Maturity matters for movement and metabolic rate: trait dynamics across the early adult life of red flour beetles

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Article info

Transitioning between life stages involves significant changes to the physiology, structural morphology, biochemistry and behaviour of an organism. Eclosion, metamorphosis and the onset of sexual maturity have consequences for the life history evolution of an organism by initiating reproductive and dispersal-related behaviours that are both energetically costly and directly related to fitness. Animal movement, particularly dispersal when sexually mature, is critical for mate location, controlling population density and promoting gene flow. Here we examined changes in dispersal-related and physiological traits during a significant transitional period in red flour beetles, Tribolium castaneum. We measured the metabolic rate, spontaneous activity, body size and movement behaviour traits of individuals of known age and sex. Traits were compared between immature and sexually mature adults as well as during early adult life when there is a strong tendency to disperse and reproduce. Spontaneous activity, movement speed and metabolic rate were distinctly reduced in immature adults prior to the onset of sexual maturity, and immature individuals moved more intermittently than mature ones. We found that these traits increased with age following eclosion, up to a relatively stable mean once sexual maturity was attained. The reduced metabolic expenditure found in immature individuals was attributable to a reduced energy demand due to relative inactivity, which we hypothesize to be a protective mechanism from conspecifics while the cuticle is undergoing sclerotization. Understanding the precise developmental trajectories of behavioural and physiological traits allows us to interpret the trait syndromes that underlie dispersal and their evolution.

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For organisms with complex life cycles, specific age and life stages are specialized to perform differently. In many insects, development involves several larval instars designed for intense growth, followed by a distinct metamorphic pupa stage and then finally an adult stage specialized for dispersal and reproduction (Wilbur, 1980). While the preadult stages are significant to the transition and resulting fitness of adults (De Block & Stoks, 2005), it is only in the adult stage that this fitness potential can be realized through the processes of dispersal and reproduction.

Several adult holometabolous insect species have a short nonreproductive adult transitional period following emergence and before the onset of sexual maturity. This transitional period, here defined as maturation, occurs over variable lengths of time within and between species, from hours to many days following imaginal eclosion (e.g. Happ, 1970; Norris, 1954; Pitnick, Markow, & Spicer, 1995; Teal & Gomez-Simuta, 2002). Maturation induces behaviours associated with reproduction and dispersal, which have potential risks including aggression, predation, adverse environmental conditions, unsuitable mates or resources and failure to reproduce or survive. During the transition to maturity, we expect selection to be strong, because this critical life history transition is when individuals are more active and therefore exposed to predation and risks associated with conspecific interaction through dispersive and reproductive behaviours (De Block & Stoks, 2005; Werner & Anholt, 1993).

Locomotor activity is involved in nearly all behavioural activities from basic locomotion to complex courtship behaviours (Martin, 2003) and is tightly linked with energy expenditure and metabolism. The locomotor musculature that an organism uses to conduct its daily foraging and activities is a substantial contributor to whole-animal metabolic rate during activity (White & Kearney, 2013). Whole-animal metabolic rate is a fundamental physiological trait that provides an estimate of the essential costs of living for...
an organism (Chown & Gaston, 1999; Kleiber, 1961). While size, activity, phylogeny and temperature are all considered to be causes of variation in metabolic rate (Konarzewski & Książek, 2013; White & Kearney, 2013), the influence of other intrinsic factors such as maturity, age and sex on metabolic rate have not been studied over a wide range of insect species.

Metabolic rate, energy dynamics and the individual’s physiological state strongly influence the resources available for animal movement and the nature of the movement path itself, which is critical for dispersal, reproduction and fitness (Bell, 1990). Characteristics of movement such as speed, step length, path length and tortuosity are dependent on the biological requirements for an individual, which vary with life stage, age and body size (Mancinelli, 2010; Potenza & Mancinelli, 2010; With, 1994). Behavioural intermittence of an animal’s movement is the combination of forces acting to interrupt otherwise continuous movement, such as pauses to change orientation or take sharp turning angles, adjustment of behaviour to sensory inputs and the dynamics of acceleration (Bartumeus, 2009). The discontinuous aspects of animals’ movement paths, particularly turning, can have significant costs for those animals’ net energy gain (Wilson et al., 2012) and also alters their exposure to potential predation (Anholt & Werner, 1995; Cuddington & Yodzis, 2002; Yoder, Marschall, & Swanson, 2004). Direct measurement of complex behaviours and traits in animals (such as dispersal ability, reproductive fitness or personality) is often difficult, and therefore the use of more simplified proximal traits is required (e.g. Heidinger, Hein, & Bonte, 2010; Sekar, 2012; Stevens et al., 2013). Integrating multiple movement behaviour traits with physiology and activity provides a relevant and useful proxy for more complex behaviours.

Here, we aimed to determine behavioural and physiological trait changes during the transition from immature adults to sexually mature adults (approximately 3 days posteclosion; Sokoloff, 1974) and during the early mature life of adult red flour beetles, Tribolium castaneum. Complex behaviours, including movement, dispersal and reproduction are associated with the onset of sexual maturity in Tribolium species (e.g. Arnaud & Haubruge, 1999; Perez-Mendoza, Campbell, & Throne, 2011; Soliman, 1987; Ziegler, 1976). We studied the dynamics of quantitative traits (activity, movement characteristics, body size and metabolic rate) that fundamentally underlie animal movement, dispersal and reproduction. Specifically, we tested the hypothesis that immature beetles would be less active than mature ones, and as sexual maturity is reached, movement characteristics would change to reflect the onset of reproductive behaviour and increased dispersal tendencies. We also predicted that males would move more actively than same-aged females, at least around the onset of maturity, because males tend to disperse and attempt to mate earlier than females (Arnaud & Haubruge, 1999; Ogden, 1970; Prus, 1966). Finally, we predicted that metabolic rate would be positively correlated with body size, activity and movement speed, due to the strong association between energy expenditure and locomotive structures.

METHODS

Animals and Housing

A stock population of T. castaneum sourced from the Postharvest Grain Protection Team (Department of Agriculture, Fisheries and Forestry: Brisbane, QLD, Australia) was used to establish stocks for a series of experiments. This stock was a wild-type susceptible line (QTC4) which was maintained in 1-litre cylindrical containers containing 200 g of medium (95% wholemeal stone-ground wheat flour supplemented with 5% torula yeast) under laboratory conditions of 29.5 ± 1 °C and moderate humidity. The original stock was bulked up, divided and maintained as two subsstocks which were cultured fortnightly.

Beetles were collected as pupae and sexed by examining the external dimorphic genitalia (Halstead, 1963) under a stereomicroscope (Olympus SZ61; Olympus Australia Pty. Ltd., Notting Hill, VIC, Australia). Pupae were separated into groups of five by sex and relative day of eclosion in 70 ml containers with approximately 5 g of flour medium. The specific age of beetles was controlled to ±12 h, and the age range used for this study was from 0 to 21 days post-eclosion. Maturity was defined as the age at which mating was first commonly observed and the cuticle was entirely melanized; approximately 3 days posteclosion under the aforementioned laboratory conditions. At least 24 h prior to trait measurements, beetles were separated and individually placed in containers to fast before respirometry, with the exception of age 0 beetles which were not provided with flour before measurement. Trait measurement proceeded for all individuals in the following order: metabolic rate and spontaneous activity, movement behaviour, fresh mass and then morphometrics. Total sample size was 220 across 22 ages, such that five males and five females were measured for each age.

Movement Behaviour in a Complex Environment

Movement behaviour of individuals was assessed by placing individual beetles in a complex artificial maze environment. The custom-built maze (Cottage Plastics, Sumner Park, QLD, Australia) was constructed from 5 mm thick acrylic sheeting, based on a design of 12 square passageways radiating from the central point (Fig. 1). The minimum distance between each passageway of the maze was approximately equal such that progression through the maze was linear. The base of the maze was constructed from white
the number of frames to reach the finish line (1280 × 720 pixels) webcam at 10 frames/s (Microsoft LifeCam Studio; Microsoft Corporation, Redmond, WA, U.S.A.). Filming conditions were under ambient and nondirect lighting in a controlled temperature room at 29.5 ± 1 °C. The resulting video recordings of each beetle's run were frame cropped from the first distinctive movement away from the centre (frame 1) to 3 min after introduction (frame 1801). The tracking of beetle movement was conducted in MATLAB software (MATLAB R2013A; The Mathworks, Inc., Natick, MA, U.S.A.), running a digitizing tracking script (DLDtv5; Hedrick, 2008). The tracking script utilized an extended Kalman filter to precisely track the approximate path (frame-by-frame to produce x and y points) taken by a beetle automatically within a user-defined residual threshold. The movement characteristics calculated from the tracking analysis were average speed (± upper and lower 5th percentiles), total path length and behavioural intermittence (stopping frequency: the number of frames in which the animal was not detected moving). The time for an individual to complete the maze was calculated as the number of frames to reach the final maze passageway (maximum time is 1801 frames). If an individual did not complete the maze within this time, it was assigned a censored value of 1801 for the Cox proportional hazards model analysis. For linear model analyses that required discrete values these individuals were assigned the whole number value of 2000 frames to represent that they did not complete the maze.

Metabolic Rate and Spontaneous Activity

The metabolic rate of individual beetles was measured to determine whether maturity status, age and sex affect the routine energy usage in these animals. Flow-through respirometry was used to measure the rate of CO₂ production of individual adult beetles as a proxy for metabolic rate (MR) (Lighton, 2008). Air drawn into the system was first chemically scrubbed using columns containing soda lime (Ajax Finechem Pty. Ltd., Taren Point, NSW, Australia) and Drierite (Sigma-Aldrich Co., St. Louis, MO, U.S.A.) to remove CO₂ and water vapour, respectively. The flow rate of the incoming air was regulated by mass flow controllers for four channels (GFC17; Aalborg Instruments & Controls, Inc., Orangeburg, NY, U.S.A.) set to a nominal flow rate of approximately 25 ml/min (standard temperature and pressure, dry). Air was then passed through four custom metabolic chambers housing beetles individually before being passed through a pair of two-channel LI-7000 CO₂/H₂O analysers (Li-COR Inc., Lincoln, NE, U.S.A.). LI-COR software was used to plot and record gas traces with a resolution of 0.1 ppm at a recording frequency of 1 Hz.

Chambers were approximately 2 ml airtight glass cylinders contained in a multichannel locomotion activity detector (LAM10H; TriKinetics Inc., Waltham, MA, U.S.A.) which used nine infrared LED emitter-detector pairs to detect motion and count each instance that an infrared beam was interrupted by movement of the animal. This allowed spontaneous activity to be measured synchronously with metabolic rate, so that activity could be included as a covariate in the analysis of metabolic rate. The respirometry chambers were housed in a controlled temperature incubator at 30 ± 1 °C in darkness to ensure temperature stability and to remove light stimulus. Adult beetles were fasted for approximately 24 h prior to MR measurement, placed in the respirometry chambers, and then measured continuously for 1 h. All basic assumptions of resting metabolic rate (standardized thermal and dark environment to reduce activity, and animals were in a postabsorptive and nonreproductive state) were met by experimental conditions. Metabolic rate, as measured in this study, was appropriately defined as routine metabolic rate (routine MR) due to the presence of spontaneous activity (Mathot & Dingemanse, 2015).

Mass and Morphometrics

Individual beetles were weighed using a precision microbalance (XS3DU microbalance; Mettler-Toledo, Columbus, OH, U.S.A.) to measure fresh mass (to 0.01 mg), and morphometrics were taken as alternative measurements of body size. Morphological measurements were obtained using a microscope-mounted camera (PL-B686; PixelLINK, Ottawa, ON, Canada) to capture dorsal and ventral images of each beetle. The following metrics were then extracted using ImageJ software (National Institutes of Health, Bethesda, MD, U.S.A.): length of the elytron (from apex to base), width of the elytron (at the widest point), length and width of the pronotum (at the widest point) and the length of the femur on the hind leg.

Statistical Analysis

Data were analysed using the R software environment for statistical and graphical computing version 2.15.3 (R Core Team, 2014) utilizing the R packages lme4 (Bates, Maechler, Bolker, & Walker, 2014) and survival (Therneau, 2014). Our analysis investigated multiple quantitative traits over the age range of 0–21 days post-emergence at 1–day intervals. Principal components analysis was used to derive the measures for movement speed and body size from multiple correlated predictor variables. The first principal component (PC1) of movement speed accounted for approximately 95% of variance among speed traits (the average, lower and upper 5th percentiles of movement speed). PC1 of body size accounted for approximately 85% of the variance among the size traits (elytron length, elytron width, pronotum length, pronotum width, rear femur length and fresh body mass). Linear mixed-effects regression (LMER) models where maturity status, age and sex were the principal predictor variables of interest were used to analyse movement speed, behavioural intermittence, spontaneous activity and routine MR as response variables. Each model tested the effect of principal predictors on a response variable, while accounting for all other covariate measurements. A Cox proportional hazards model was used to analyse the proportion of individuals that completed the maze as the response variable. Random effects of respirometry channel and stock were included in all models. Full models including interaction terms were fitted initially, with nonsignificant interaction terms removed to simplify models according to the principle of parsimony.

Ethical Note

We conducted this study according to the ASAB/ABS guidelines for the ethical treatment of animals. All animals used in this study were kept at low densities in large containers (70 ml relative to a 2–3 mg animal), and provided with adequate flour medium (5 g, changed regularly) to consume ad libitum and burrow for shelter. Handling of beetles was minimized throughout the study, and only enough beetles to provide an adequate sample size for each age group were utilized.
RESULTS

Speed Increases and Behavioural Intermittence Decreases during Maturation

Consistent patterns of increasing movement characteristics with age, particularly during maturation are observable in the lower 5th percentile of speed (Fig. 2a), average speed (Fig. 2b) and upper 5th percentile of speed (Fig. 2c). Age was significantly positively correlated with movement speed; however, maturity status, routine MR and spontaneous activity were not (Table 1). The increased spontaneous activity found in males was not expressed in their movement behaviour, as sex was not significantly correlated with movement speed. Behavioural intermittence and maze completion were also significantly related to movement speed, where individuals that moved more frequently were able to move further with greater speed to successfully complete the maze (Table 1).

Behavioural intermittence was significantly correlated with age and maturity status (Table 2), where individuals aged 3 days posteclosion and older moved significantly more frequently than younger immature individuals of either sex (Fig. 3). Behavioural intermittence was also significantly correlated with movement speed (Table 2), as expected by the intuitive relationship between the time an individual spends immobile and its average speed, which would consequently reduce the distance able to be travelled.

The proportion of individuals that completed the maze was significantly greater in mature individuals, and these also completed the maze in a shorter time than immature individuals (Fig. 4). Both maturity state and age were significantly related to completing the maze (Table 3), such that a lower proportion of immature individuals (males: 0.4; females: 0.27) were able to complete the maze within the set time than mature individuals (males: 0.86; females: 0.87).

Spontaneous Activity and Metabolic Rate Increase at Maturity

Spontaneous activity as a response variable was found to be significantly correlated with both sex and maturity status (Table 4). Generally, male beetles were more active than females during respirometry; however, there was substantial variation in activity counts across ages and particularly within males (Fig. 5). Spontaneous activity was considerably reduced in immature individuals, thereafter increasing from the onset of sexual maturity (Fig. 5) in a pattern similar to that of routine MR (Fig. 6). As expected, the reciprocal relationship between spontaneous activity and routine MR was positive; however, the effect was not significant, probably because of the wide variance within this trait (Table 4).

Routine MR was significantly different between immature and mature adults (Fig. 6, Table 5). Routine MR was neither significantly correlated with age nor significantly different between males and females. Routine MR scaled positively with body size, which was a significant covariate (Table 5). Spontaneous activity was not significantly correlated with routine MR (Table 5).

DISCUSSION

Effects of Maturation on Movement, Activity and Metabolism

Our results have demonstrated that the process of maturing has a significant effect on individuals in terms of their behaviour and physiology. Most aspects of movement behaviour as well as spontaneous activity and metabolic rate are significantly reduced in younger individuals prior to the onset of sexual maturity. Movement speed was predicted to be related to spontaneous activity, routine MR and body size due to the link between locomotive structures and energy expenditure; however, surprisingly these traits were not strongly correlated with movement speed. Patterns of increasing movement speed from eclosion through to
Figure 3. Mean behavioural intermittence count (stopping frequency; the number of frames in which the individual was not detected moving) during movement of individual adult T. castaneum aged from eclosion (age 0) to 21 days posteclosion. Each age had five males and five females, for a total of N = 220 data points. Data presented are means ± SE.

attaining sexual maturity emerged (Fig. 2), particularly in average speed and the upper 5th percentile of speed (Fig. 2b, c). These results support the nonexclusive hypotheses that T. castaneum that are not yet sexually mature either limit their energy expenditure by remaining relatively inactive or have a limited physiological ability to move prior to reaching maturity. By introducing individual beetles into the maze used to assess their movement characteristics this may bias results, therefore we do not have a strong drive to seek mates, conspecifics or even to find food,

Table 1
LMER model of the response of movement speed (PC1) to maturity status, age, sex, log-routine MR, spontaneous activity, body size, behavioural intermittence and maze completion of beetles aged from eclosion to 21 days posteclosion

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate±SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>25.794±5.305</td>
<td>4.863</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maturity status</td>
<td>0.602±0.821</td>
<td>0.733</td>
<td>0.463</td>
</tr>
<tr>
<td>Age</td>
<td>0.187±0.035</td>
<td>5.297</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.168±0.384</td>
<td>-0.437</td>
<td>0.651</td>
</tr>
<tr>
<td>Log-routine MR</td>
<td>1.591±2.305</td>
<td>0.69</td>
<td>0.282</td>
</tr>
<tr>
<td>Activity</td>
<td>0.000±0.001</td>
<td>0.112</td>
<td>0.912</td>
</tr>
<tr>
<td>Body size (PC1)</td>
<td>0.431±1.615</td>
<td>0.267</td>
<td>0.789</td>
</tr>
<tr>
<td>Intermittence</td>
<td>-0.023±0.002</td>
<td>-11.338</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maze completion</td>
<td>-0.003±0.0004</td>
<td>-6.915</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Random effects were respirometry channel (variance = 0.28, SD = 0.53), stock (variance = 0.23, SD = 0.48) and residual (variance = 6.54, SD = 2.56). Significant P values are in bold.

Figure 4. Mean proportion and total time taken by individuals to reach the final passageway of the complex maze environment (successful movement through the maze). Immature (age 0 to 3 days posteclosion) and mature (age 4 to 21 days posteclosion) life stages are indicated on the graph, and divided into male and female groups. Each age had five males and five females, for a total of N = 220 data points.

Table 2
LMER model of the response of behavioural intermittence to maturity status, age, sex, log-routine MR, spontaneous activity, body size, movement speed and maze completion of beetles aged from eclosion to 21 days posteclosion

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate±SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>842.526±134.384</td>
<td>6.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maturity status</td>
<td>-80.92±20.525</td>
<td>-3.943</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>1.429±0.975</td>
<td>1.466</td>
<td>0.143</td>
</tr>
<tr>
<td>Sex</td>
<td>3.263±10.045</td>
<td>0.325</td>
<td>0.745</td>
</tr>
<tr>
<td>Log-routine MR</td>
<td>-46.027±56.738</td>
<td>-0.811</td>
<td>0.417</td>
</tr>
<tr>
<td>Activity</td>
<td>-0.007±0.032</td>
<td>-0.222</td>
<td>0.824</td>
</tr>
<tr>
<td>Body size (PC1)</td>
<td>-39.125±42.138</td>
<td>-0.929</td>
<td>0.353</td>
</tr>
<tr>
<td>Speed (PC1)</td>
<td>-15.996±1.379</td>
<td>-11.597</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maze completion</td>
<td>0.075±0.012</td>
<td>0.622</td>
<td>0.534</td>
</tr>
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</table>

Random effects were respirometry channel (variance = 0.00, SD = 0.00), stock (variance = 0.00, SD = 0.00) and residual (variance = 4523, SD = 67.26). Significant P values are in bold.

Table 3
Cox proportional hazards model of the response of maze completion to maturity status, age, sex, log-routine MR, spontaneous activity and body size of beetles aged from eclosion to 21 days posteclosion

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate±SE</th>
<th>Hazard ratio (95% CI)</th>
<th>z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maturity status</td>
<td>1.403±0.199</td>
<td>4.067 (1.860–8.893)</td>
<td>3.515</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>0.028±0.014</td>
<td>1.028 (1.001–1.057)</td>
<td>2.019</td>
<td>0.043</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.078±0.163</td>
<td>0.925 (0.672–1.274)</td>
<td>-0.476</td>
<td>0.634</td>
</tr>
<tr>
<td>Log-routine MR</td>
<td>0.218±1.243</td>
<td>1.243 (0.214–7.217)</td>
<td>0.243</td>
<td>0.808</td>
</tr>
<tr>
<td>Activity</td>
<td>-0.0006±0.999</td>
<td>0.999 (0.998–1.000)</td>
<td>-1.135</td>
<td>0.256</td>
</tr>
<tr>
<td>Body size (PC1)</td>
<td>-0.207±0.813</td>
<td>0.813 (0.208–3.181)</td>
<td>-0.298</td>
<td>0.766</td>
</tr>
</tbody>
</table>

Significant P values are in bold.

Table 4
LMER model of the response of spontaneous activity to maturity status, age, sex, body size, log-routine MR, movement speed, behavioural intermittence and maze completion of beetles aged from eclosion to 21 days posteclosion

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate±SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-371.12±314.12</td>
<td>-1.181</td>
<td>0.237</td>
</tr>
<tr>
<td>Maturity status</td>
<td>127.24±45.527</td>
<td>2.795</td>
<td>0.005</td>
</tr>
<tr>
<td>Age</td>
<td>0.601±2.12</td>
<td>0.283</td>
<td>0.777</td>
</tr>
<tr>
<td>Sex</td>
<td>46.316±21.413</td>
<td>2.163</td>
<td>0.031</td>
</tr>
<tr>
<td>Body size (PC1)</td>
<td>69.22±91.014</td>
<td>0.761</td>
<td>0.447</td>
</tr>
<tr>
<td>Log-routine MR</td>
<td>191.48±128.555</td>
<td>1.49</td>
<td>0.136</td>
</tr>
<tr>
<td>Speed (PC1)</td>
<td>0.555±3.849</td>
<td>0.144</td>
<td>0.885</td>
</tr>
<tr>
<td>Intermittence</td>
<td>-0.036±0.148</td>
<td>-0.246</td>
<td>0.806</td>
</tr>
<tr>
<td>Maze completion</td>
<td>0.024±0.026</td>
<td>0.941</td>
<td>0.347</td>
</tr>
</tbody>
</table>

Random effects were respirometry channel (variance = 709.7, SD = 26.64), stock (variance = 0.00, SD = 0.00) and residual (variance = 20849.3, SD = 144.4). Significant P values are in bold.
Random effects were respirometry channel (variance = 0.002, SD = 0.044), stock (variance = 0.00, SD = 0.006) and residual (variance = 0.005, SD = 0.07). Significant P values are in bold.

Sex-specific Effects of Maturation on Traits

Dispersive behaviours have been previously found to occur earlier in males than in females in Tribolium species (Ogden, 1970; Prus, 1966); therefore we expected that movement behaviour...
would potentially differ between the sexes. Dispersal of both sexes in T. castaneum occurs shortly after sexual maturity is attained (Ziegler, 1976). Unmated male T. castaneum are generally more likely to emigrate than unmated females in a laboratory dispersal apparatus (Ogden, 1970; Prus, 1966), but not all do so (Ziegler, 1976). Males are, however, more exploratory when unmated, relative to unmated females (Ziegler, 1976). In our study, movement behaviour did not differ between the sexes, with the exception of higher spontaneous activity in males. The general consensus that unmated males (as in our study) would be more actively exploring to seek females is supported by the finding that around 85% of male-female contacts are initiated by the male (Arnaud & Haubruege, 1999). The higher spontaneous activity in males we observed adheres to this prediction that males would be more active; however, movement behaviour, which may be a good proxy for dispersal behaviour, does not. Our results suggest that both sexes are responding relatively equally (with substantial individual variation) in terms of exploratory behaviour within the maze following sexual maturity. This response could be a result of both sexes responding to a stimulus from the novel environment, either perceiving it as a hostile environment or seeking out food, conspecifics or shelter. To improve our understanding of the exploratory response in T. castaneum, it would be useful to compare the movement or dispersal of mated and unmated mature individuals kept in mixed- or single-sex cohorts prior to measurements. Furthermore, providing alternative environments with and without a food source, conspecifics and suitable shelter could improve the ecological relevance of the exploratory behaviour assessment.

Conclusions

The process of maturation has significant effects on many behavioural and physiological traits of individuals. The reduced energy expenditure of immature individuals is likely to be a result of the combination of lower energy requirements until the onset of sexual maturity and being relatively inactive. This inactivity is also likely to provide protection from conspecifics during the cuticle sclerotization and maturation process. Certainly, from eclosion to the onset of sexual maturity there are significant biological changes occurring within the individual, many of which we have demonstrated through their movement behaviour, physiology and morphology. For behavioural, evolutionary, and dispersal-based studies, taking into account the timing and effect of maturity status and age when applying experimental treatments or selection is clearly important. To understand behavioural syndromes and their underlying physiological mechanisms, it is essential to be informed about the trajectories of the traits of interest throughout the portion of adult life in which dispersal and reproduction occur.

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