length and width, and mass were partially collinear; therefore, a principal components analysis (PCA) was conducted to distil these body size traits into a single trait (the 1st principal component, PC1, which explained 89.4% of the variance among the five traits; Table 1), hereafter called 'body size'. A second

**Table 1.** Principal components analysis and relative loading of morphological and movement traits onto principal components that describe 'body size', 'movement ability' (PC1) and 'movement displacement' (PC2)

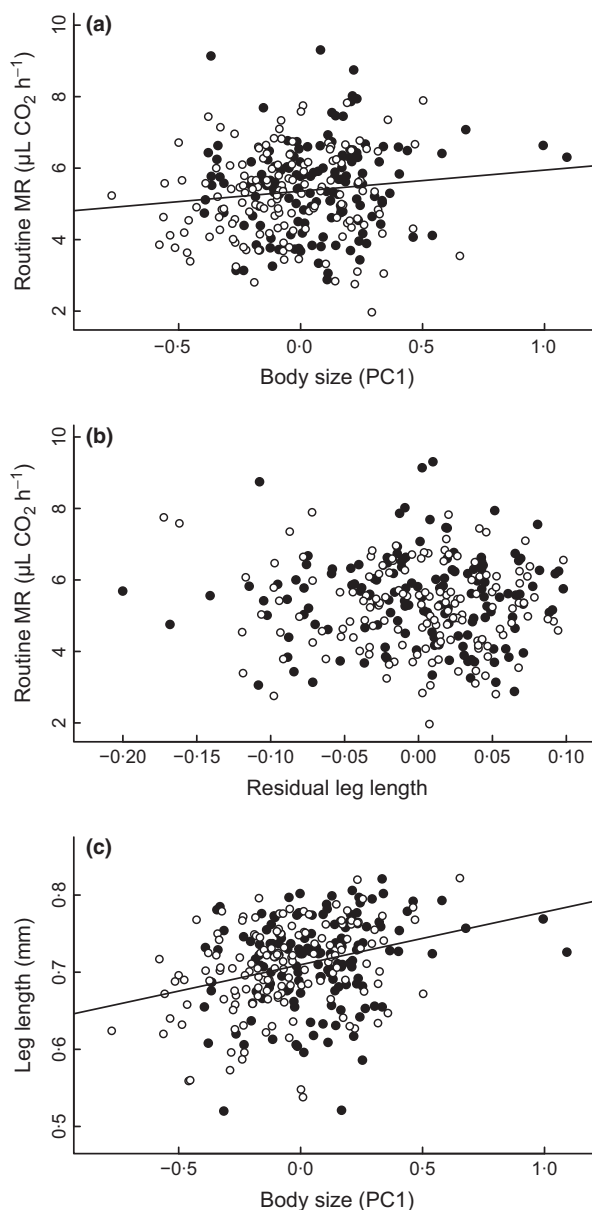
Loadings	Body size		Movement	
	Body size traits	Body size PC1	Movement traits	Movement PC1 PC2
Elytron length		0.302	Path length	0.492 -0.153
Elytron width		0.154	Linear distance travelled	0.221 0.753
Pronotum length		0.098	Minimum speed	0.384 -0.409
Pronotum width		0.153	Maximum speed	0.463 -0.012
Body mass		0.923	Behavioural intermittence	-0.471 0.192
–	–	–	Time to maze edge	-0.355 -0.454
Eigenvalue		0.0644		3.737 1.063
Proportion variance		89.45%		62.29% 17.72%

PCA was conducted to condense path length, linear distance, minimum speed, maximum speed, behavioural intermittence and time to maze edge into two traits (Table 1). PC1 explained 62.3% of the total variance among the movement traits and was loaded most strongly by path length and both measures of speed (positively) and behavioural intermittence (negatively) and is therefore called 'movement ability' hereafter. PC2 explained a further 17.7% of the variance among movement traits and was loaded most strongly by linear distance travelled (positively) and time to maze edge (negatively) and is therefore called 'movement displacement' hereafter. Leg length and routine MR are both presented as body-size-corrected residuals. Data analyses were conducted in the R software environment version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria), utilizing the linear mixed-effects model 'lme4' package v1.0.4 (Bates *et al.* 2014), the multi-model interference 'MuMIn' 1.15.6 package (Bartoń 2012). Linear mixed-effects regression (LMER) models were fitted, and then, models were simplified using conditional model averages based on Akaike weights of  $>0.004$  to subset the model and then removal of near-zero importance models by fitting a cumulative sum of Akaike weights to  $\leq 0.995$ . No interactions were significant after the model simplification process.

## Results

### ASSOCIATIONS AMONG BODY SIZE, LEG LENGTH AND MR

Body size is typically a strong predictor of MR, and here, we found that routine MR was significantly positively associated with body size (Fig. 2a;  $Z = 2.17$ ,  $P = 0.03$ ), but had no relationship with relative leg length (Fig. 2b;  $Z = 0.68$ ,  $P = 0.496$ ). Unsurprisingly, leg length was significantly positively associated with body size (Fig. 2c;  $Z = 5.81$ ,  $P < 0.001$ ) as larger individuals typically also had longer legs. Given the scaling of routine MR and leg length with body size, these traits were corrected for body size in further data presentation. Descriptive statistics for all traits are shown in Table 2.



**Fig. 2.** Associations among physiological and morphological traits: (a) body size and routine MR, (b) residual leg length (adjusted for body size) and routine MR, and (c) body size and leg length. Regression lines represent significant associations only. Males ( $n = 145$ ) are represented by unfilled circles (○), and females ( $n = 145$ ) are represented by filled circles (●).

### BODY SIZE AND MOVEMENT

Body size was not positively associated with any measurement of movement, contrary to our prediction that larger individuals should be able to move farther and faster than smaller individuals. Body size was not a significant predictor of movement ability ( $Z = 1.39$ ,  $P = 0.166$ ; Fig. 3a) or movement displacement ( $Z = 0.46$ ,  $P = 0.643$ ; Fig. 3d). However, there was a significant negative relationship between body size and spontaneous activity ( $Z = 2.04$ ,  $P = 0.04$ ; Fig. 3g), where larger individuals were typically less active than smaller ones.



**Table 2.** Descriptive statistics (means  $\pm$  95% confidence intervals) for all traits that were measured and calculated in this study

Trait	Male Mean ( $\pm$ 95% CI)	Female Mean ( $\pm$ 95% CI)
Age (days post-eclosion)	35.14 (30.1 to 40.2)	35.14 (30.1 to 40.2)
Routine MR ( $\mu\text{L CO}_2 \text{ h}^{-1}$ )	5.23 (5.03 to 5.42)	5.49 (5.29 to 5.69)
Spontaneous activity (counts $\text{h}^{-1}$ )	182.4 (156.1 to 208.7)	134.1 (114.5 to 153.6)
Mass (mg)	2.392 (2.354 to 2.430)	2.504 (2.467 to 2.540)
Elytron length (mm)	2.526 (2.509 to 2.542)	2.590 (2.576 to 2.605)
Elytron width (mm)	1.290 (1.281 to 1.298)	1.313 (1.301 to 1.320)
Pronotum length (mm)	0.808 (0.801 to 0.814)	0.824 (0.817 to 0.831)
Pronotum width (mm)	1.181 (1.173 to 1.188)	1.206 (1.199 to 1.213)
Femur length (mm)	0.705 (0.695 to 0.714)	0.715 (0.705 to 0.724)
Path length (mm)	1769 (1701 to 1839)	1814 (1737 to 1891)
Linear distance travelled (mm)	192.9 (184.7 to 200.0)	197.4 (189.4 to 205.4)
Average speed (mm $\text{s}^{-1}$ )	9.83 (9.45 to 10.22)	10.08 (9.65 to 10.50)
Minimum speed (mm $\text{s}^{-1}$ )	4.13 (3.91 to 4.35)	4.21 (3.97 to 4.45)
Maximum speed (mm $\text{s}^{-1}$ )	16.86 (16.21 to 17.51)	17.27 (16.53 to 18.00)
Behavioural intermittence (frequency)	310.0 (291.3 to 328.7)	297.3 (282.2 to 312.4)
Time to maze edge (s)	115.9 (108.0 to 123.9)	112.3 (104.4 to 120.2)
Body size (PC1)	-0.066 (-0.107 to -0.025)	0.066 (0.027 to 0.105)
Movement ability (PC1)	-0.110 (-0.430 to 0.211)	0.110 (-0.199 to 0.419)
Movement displacement (PC2)	-0.020 (-0.172 to 0.132)	0.020 (-0.163 to 0.203)

#### RELATIVE LEG LENGTH AND MOVEMENT

Leg length relative to body size was significantly associated with two measurement of movement. Residual leg length was positively related to movement ability ( $Z = 5.97$ ,  $P < 0.001$ ; Fig. 3b), which is consistent with our prediction that individuals with longer legs relative to their body size would move farther and faster. However, residual leg length was also significantly negatively associated with movement displacement ( $Z = 2.42$ ,  $P = 0.015$ ; Fig. 3e); individuals with relatively longer legs ultimately moved less distance away from their starting position. Relative leg length was not significantly related to spontaneous activity ( $Z = 1.13$ ,  $P = 0.257$ ; Fig. 3h).

#### ROUTINE MR AND MOVEMENT

Routine MR corrected for body size was only significantly associated with one measurement of movement. Routine MR was not significantly related to movement ability ( $Z = 1.18$ ,  $P = 0.236$ ; Fig. 3c), or to movement displacement ( $Z = 1.95$ ,  $P = 0.051$ ; Fig. 3f); however, this association was marginally significant and trends negatively. Routine MR was significantly positively related to spontaneous activity ( $Z = 4.02$ ,  $P < 0.001$ ; Fig. 3i), which is unsurprising given that the two traits were measured concurrently, and that increased activity increases MR.

#### SPONTANEOUS ACTIVITY AS A PROXY FOR MOVEMENT

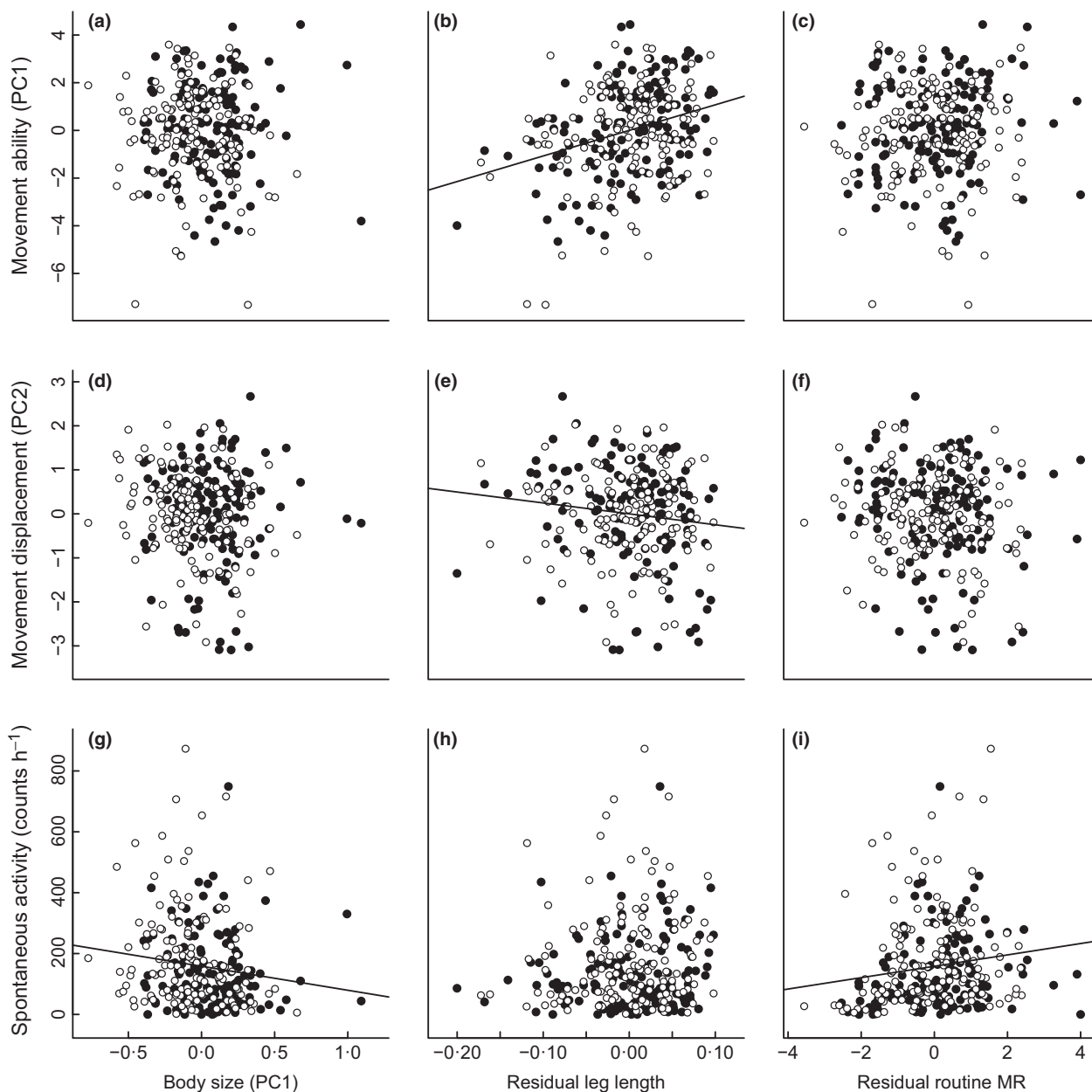
Spontaneous activity has been suggested to be a simple measure for complex movement behaviour. But here spontaneous activity did not relate significantly to movement ability ( $Z = 1.55$ ,  $P = 0.120$ ; Fig. 4a) or movement

displacement ( $Z = 0.84$ ,  $P = 0.402$ ; Fig. 4b). Full tables of averaged LMER models with the movement traits as response variables, including covariates, are presented in Supporting Information (see Tables S1 and S2, Supporting Information).

#### Discussion

The present study demonstrates that while the associations among physiological, morphological and behavioural traits associated with the dispersal phenotype may seem relatively intuitive, the strength, variance and even the direction of relationships may not be. Certainly, the well-established relationships of routine MR and leg length scaling with body size were significantly positively related as expected (Kleiber 1947; White & Kearney 2014). However, contrary to some of our predictions, we found that only relative leg length, but not body size nor metabolic rate, related positively to movement ability.

Larger individuals were predicted to have a greater movement ability and displacement; however, our findings do not support this hypothesis. A positive size-dispersal relationship has been found in some species (e.g. Anholt 1990; Stevens *et al.* 2012), but in others, small- or intermediate-sized individuals may be the ones to disperse (Hanski, Peltonen & Kaski 1991; McDevitt *et al.* 2013). The size-dispersal relationship frequently depends on the context and environment that triggers dispersal events (Benard & McCauley 2008), and our results suggest that short ground-based movement may not strongly relate to within-species variation in body size. Large body size has potential to handicap movement in some species (Benton & Bowler 2012), and smaller, less competitive individuals may disperse before larger individuals to reach and exploit



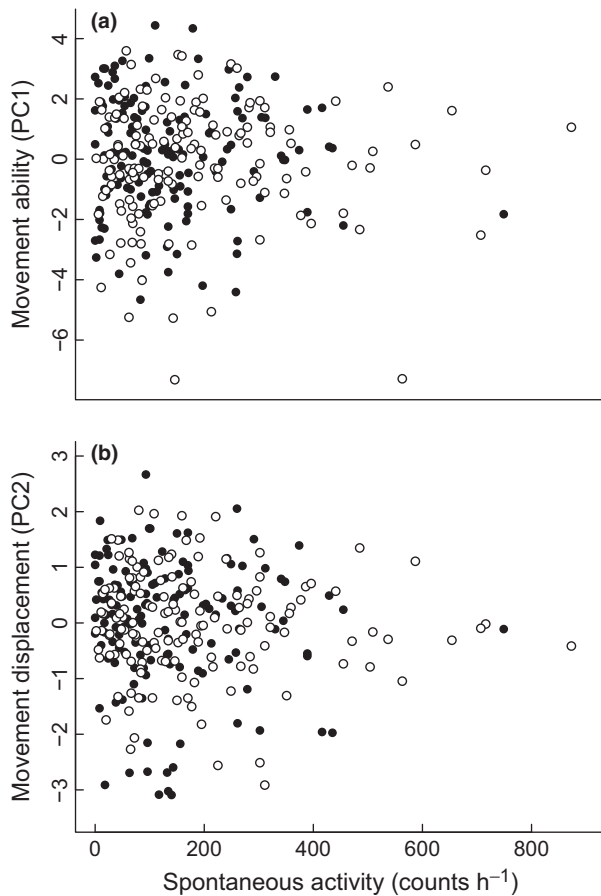
**Fig. 3.** Associations among six dispersal-related traits. Body size, residual leg length (adjusted for body size) and residual routine MR (adjusted for body size) were predictor variables for three movement traits: (a–c) movement ability (PC1), (d–f) movement displacement (PC2) and (g–i) spontaneous activity. Regression lines represent significant associations only. Males ( $n = 145$ ) are represented by unfilled circles (○), and females ( $n = 145$ ) are represented by filled circles (●).

resources earlier (Bowler & Benton 2005). Larger individuals were less active in the metabolic chambers during routine MR measurement. If the animals can perceive that the metabolic chamber is an energy-limited environment, then larger individuals may opt to conserve energy by reducing activity, as the absolute cost of movement is greater for larger individuals relative to smaller ones (Halsey 2016).

Movement ability was the most representative measure of variance among all dispersal-related traits. Individuals that scored positively along the axis of movement ability had a high minimum and maximum speed, a greater path length, less behavioural intermittence and reached the

maze edge quickly. Highly dispersive individuals have been suggested to share these movement characteristics, and this movement axis may be a suitable representation of dispersal. For example, dispersive cane toads from the edge of their range travelled farther and faster for longer periods without pausing compared to non-dispersive toads (Phillips *et al.* 2006; Alford *et al.* 2009; Llewellyn *et al.* 2010).

We found that individuals with relatively longer legs had greater movement ability. This relationship could be explained mechanistically by longer legs allowing an increased stride length, in turn allowing the animal to move a greater distance with each step taken, therefore



**Fig. 4.** Associations among three movement traits. Movement ability (PC1) and movement displacement (PC2) are intrinsically uncorrelated due to the nature of principal components analysis, therefore not shown. Associations between: (a) movement ability and spontaneous activity, and (b) movement displacement and spontaneous activity. Neither relationship is significant. Males ( $n = 145$ ) are represented by unfilled circles (○), and females ( $n = 145$ ) are represented by filled circles (●).

also achieving a greater speed (Zollikofer 1994). A long-term study of stream salamanders *Gyrinophilus porphyriticus* found that individuals with long forelimbs relative to their hindlimbs dispersed greater distance, indicating that locomotor morphology has a major role in long-distance dispersal and fitness (Lowe & McPeck 2012). Evolutionary mechanisms can select for individuals that have phenotypic traits that allow them to move farther, which may indicate the presence of a dispersal phenotype (e.g. Phillips *et al.* 2006; Laparie *et al.* 2013). Whether selection for greater movement or dispersal increases the length of locomotor structures independent of body size can only be directly determined through experimental evolution. Our finding of the significant negative relationship between relative leg length and movement displacement may be a continued searching behaviour. That is, individuals with relatively longer legs have a greater path length and reach the maze edge quickly, but upon reaching the edge, these individuals may back-track and return towards the maze centre, reducing their overall displacement.

We predicted that routine MR would be associated with movement, supporting either the performance or allocation hypotheses. Routine MR was not significantly related to either movement ability or displacement, but was with spontaneous activity. This relationship is intuitive; the more an individual moves during measurement of its energy expenditure, the greater its energy expenditure will be (Mathot & Dingemanse 2015) and thus may not be representative of movement or dispersal. Under the performance model of energy expenditure, individuals with greater performance (movement, in the present case) should have a higher MR (Careau *et al.* 2008). Under the allocation model, individuals with a higher resting MR have less energy remaining to allocate to activity (Careau *et al.* 2008). The marginally significant negative relationship between movement displacement and routine MR suggests that individuals with a high routine MR travelled less distance overall. The data describing this relationship were highly variable, and the allocation model cannot be assessed directly in our study as both maintenance energy and activity are confounded in the measure of routine MR. Resting MR has been previously found to be higher in individuals with greater movement propensity (Niitepõld *et al.* 2009; Myles-Gonzalez *et al.* 2015) and a recent study found that genes underlying metabolic processes are upregulated in dispersive cane toads (Rollins, Richardson & Shine 2015). Further investigations into the association between MR and movement are warranted, particularly given the potential involvement of genetic and epigenetic mechanisms (Rollins, Richardson & Shine 2015).

Our data do not support either the performance model or the allocation model of energy expenditure and add to a collection of studies demonstrating that MR does not always correlate with components of performance or fitness (e.g. life span: Hulbert *et al.* 2004; growth and survival: Burton *et al.* 2011; reproductive performance: Schimpf, Matthews & White 2012; locomotor performance: Le Galliard *et al.* 2013; aerobic performance: Merritt, Matthews & White 2013; scaling: White & Kearney 2013; behaviour: Royauté *et al.* 2015). Under different environmental contexts, the relationship between MR and other traits or between MR at rest and during activity (i.e. maximum or flight MR) can change and must be cautiously interpreted (e.g. Burton *et al.* 2011; Killen *et al.* 2012, 2013; Lebeau, Wesselingh & Van Dyck 2016; Metcalfe, Van Leeuwen & Killen 2016). Variation in MR is maintained in most natural populations allowing for different strategies for growth, behaviours and reproduction in specific contexts (Burton *et al.* 2011). Additionally, historical findings that established distinct paradigms of trait associations, including with MR, tend to be based on small sample sizes and therefore low statistical power, which may undermine identification of the true relationship (Button *et al.* 2013).

Our final prediction considered that spontaneous activity may be a useful approximate measure of complex movement traits. Spontaneous locomotor activity is

thought to represent the activity response of an animal when not specifically stimulated (Ewing 1963) and is the basis of many complex behaviours (Martin 2003). In the present study, neither movement ability nor displacement had any discernible association with spontaneous activity, and the relationship between morphological and physiological traits and movement was not similar to those observed with spontaneous activity. Therefore, at least for *T. castaneum*, spontaneous activity was not a useful proximate trait for complex movement traits.

Our results demonstrate that associations among traits are not always intuitive, and do not always conform to expectations from literature paradigms. We found that much of the variance among different movement traits can be described along a dominant axis of variation, movement ability. We have shown that movement ability (which potentially represents dispersal) is more strongly linked with locomotor morphology than body size or MR. These findings support the hypotheses that locomotor morphology could be a strong driver distributing individuals through a landscape by spatial sorting or natural selection, thereby accelerating rates of range expansion in invasive species (Shine, Brown & Phillips 2011). The movement ability axis and the significant trait associations identified here can be used as a foundation for studying variation in movement and dispersal, and *T. castaneum* represents an ideal opportunity to test this hypothesis using experimental evolution in a laboratory setting.

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## Data accessibility

Data have been deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.t3d52> (Arnold, Cassey & White 2016b).

## References

- Alford, R.A., Brown, G.P., Schwarzkopf, L., Phillips, B.L. & Shine, R. (2009) Comparisons through time and space suggest rapid evolution of dispersal behaviour in an invasive species. *Wildlife Research*, **36**, 23–28.
- Anholt, B.R. (1990) Size-biased dispersal prior to breeding in a damselfly. *Oecologia*, **83**, 385–387.
- Arnold, P.A., Cassey, P. & White, C.R. (2016a) Maturity matters for movement and metabolic rate: trait dynamics across the early adult life of red flour beetles. *Animal Behaviour*, **111**, 181–188.
- Arnold, P.A., Cassey, P. & White, C.R. (2016b) Data from: Functional traits in red flour beetles: the dispersal phenotype is associated with leg length but not body size nor metabolic rate. *Dryad Digital Repository*, <http://dx.doi.org/10.5061/dryad.t3d52>.
- Bartoń, K. (2012) Package 'MuMIn'. Multi-model interference. <https://cran.r-project.org/web/packages/MuMIn/index.html>, R package version 1.15.6.
- Bates, D., Maechler, M., Bolker, B.M. & Walker, S. (2014) lme4: linear mixed-effects models using Eigen and S4. <http://cran.r-project.org/package=lme4>, R package version 1.1-7.
- Bazazi, S., Bartumeus, F., Hale, J.J. & Couzin, I.D. (2012) Intermittent motion in desert locusts: behavioural complexity in simple environments. *PLoS Computational Biology*, **8**, e1002498.
- Benard, M.F. & McCauley, S.J. (2008) Integrating across life-history stages: consequences of natal habitat effects on dispersal. *The American Naturalist*, **171**, 553–567.
- Benton, T.G. & Bowler, D.E. (2012) Dispersal in invertebrates: influences on individual decisions. *Dispersal Ecology and Evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 41–49. Oxford University Press, Oxford, UK.
- Blanckenhorn, W.U. (2000) The evolution of body size: what keeps organisms small? *The Quarterly Review of Biology*, **75**, 385–407.
- Bowler, D.E. & Benton, T.G. (2005) Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews*, **80**, 205–225.
- Bowler, D.E. & Benton, T.G. (2009) Variation in dispersal mortality and dispersal propensity among individuals: the effects of age, sex and resource availability. *Journal of Animal Ecology*, **78**, 1234–1241.
- Brown, G.P., Phillips, B.L. & Shine, R. (2014) The straight and narrow path: the evolution of straight-line dispersal at a cane toad invasion front. *Proceedings of the Royal Society of London B: Biological Sciences*, **281**, 20141385.
- Burton, T., Killen, S.S., Armstrong, J.D. & Metcalfe, N.B. (2011) What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences*, **278**, 3465–3473.
- Button, K.S., Ioannidis, J.P.A., Mokrysz, C., Nosek, B.A., Flint, J., Robinson, E.S.J. *et al.* (2013) Power failure: why small sample size undermines the reliability of neuroscience. *Nature Reviews Neuroscience*, **14**, 365–376.
- Campbell, J.F. & Hagstrum, D.W. (2002) Patch exploitation by *Tribolium castaneum*: movement patterns, distribution, and oviposition. *Journal of Stored Products Research*, **38**, 55–68.
- Careau, V., Thomas, D., Humphries, M.M. & Réale, D. (2008) Energy metabolism and animal personality. *Oikos*, **117**, 641–653.
- Choi, I., Shim, J.H. & Ricklefs, R.E. (2003) Morphometric relationships of take-off speed in anuran amphibians. *Journal of Experimental Zoology A*, **299**, 99–102.
- Clarke, A. & Fraser, K.P.P. (2004) Why does metabolism scale with temperature? *Functional Ecology*, **18**, 243–251.
- Clobert, J., Le Galliard, J.-F., Cote, J., Meylan, S. & Massot, M. (2009) Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology Letters*, **12**, 197–209.
- Cote, J., Fogarty, S., Weinersmith, K., Brodin, T. & Sih, A. (2010) Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1571–1579.
- Crnokrak, P. & Roff, D.A. (2002) Trade-offs to flight capability in *Gryllus firmus*: the influence of whole-organism respiration rate on fitness. *Journal of Evolutionary Biology*, **15**, 388–398.
- Cutts, C.J., Metcalfe, N.B. & Taylor, A.C. (1998) Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. *Journal of Fish Biology*, **52**, 1026–1037.
- Delgado, M.M., Penteriani, V., Revilla, E. & Nams, V.O. (2010) The effect of phenotypic traits and external cues on natal dispersal movements. *Journal of Animal Ecology*, **79**, 620–632.
- Ducatez, S., Legrand, D., Chaput-Bardy, A., Stevens, V.M., Fréville, H. & Baguette, M. (2012) Inter-individual variation in movement: is there a mobility syndrome in the large white butterfly *Pieris brassicae*? *Ecological Entomology*, **37**, 377–385.
- Ewing, A.W. (1963) Attempts to select for spontaneous activity in *Drosophila melanogaster*. *Animal Behaviour*, **11**, 369–378.
- Glazier, D.S. (2005) Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. *Biological Reviews*, **80**, 611–662.
- Haag, C.R., Saastamoinen, M., Marden, J.H. & Hanski, I. (2005) A candidate locus for variation in dispersal rate in a butterfly metapopulation. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 2449–2456.
- Halsey, L.G. (2016) Terrestrial movement energetics: current knowledge and its application to the optimising animal. *Journal of Experimental Biology*, **219**, 1424–1431.
- Halstead, D.G.H. (1963) External sex differences in stored-product Coleoptera. *Bulletin of Entomological Research*, **54**, 119–134.



- Hanski, I., Peltonen, A. & Kaski, L. (1991) Natal dispersal and social dominance in the common shrew *Sorex araneus*. *Oikos*, **54**, 119–134.
- Hedrick, T.L. (2008) Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspiration & Biomimetics*, **3**, 034001.
- Honěk, A. (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, **66**, 483–492.
- Hulbert, A.J., Clancy, D.J., Mair, W., Braeckman, B.P., Gems, D. & Partridge, L. (2004) Metabolic rate is not reduced by dietary-restriction or by lowered insulin/IGF-1 signalling and is not correlated with individual lifespan in *Drosophila melanogaster*. *Experimental Gerontology*, **39**, 1137–1143.
- Jennions, M.D. & Petrie, M. (1997) Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews*, **72**, 283–327.
- Killen, S.S., Marras, S., Ryan, M.R., Domenici, P. & McKenzie, D.J. (2012) A relationship between metabolic rate and risk-taking behaviour is revealed during hypoxia in juvenile European sea bass. *Functional Ecology*, **26**, 134–143.
- Killen, S.S., Marras, S., Metcalfe, N.B., McKenzie, D.J. & Domenici, P. (2013) Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology & Evolution*, **28**, 651–658.
- Kleiber, M. (1947) Body size and metabolic rate. *Physiological Reviews*, **27**, 511–541.
- Laparie, M., Renault, D., Lebouvier, M. & Delattre, T. (2013) Is dispersal promoted at the invasion front? Morphological analysis of a ground beetle invading the Kerguelen Islands, *Merizodus soledadinus* (Coleoptera, Carabidae). *Biological Invasions*, **15**, 1641–1648.
- Le Galliard, J.-F., Paquet, M., Cisel, M. & Montes-Poloni, L. (2013) Personality and the pace-of-life syndrome: variation and selection on exploration, metabolism and locomotor performances. *Functional Ecology*, **27**, 136–144.
- Lebeau, J., Wesselingh, R.A. & Van Dyck, H. (2016) Nectar resource limitation affects butterfly flight performance and metabolism differently in intensive and extensive agricultural landscapes. *Proceedings of the Royal Society of London B: Biological Sciences*, **283**, 20160455.
- Lighton, J.R.B. (2008) *Measuring Metabolic Rates: A Manual for Scientists*. Oxford University Press, Oxford, UK.
- Llewellyn, J., Phillips, B.L., Alford, R.A., Schwarzkopf, L. & Shine, R. (2010) Locomotor performance in an invasive species: cane toads from the invasion front have greater endurance, but not speed, compared to conspecifics from a long-colonised area. *Oecologia*, **162**, 343–348.
- Lomnicki, A. (2006) Population regulation by dispersal under selection pressure for and against dispersal: an experimental test with beetles, *Tribolium confusum*. *Evolutionary Ecology Research*, **8**, 63–73.
- Lowe, W.H. & McPeck, M.A. (2012) Can natural selection maintain long-distance dispersal? Insight from a stream salamander system. *Evolutionary Ecology*, **26**, 11–24.
- Martin, J.-R. (2003) Locomotor activity: a complex behavioural trait to unravel. *Behavioural Processes*, **64**, 145–160.
- Mathot, K.J. & Dingemanse, N.J. (2015) Energetics and behavior: unrequited needs and new directions. *Trends in Ecology & Evolution*, **30**, 199–206.
- McDevitt, A.D., Oliver, M.K., Piertney, S.B., Szafranska, P.A., Konarzewski, M. & Zub, K. (2013) Individual variation in dispersal associated with phenotype influences fine-scale genetic structure in weasels. *Conservation Genetics*, **14**, 499–509.
- Melbourne, B.A. & Hastings, A. (2009) Highly variable spread rates in replicated biological invasions: fundamental limits to predictability. *Science*, **325**, 1536–1539.
- Merritt, L., Matthews, P.G. & White, C.R. (2013) Performance correlates of resting metabolic rate in garden skinks *Lampropholis delicata*. *Journal of Comparative Physiology B*, **183**, 663–673.
- Metcalfe, N.B., Van Leeuwen, T.E. & Killen, S.S. (2016) Does individual variation in metabolic phenotype predict fish behaviour and performance? *Journal of Fish Biology*, **88**, 298–321.
- Myles-Gonzalez, E., Burness, G., Yavno, S., Rooke, A. & Fox, M.G. (2015) To boldly go where no goby has gone before: boldness, dispersal tendency, and metabolism at the invasion front. *Behavioral Ecology*, **26**, 1083–1090.
- Naylor, A.F. (1961) Dispersal in the red flour beetle *Tribolium castaneum* (Tenebrionidae). *Ecology*, **42**, 231–237.
- Niitepöld, K., Smith, A.D., Osborne, J.L., Don, R.R., Carreck, N.L., Martin, A.P. *et al.* (2009) Flight metabolic rate and *Pgi* genotype influence butterfly dispersal rate in the field. *Ecology*, **90**, 2223–2232.
- Phillips, B.L., Brown, G.P., Webb, J.K. & Shine, R. (2006) Invasion and the evolution of speed in toads. *Nature*, **439**, 803.
- Rehage, J.S. & Sih, A. (2004) Dispersal behavior, boldness, and the link to invasiveness: a comparison of four *Gambusia* species. *Biological Invasions*, **6**, 379–391.
- Roff, D.A. & Fairbairn, D.J. (1991) Wing dimorphisms and the evolution of migratory polymorphisms among the Insecta. *American Zoologist*, **31**, 243–251.
- Rollins, L.A., Richardson, M.F. & Shine, R. (2015) A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). *Molecular Ecology*, **24**, 2264–2276.
- Ronce, O. & Clobert, J. (2012) Dispersal syndromes. *Dispersal Ecology and Evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 119–138. Oxford University Press, Oxford, UK.
- Royauté, R., Greenlee, K., Baldwin, M. & Dochtermann, N.A. (2015) Behaviour, metabolism and size: phenotypic modularity or integration in *Acheta domesticus*? *Animal Behaviour*, **110**, 163–169.
- Schimpf, N.G., Matthews, P.G.D. & White, C.R. (2012) Standard metabolic rate is associated with gestation duration, but not clutch size, in speckled cockroaches *Nauphoeta cinerea*. *Biology Open*, **1**, 1185–1191.
- Sekar, S. (2012) A meta-analysis of the traits affecting dispersal ability in butterflies: can wingspan be used as a proxy? *Journal of Animal Ecology*, **81**, 174–184.
- Shaw, A.K. & Kokko, H. (2014) Mate finding, Allee effects and selection for sex-biased dispersal. *Journal of Animal Ecology*, **83**, 1256–1267.
- Shine, R., Brown, G.P. & Phillips, B.L. (2011) An evolutionary process that assembles phenotypes through space rather than through time. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 5708–5711.
- Socha, R. & Zemek, R. (2003) Wing morph-related differences in the walking pattern and dispersal in a flightless bug, *Pyrrhocoris apterus* (Heteroptera). *Oikos*, **100**, 35–42.
- Speakman, J.R. & Selman, C. (2003) Physical activity and resting metabolic rate. *The Proceedings of the Nutrition Society*, **62**, 621–634.
- Stevens, V.M., Trochet, A., Van Dyck, H., Clobert, J. & Baguette, M. (2012) How is dispersal integrated in life histories: a quantitative analysis using butterflies. *Ecology Letters*, **15**, 74–86.
- Stevens, V.M., Whitmee, S., Le Galliard, J.-F., Clobert, J., Böhning-Gaese, K., Bonte, D. *et al.* (2014) A comparative analysis of dispersal syndromes in terrestrial and semi-terrestrial animals. *Ecology Letters*, **17**, 1039–1052.
- Vaanholt, L.M., De Jong, B., Garland, T., Daan, S. & Visser, G.H. (2007) Behavioural and physiological responses to increased foraging effort in male mice. *Journal of Experimental Biology*, **210**, 2013–2024.
- White, C.R. (2011) Allometric estimation of metabolic rates in animals. *Comparative Biochemistry and Physiology A*, **158**, 346–357.
- White, C.R. & Kearney, M.R. (2013) Determinants of inter-specific variation in basal metabolic rate. *Journal of Comparative Physiology B*, **183**, 1–26.
- White, C.R. & Kearney, M.R. (2014) Metabolic scaling in animals: methods, empirical results, and theoretical explanations. *Comprehensive Physiology*, **4**, 231–256.
- Whitmee, S. & Orme, C.D.L. (2013) Predicting dispersal distance in mammals: a trait-based approach. *Journal of Animal Ecology*, **82**, 211–221.
- Ziegler, J.R. (1976) Evolution of the migration response: emigration by *Tribolium* and the influence of age. *Evolution*, **30**, 579–592.
- Zirkle, D.F., Dawson, P.S. & Lavie, B. (1988) An experimental analysis of the genetic relationships among life-history traits and emigration behavior in *Tribolium castaneum*. *Oikos*, **53**, 391–397.
- Zolliker, C. (1994) Stepping patterns in ants - influence of body morphology. *Journal of Experimental Biology*, **192**, 107–118.

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## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Table S1.** Full LMER output of movement ability as the response variable.

**Table S2.** Full LMER output of movement displacement as the response variable.