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Nutrition affects onset and duration of wandering behavior in tobacco hornworms

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Abstract

Insects that undergo complete metamorphosis (including egg, larval, pupal, and adult stages) obtain no nutrition in the time between the last larval stage and emergence from the pupa as an adult. In the final larval stage of burrowing insects, such as the tobacco hornworm (*Manduca sexta*), larvae wander to find soil or detritus in which to burrow, and then burrow, pupate, emerge as fully-formed adults, and return to the surface, relying solely on energy stored during the larval stage. I examined the effects of larval nutrition on wandering behavior and expected that dietary manipulation would delay wandering in both underfed and overfed treatments. I anticipated that duration of wandering behavior decreases in underfed larvae because they need to reserve energy. I predicted increased wandering behavior in overfed larvae because they could use excess stored energy to be more selective in where they burrow. To manipulate energy reserves, I manipulated diet and measured onset and duration of wandering behavior. Weight varied significantly across treatments, with overfed weighing the most and underfed weighing the least. Onset of wandering also differed, occurring first in the control animals and last in overfed larvae. Underfed larvae wandered for a lower percentage of time than both control and overfed larvae. This study confirmed that nutrition impacts wandering behavior and demonstrates the balance between energetic cost and nutritional benefit.

Introduction

All animals must balance metabolic costs with metabolic gains met through feeding. Insects have the added energetic expense of molting paired with concurrent fasting (Dominick & Truman, 1984). If costs outweigh gains, then functions, systems, or growth may be impacted negatively (Plaistow & Silvia-Jothy, 1999; Xu, Held, & Hu, 2013). If energy stores cannot offset metabolic costs associated with shedding their exoskeleton, they will not molt successfully, either failing to pupate fully, not forming an adult, or not completing eclosion, each resulting in death (Davidowitz, D'Amico, & Nijhout, 2003).

Research has linked nutrition to growth, development, and fitness across many species (Davidowitz, D'Amico, & Nijhout, 2003; Plaistow & Silvia-Jothy, 1999; Xu, Held, & Hu, 2013). However, to my knowledge, no research has examined links between diet and wandering behaviors. If energy allotted to wandering depends upon energy stored prior to the last larval stage, I predict that a manipulated diet alters duration of wandering behavior.

Juvenile hormone (JH) and ecdysteroid are two critical hormones in the metamorphic process, and a drop in JH signals the start of wandering (Dominick & Truman, 1984). Hydroprene is a JH mimic and, when added to the diet, delays the onset of wandering and allows the animal to eat more than it would in nature (Hrdý,, Kuldová, Hanus, & Wimmer, 2006).

I studied tobacco hornworms, *Manduca sexta*, throughout their 5th instar and into pupation. The hornworm is an ideal candidate for this study because its wandering behaviors are well-documented (Dominick & Truman, 1984; Reinecke, Buckner, & Grugel, 1980). Additionally, they are large, which increases visibility for observations. Finally, the artificial food fed to *Manduca* in a laboratory setting is easily manipulated for experimentation.

Predictions

1) As diets will be manipulated, overfed larvae will weigh the most and underfed will weigh the least.

2) Underfed larvae will delay wandering because they will not yet have reached the minimum weight that signals wandering.

3) Overfed larvae will spend more time wandering because their larger energy stores enables them to expend more energy being selective in choosing a pupation location.

4) Underfed larvae will spend less time wandering to conserve limited energy stores for metamorphosis.

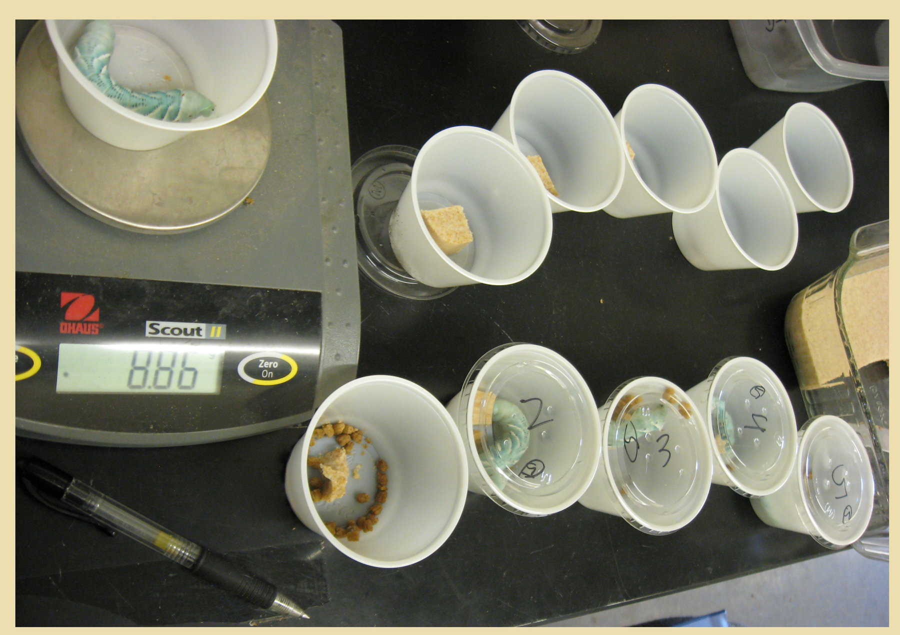
Methods

Rearing Conditions

I maintained *Manduca sexta* larvae in a controlled environment of 26°C, relative humidity of 20-35%, and a 15h:9h light:dark cycle in the Science Building of the University of Southern Maine. I fed each larva an artificial base food diet *ad libitum*. At the start of ecdysis between 4th and 5th instars, I removed food for 24h to ensure a uniform start of wandering behavior within the group. I then returned control group (n = 16) larvae to base food. To simulate higher energy reserves, I fed another group (n = 15) food containing 30 μL hydroprene, which delays onset of wandering and allows larvae to feed longer prior to pupation. To simulate lower energy reserves, I returned a group (n = 12) to the base food for 42h and then withheld food for the remainder of the experiment.

Data Collection

Each day, I weighed each larva on a digital scale to 0.01 g to monitor growth, and I also observed them for visual cues (body-coating, visible dorsal vein, and disruption of frass and food) that indicate wandering has begun (Dominick & Truman, 1984). After onset of wandering, I placed larvae individually into an arena of segregated, 10 x 15 cm plastic containers with air holes in the sides, topped with a Plexiglas cover. The arena enabled maximum wandering potential, as there was nowhere to burrow. I positioned a Kodak PlaySport (Zx5) HD camera to record video from above, and I used focal sampling to measure duration of wandering movement. Two larvae in the underfed group showed only minimal movement during observation in the arena, and I did not include their data in the percentage of time spent wandering.



Images 1 and 2. Weighing *Manduca sexta* and Manduca wandering in the observation arena. The dorsal vein, which becomes visible during wandering, can be seen in those larvae in the arena.

Data Analysis

Larvae periodically stopped wandering and remained still for some time before moving again and eventually settling permanently in one position. Because total duration differed for each animal, I converted time spent wandering into a percentage for each larva. For each treatment, I calculated mean weight at wandering, mean time until onset of wandering, and mean percentage of time wandering. I used ANOVA and Tukey-Kramer HSD post-hoc tests to determine significance at $p < 0.05$. All tests were performed using JMP software (SAS Institute, Inc., Cary, NC).

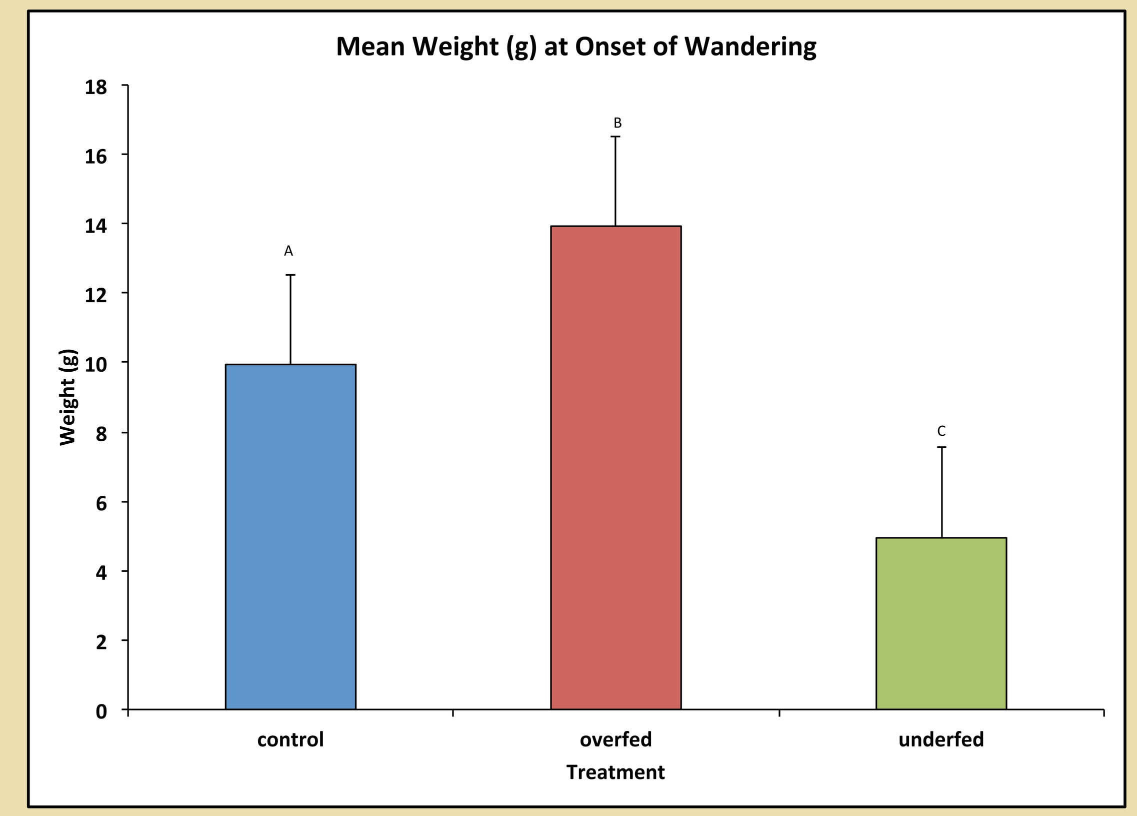


Figure 1. Mean (+SE) weight at onset of wandering for 3 groups of *Manduca sexta* larvae. Mean weights differed significantly across treatments ($F_{2, 40} = 414.01$, $p < 0.0001$). Overfed larvae weighed significantly more than controls and underfed larvae, and underfed larvae weighed significantly less than the other treatments (Tukey post hoc comparisons, $p < 0.0001$).

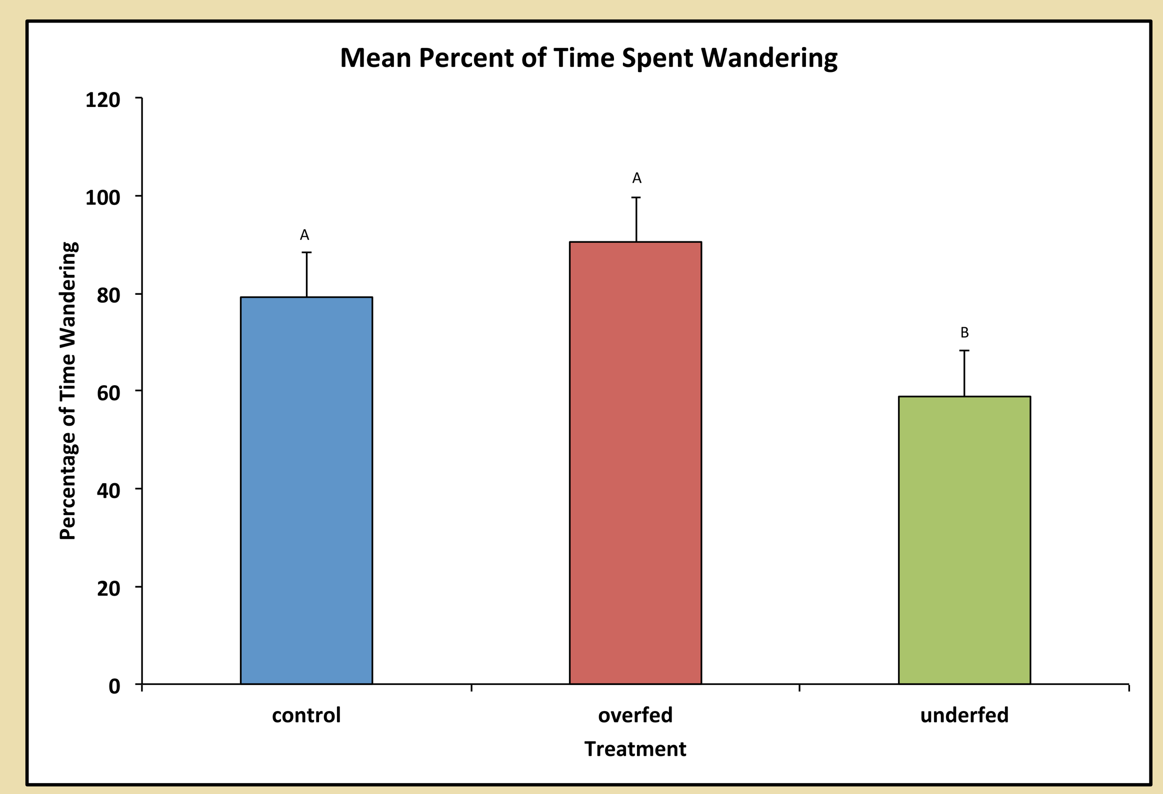


Figure 2. Mean (+ SE) time until the onset of wandering varied significantly among the 3 groups ($F_{2, 40} = 316.39$, $p < .0001$). Control larvae wandered first, followed by underfed larvae, and overfed larvae wandered last (Tukey post hoc comparisons, $p < 0.0005$).

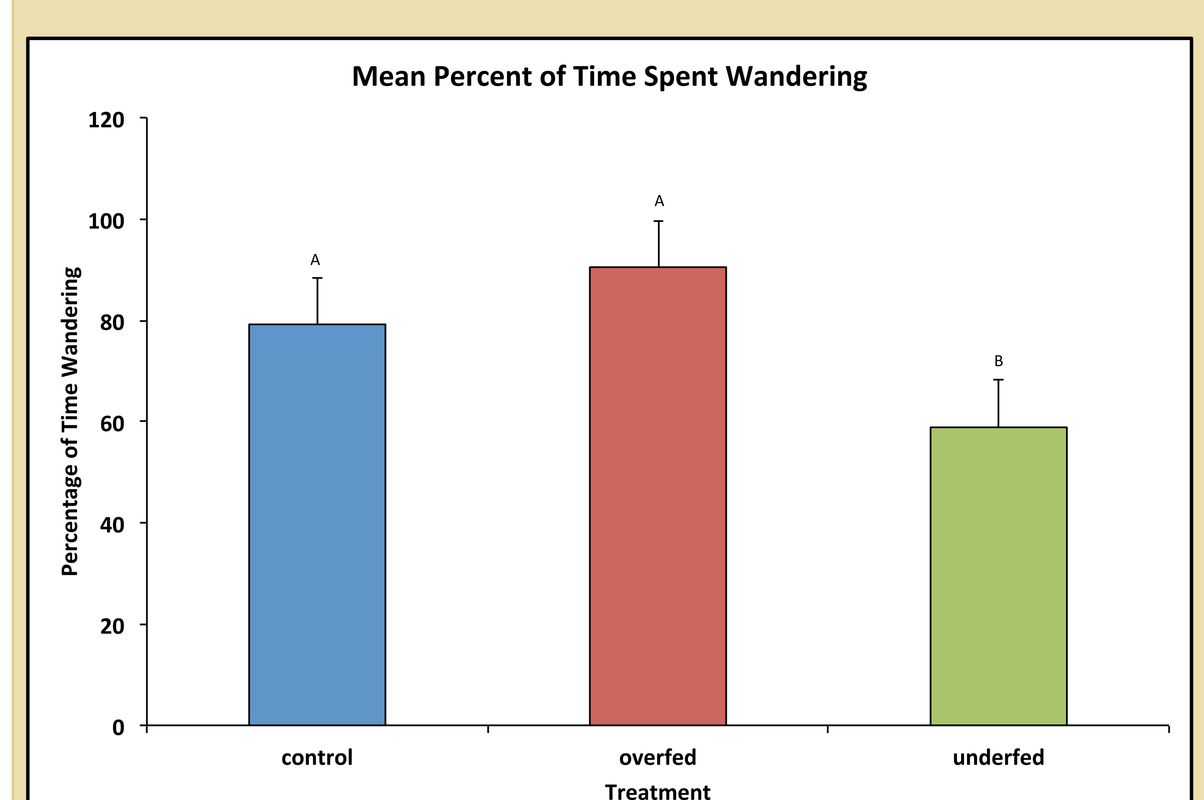


Figure 3. Mean (+SE) percentage of time spent wandering differed significantly across treatments ($F_{2, 38} = 7.94$, $p = 0.0013$). Overfed larvae wandered for a significantly higher percentage of time than underfed animals (Tukey post hoc comparisons, $p < 0.001$). Underfed larvae also wandered for a significantly lower percentage of time than the control group ($p = 0.0353$). However, overfed and control treatments did not differ ($p = 0.2521$).

Discussion

I predicted that overfed larvae would weigh the most and underfed larvae would weight the least as a result of eating a manipulated diet, which the data supported. When animals eat more, they grow to larger sizes, and when nutrients are limited, those animals grow less (Davidowitz, D'Amico, & Nijhout, 2003; Sears, Kerkhoff, Messerman, & Itagaki, 2012; Stern, 2011).

I anticipated that both underfed and overfed larvae would delay onset of wandering, which the data supported. The overfed group experienced a delay due to the presence of hydroprene in their systems. Underfed larvae had not yet met the minimum weight that usually triggers the onset of wandering, so the lapse may reflect a balance between the cost of wandering at a smaller size and securing enough food to reach the minimum weight, resulting in a later onset than observed in the control group.

I predicted that manipulating diet would affect duration of wandering behavior, and the data partially supported my predictions. Underfed larvae spent less time wandering compared to both control and overfed groups. With lower energy reserves, underfed larvae perhaps allocate energy to pupation and transformation into the adult moth rather than to locomotion.

I also expected the overfed group to spend more time wandering, but they did not differ from controls. Perhaps overfed larvae continue to conserve their energy in case they need it later in metamorphosis. Larger larvae result in larger pupae, which result in larger moths (M. Bentzel, pers. obs.), so a benefit may exist in conserving energy to form a larger adult.

Future studies could increase sample sizes, length of observation, and amount of hydroprene to see if larvae differ in time spent wandering. Additionally, one could examine wandering behaviors in a more natural arena, manipulate protein and caloric content of diet to determine whether general weight or a specific element of the diet affects behaviors, or induce diapauses to investigate underlying nutritional effects on wandering behavior.

Results of this study show how *Manduca* respond to varied nutrition when faced with energetic costs of wandering, pupation, and metamorphosis. If these needs are not balanced with stored energy, the insect could die. This relationship between energetic costs and nutritional benefits occurs across species in the animal kingdom, and further studies could glean information from the findings revealed in this study.

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