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Sex-specific differences in nutrient regulation in a capital breeding caterpillar, *Spodoptera litura* (Fabricius)

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ABSTRACT

Nutrient requirements by male and female insects are likely to differ, but relatively little is known regarding how sexes differ in their regulation of macronutrient acquisition. The present study reports the results from a laboratory experiment in which behavioural and physiological components of nutrient regulation were compared between male and female caterpillars of *Spodoptera litura* (Fabricius). When provided with choices between two nutritionally complementary foods (one is a protein-biased diet and the other a carbohydrate-biased diet), both males and females adjusted their food selection to defend an intake target. However, the composition of diet preferred by the two differed, with females selecting significantly more protein than males with no difference in carbohydrate intake between the two. When confined to single diets with varying mixtures of protein and carbohydrate [P:C ratios, expressed as the percentage of diet by dry mass: protein 42%:carbohydrate 0% (p42:c0), p35:c7, p28:c14, p21:c21, p14:c28, p7:c35], females consumed more macronutrients than did males across on all P:C diets except the extremely carbohydrate-biased diet (p7:c35). Under both choice and no-choice feeding condition, such sex differences in nutrient intake were not expressed until late in the feeding stage of the final stadium. Sexes also differed in post-ingestive utilization of ingested nutrients. Females utilized ingested protein for body growth with greater efficiency compared to males, presumably reflecting provisioning their adult needs for protein to develop eggs, whereas males were more efficient at depositing lipids from carbohydrate intake than females.

1. Introduction

Various aspects of morphology, physiology, behaviour, and life-history differ considerably between males and females, as a result of differences in their reproductive strategies (Andersson, 1994). For most insects, the reproductive success of females is critically dependent upon the level of nourishment for ovarial development and yolk production (Wheeler, 1996). Yolk is rich in lipids and proteins, and female reproduction is limited by the supply of these specific nutrients (Chapman, 1998). Male reproduction is no less nutrient-dependent than females, with the expressions of many male-specific sexual traits being condition-dependent (Emlen, 1994; Kotiaho, 2002; Hunt et al., 2004; Maklakov et al., 2008). Thus, the reproductive success of both sexes rests on how efficient they are at acquiring nutritional and energetic resources but males and females require different amounts and mixtures of multiple nutrients to maximize their respective reproductive performance (Behmer and Joern, 1994; Clarebrough et al., 2000; Carrel and Tanner, 2002; Morehouse et al., 2010). Recently, the latter was elegantly demonstrated in an income breeder, adults of the Australian field cricket, *Teleogryllus commondus* (Maklakov et al., 2008).

Larval and adult diets of most holometabolous insects are different and the extreme case of such disparity is found in capital breeding species of many Lepidoptera. Because their adult diets (nectar) are relatively deficit in protein or amino acids, females must rely on their larval diet to provision sufficient protein pool for vitellogenesis, which occurs at or just after adult emergence (Boggs, 2009). However, relatively little is known concerning how male and female larvae of capital breeding insects differ in behavioural and physiological processes that regulate nutrient acquisition (but see Stockhoff, 1993; Telang et al., 2001, 2003).

A central aim of the present study is to determine sex-specific differences in nutrient regulatory responses in a capital breeding caterpillar of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), a species which is known for its highly polyphagous feeding habit. There are two steps through which insects achieve nutritional homeostasis; the first is behavioural, through adjustment of feeding, and the second is post-ingestive, involving alteration of the efficiency of converting ingested nutrients to body growth (reviewed by Simpson and Simpson, 1990; Waldbauer and Friedman, 1991; Slansky, 1993; Simpson et al., 1995; Clissold et al., 2010). The present study tests three predictions with respect to sex

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differences in the mechanisms involved in the acquisition of the two major macronutrients independently regulated by most insects, protein and digestible carbohydrate (Simpson and Simpson, 1990; Simpson et al., 1995). First, given their greater protein requirement for egg production, female caterpillars are predicted to select a higher protein diet than male caterpillars when given choices between nutritionally complementary diets. Second, if caterpillars are denied from reaching their preferred intake under a no-choice feeding regime, females are predicted to consume more macronutrients than males to match their high protein requirement at the expense of incidentally eating more carbohydrate. Third, females are expected to be more efficient at converting protein intake to body protein pool than males (i.e., post-ingestive utilization for protein), which may be destined for egg production. To test for these predictions, the two sexes of *S. litura* caterpillars were compared in experiments designed using the Geometric Framework, which has been proven as a powerful analytic tool for studying nutritional biology in insects and other animals (Simpson and Raubenheimer, 1993; Raubenheimer and Simpson, 1997, 1999, 2004; Raubenheimer et al., 2009).

2. Materials and methods

2.1. Experimental diets

Seven dry, granular, chemically defined synthetic diets were prepared based on a protocol described in Simpson and Abisgold (1985). All seven diets contained a fixed amount of protein plus digestible carbohydrate (42% by dry mass) but each varied in the mixture of protein to digestible carbohydrate concentrations (dietary P:C ratio, hereafter): 42% protein with 0% digestible carbohydrate (p42:c0), p35:c7, p28:c14, p21:c21, p14:c28, p7:c35, and p0:c42. This range of ratios has been used in previous studies on other caterpillars (Lee et al., 2002, 2003, 2004, 2006; Simpson et al., 2004; Despland and Noseworthy, 2006; Telang et al., 2001, 2002, 2003; Merkx-Jacques et al., 2008; Thompson and Redak, 2005; Warbrick-Smith et al., 2006, 2009). The protein component of the diets was a 3:1:1 mixture of casein (Sigma C7078), peptone (Fluka 82962) and albumen (Sigma A5253), and digestible carbohydrate comprised sucrose (Sigma S9378). All diets contained 4% of essential micronutrients (salts, vitamins, cholesterol and linoleic acid; see Lee et al., 2002) and 54% of indigestible cellulose powder (Sigma C8002). The complete mixture of dry ingredients was blended with 1% agar solution in a 1:6 dry diet: agar solution ratio (ca. 86% water content). This agar-blended, fresh diet was sliced into blocks just before being presented to insects.

2.2. Experimental design

Two separate experiments were performed with a total of 100 final-instar (6th) *S. litura* caterpillars (a total of 51 males and 49 females). The first experiment was a food choice test, which was designed to determine sex-specific difference in the preferred dietary mixture of protein and digestible carbohydrate. In this choice experiment, 40 caterpillars (23 males and 17 females) were each provided with one of four food choices, in which one of two protein-biased diets (p42:c0 or p35:c7) was paired with one of two carbohydrate-biased diets (p0:c42 or p7:c35): p42:c0 vs. p0:c42 (treatment PC), p42:c0 vs. p7:c35 (treatment Pc), p35:c7 vs. p0:c42 (treatment pC), and p35:c7 vs. p7:c35 (treatment pc). The second experiment was a no-choice test, where 60 caterpillars (28 males and 32 females) were restricted to one of six single diets differing in their P:C ratio (p42:c0, p35:c7, p28:c14, p21:c21, p14:c28 or p7:c35). The aim of the second experiment was to investigate how the two sexes differ in their feeding and post-ingestive responses when exposed to nutritional circumstances forcing them to eat too much of one nutrient and too little of the other.

2.3. Experimental protocol

S. litura caterpillars were derived from a laboratory culture maintained at Seoul National University, Republic of Korea, and were raised on a wheat germ-soy based semi-artificial diet at 25 °C with a 12:12 light:dark photo regime. During this pre-experimental culturing period, the insects were reared at a density of 10–15 per each rearing arena (9-cm diameter Petri dish) until they had reached final larval stadium.

Both experiments started when the caterpillars had ecdysed to the final larval stadium. Upon moulting, individual caterpillars were weighed to the nearest 0.1 mg (obtaining initial fresh mass), and each was placed in its own experimental arena (9-cm diameter Petri dish with five 1 mm-diameter perforations in the upper lid allowing ventilation) where they receive either two blocks (choice experiment) or a single block of food (no-choice experiment). Each food block was pre-weighed to the nearest 0.1 mg within the range of 800–1200 mg (by fresh mass). This range was carefully chosen to avoid the risk of measurement error that would result from providing food blocks far in excess of the amount consumed by individual insects (Schmidt and Reese, 1986). Once the food and insects were inside, feeding dishes were sealed with a strip of Parafilm, and kept in an incubator set at 25 °C under a 12:12 light:dark photo regime throughout the experiment. After 24 h, any uneaten food was removed and was replaced by fresh, pre-weighed food blocks. Removed food was dried to a constant mass in a drying oven set at 50 °C, and its dried mass was weighed to the nearest 0.1 mg. This procedure was repeated until each caterpillar had ceased feeding before entering the prepupal wandering stage.

2.4. Nutrient intake measurements

To estimate daily food intake, at least 12 control arenas were established that only contained pre-weighed food blocks for each dietary treatment. These controls were run concurrently with the experimental arenas throughout the experiment. Food blocks from these control arenas were collected each day, dried to a constant mass at 50 °C, and weighed to the nearest 0.1 mg. By using these controls, linear regression equations were constructed for each dietary treatment (R^2 values all above 0.98), from which the dry mass of food blocks initially provided to each caterpillar was estimated. Daily food intake was calculated as the difference in dry mass between initial dry food mass and final dry food mass remained uneaten by caterpillar. The intake of protein and carbohydrate was calculated as the product of dry mass of food intake and the concentration of respective nutrient in the food.

2.5. Performance and body nutrient growth measurements

The duration of the final larval stadium (stadium duration, hereafter) was measured to the nearest 6 h from pupation. Once pupal cuticle was fully tanned, pupae were sexed according to the morphology of the abdominal terminal segments (Butt and Cantu, 1962), and were weighed to within 0.1 mg (fresh pupal mass). These pupae were killed by freezing at −20 °C, dried for 3 days at 50 °C, and then weighed to the nearest 0.1 mg to obtain dry pupal mass. To extract lipids from pupae, dried pupae were placed individually in a 10 ml vial and soaked in chloroform for 24 h. After 24 h, chloroform was removed from the vial and replenished with fresh chloroform. This procedure was repeated for two more consecutive days, after which lean pupae were re-dried and re-weighed. Lipid content was calculated as mass change (dry mass) before and after the three, 24-h changes of chloroform (Simpson,