Distinct effects of two separately applied stressors on behavior in the red flour beetle

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ARTICLE INFO

ABSTRACT

Stress is ubiquitous in nature and organisms have evolved mechanisms to cope with it. Stress responses nevertheless vary based on the type of stress, its duration, magnitude, and the organism's physiological status. We studied here the effect of two separately applied stress types, differing also in their duration, on various behavioral responses of the red flour beetle (*Tribolium castaneum*). The two stress types applied were short-term cold stress and long-term low protein stress. The behavioral response variables were movement activity, preference to move along the test arena edges, latency to emerge from shelter, and preference for dark microhabitat, measured at two stages of the beetles' lives, as young adults after eclosion and one month later. Behavioral responses were stress-specific. Cold-stressed beetles moved over the longest distances but were also the most reluctant to leave an accessible shelter. We interpret this to mean that cold-stressed beetles were the most active under unfavorable conditions and least bold under more favorable, protected conditions. As beetles aged, their movement activity decreased and latency to emerge from shelter increased. Non-stressed beetles were the most consistent in their behavior across all behavioral tests. This finding is similar to those in previous studies and suggests that moderate stress reduces behavioral consistency. While all beetles gained mass during the study period, the two stress types led to different patterns of body mass increase, which was more moderate for cold-stressed than for protein-stressed beetles.

1. Introduction

Stress is a state induced by suboptimal conditions (stressors) that cause deviations from the ecological niche of an organism (Steinberg, 2012, ch. 1). Organisms in nature are rarely exposed to constant conditions and experience episodes of stress, such as food shortage, temperatures exceeding their thermal preference, and threats by predators. Organisms have therefore evolved various mechanisms by which to tolerate, at least temporally, such unfavorable situations. For example, animals can improve their starvation tolerance by reducing their metabolic rate and activity until regaining access to food (Djawdan et al., 1997; McCue, 2010). In the presence of predators, animals reduce activity, improve their escape behavior, shift their preferred habitat and foraging timing, or develop specific morphologies that impede predation (Kotler et al., 1994; Relyea, 2001; Schmitz and Suttle, 2001; Hawlena et al., 2011). Detrimental effects of stress are common, because energy reserves are limited and must be diverted from other important demands in order to cope with stress. The price for this could be paid later on in life, in longer development time, shorter longevity, impaired reproduction, or lower quality of the offspring (Krebs and

Feder, 1998; Boggs and Freeman, 2005; Hawlena et al., 2011). That said, moderate levels of stress can sometimes lead to long-term improvement, a process termed "hormesis" (Le Bourg, 2009).

Studies on the behavioral responses to stressors usually focus on a single stress and a behavioral response. It is nevertheless important to determine how stress affects multiple responses, in order to better understand its consequences. Different stressors can lead to contrasting responses, even when applied to the same organism (e.g., in fruit flies starvation improves desiccation tolerance whereas heat shock impairs it; Bubliy et al., 2012). Even the same stressor, but varying in magnitude or duration, can have different impacts (Moskalev et al., 2015). For instance, with increasing starvation, many insect species first increase their activity in order to locate food, but at some point their activity drops to conserve energy, resulting in a hump-shaped pattern linking activity and starvation (Scharf, 2016; cf. Graf and Sokolovski, 1989; Knoppien et al., 2000). Stickleback fish exposed to predation threat first reduce their courting behavior and territorial maintenance, but with time, they increasingly engage in reproduction-related behavior, despite the ongoing predation threat (Candolin, 1998).

Understanding behavioral consistency, or how strongly repeatable

individuals are in their behavior in successive tests, is important for several reasons. First, it assists in measuring heritability levels (Boake, 1989). Second, it is a precondition for the determination of behavioral types or "animal personalities" (Bell et al., 2009). Third, it is especially interesting to compare holometabolous insects as larvae and as adults in order to understand whether some behavioral aspects are robust to the thorough change that insects undergo in the pupal stage (e.g., Müller and Müller, 2015; Wexler et al., 2016). Similar to the majority of behavioral traits, it differs both within and between populations (Sinn et al., 2010; Van Dongen et al., 2010; Herborn et al., 2014). Stress is expected to affect consistency. Stress experienced by adults induces phenotypic divergence of the offspring (Hoffmann and Merilä, 1999), which is perhaps analogous to decreasing behavioral consistency. Although such studies are rare, the findings to date indicate that moderate stress or suboptimal conditions seem to lower behavioral consistency. For instance, hungry spiders were less behaviorally consistent than satiated ones (Lichtenstein et al., 2016) and antlions kept under a suboptimal substrate were less behaviorally consistent than those kept under the preferred one (Alcalay et al., 2014). Opposite examples exist too: food stress during development led to higher behavioral consistency in adult zebra finches (Careau et al., 2014).

We studied how two different separately applied stressors, protein-poor nutrition and cold-stress, affect four important behavioral traits of the red flour beetle: movement activity, preference to move along the test arena's edges, latency to emerge from shelter, and preference for a dark microhabitat vs. a lit one. These behaviors generally represent the levels of activity and boldness (Carter et al., 2013). A previous study on the same species demonstrated changes in movement activity and preference for the arena edges with mating status and age (Wexler et al., 2016; Wexler et al., in press). Protein-poor nutrition in flour beetles is most often simulated by providing either oat meal or a low proportion of baking yeast added to the wheat flour they consume (Van Allen and Rudolf, 2013; Scharf et al., 2015). Protein-poor food modifies behavior in different ways. For example, beetles become more cannibalistic and consume more eggs to compensate for the protein shortage (Via, 1999); while recurring cold stress leads to an opposite result of reduced dispersal and movement activity (Scharf et al., in press). These two stressors differ in their duration (shorter vs. longer exposure) and in how they displace the individual from its ecological niche (nutrients vs. temperature).

We therefore expected the two stressors to differ in their effects on behavior. Keeping adults under protein-poor nutrition after being raised under rich nutrition should trigger a higher dispersal rate and hence movement activity, in order to locate new food patches (Van Allen and Bhavsar, 2014). Cold-shock should induce the opposite response (Moskalev et al., 2015), owing to potential cold injury or a decrease in metabolic rate. Latency to emerge from shelter should be shorter for beetles kept under poor nutrition, similar to other animals that leave shelter earlier when hungry (Ebert, 1998; Spanier et al., 1998). Cold shock, in contrast, should lead to longer latency to emerge from shelter. We expected beetles to move less as they aged, to move less along the test arena edges, and to increase their latency to emerge from shelter (Wexler et al., 2016; Wexler et al., in press). We also expected an interaction between the effect of time and treatment, because cold stress was an acute though single event, whereas the beetles were continuously kept under poor nutrition. Consequently, cold-stressed beetles were expected to demonstrate more similar behavior to the control beetles when tested later in life, while protein-stressed beetles were expected to deviate further from the control over time. Finally, we expected the control beetles to be more behaviorally consistent across tests than the cold-stressed and protein-stressed beetles.

2. Materials and methods

2.1. Preparation for the experiment

Our experimental animal was the red flour beetle *Tribolium castaneum* (Herbst, 1797). This is an important stored-product pest, often used as a model organism in behavioral studies (Fedina and Lewis, 2008; Miyatake et al., 2008) or responses to stress (e.g., starvation: Daglish, 2006; Sbilordo et al., 2011). The beetles were grown in the laboratory over 20 generations under 26 °C. This temperature is lower than the preferred or standard one for the beetles, which is about 30 °C (Grazer and Martin 2012; Halliday and Blouin-Demers, 2014), but we preferred to use it in order to avoid a contrast with the test room temperature, which was about the same temperature (26˚). We first allowed 160 beetles to mate for four days in two plastic boxes with 120 g wheat flour and 10% yeast. The adults were removed from the box after mating and larvae that emerged from the eggs pupated after five weeks. Following pupation, we sexed 150 pupae and allocated 25 members of each sex to each of the thermal-stress treatment, protein-stress treatment, and control. Individuals were kept singly in plastic tubes filled with 3 g of the flour-yeast mix (10:1 ratio) or oat flour for the protein-stress treatment. The cold-stressed treatment was moved 1–3 days after eclosion to a refrigerator (4 °C) for 1 h to simulate cold stress (similar to Kiyotake et al., 2014, using the same species). The protein-stressed treatment was moved from the wheat flour to oat flour upon pupation and remained in oat flour till the end of the experiment. Oat flour is a poor-quality food with low protein levels for flour beetles and is regarded as a stressful environment (Via, 1999; Van Allen and Bhavsar, 2014). The control treatment beetles were maintained under standard conditions and were hence not expected to experience any stress prior to use in experiments. We weighed all beetles 1–3 days after eclosion and again at the end of the experiment, 52 days later (see Supplementary Material, Table S1, for a timetable of the whole experiment). No permits were required to work on flour beetles in the laboratory and the cold and protein stressors applied were not lethal.

2.2. Behavioral tests

We measured four behaviors for each beetle: (1) movement activity, (2) edge preference, (3) latency to emerge from shelter, and (4) dark preference. For movement activity and edge preference we placed each beetle in a 9-cm Petri dish with paper on the bottom (similar to Wexler et al., 2016). The beetles were given 30 s of acclimation, after which we tracked their movement and position for 5 min using a webcam and the free software Buridan Tracker (Colomb et al., 2012). Movement activity was measured as the total distance covered by the subject and edge preference was measured as the proportion of time spent in the peripheral 5% of the dish. Latency to emerge from shelter was measured as the time spent by the beetle in a dark cardboard box (1 × 3 cm) before exiting to an open area. We first placed the beetle in the box, allowed 30 s for acclimation and then turned the box on its side, enabling the beetle to leave. The test was ended when the beetle was completely out of the box. If it remained in the box longer than 10 min, the test was stopped and the beetle received a score of 600 s (8.6% of the cases). To measure dark preference we used a square box (12.5 × 12.5 cm) divided in the middle into two equal sides, one exposed to light and the other to dark. We placed each beetle in the center of the box and after 30 s of acclimation recorded the time it spent in the dark side of the box, over a five-minute period.

Each behavior was tested a total of four times in four sessions, with an interval of a week between the first and second tests and the third and fourth tests, and a month between the second and third tests. We used this framework to study both earlier and later effects of stress. Within each phase (the two earlier vs. later sessions), all behaviors were tested twice to measure consistency and to increase measurement accuracy. Each session lasted three days. On day 1 we tested movement activity and edge preference; on day 2-latency to emerge from shelter; and on day 3-dark preference (timetable: Supplementary material: Table S1). The first session started on day 1 of the behavioral trials, 3–5 days after eclosion. The second session started on day 8, the third session on day 43, and the fourth session on day 50. The short-term stress treatment beetles started the behavioral tests one day later than