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Dual fitness benefits of post-mating sugar meals for female hawkmoths (*Hyles lineata*)

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ABSTRACT

The white-lined sphinx moth (*Hyles lineata*: Sphingidae) is the most widespread and abundant hawkmoth pollinator in North America and plays a major role in the reproductive biology of many plant species. *H. lineata* visits a wide range of plants, which differ in the quality and quantity (e.g. caloric content, volume) of the nectar reward that they offer in exchange for pollination services. Some of these plants represent a suitable oviposition substrate as well as a profitable nectar source, allowing mated *H. lineata* females to mix foraging and oviposition bouts. We investigated the effects of post-mating nectar intake on the reproductive success of female *H. lineata*. While all experimental females had access to a 20% sucrose solution during the pre-mating phase (avg. 2.7 days) we manipulated the post-mating diet, assigning mated females to three experimental groups (sucrose fed, water fed, or unfed). Mated females with access to sucrose lived twice as long and produced more fertile eggs at double the rate of control moths that were starved or water-fed after mating. Thus, the sugar component of floral nectar positively affects the physiology of mated *H. lineata* at multiple levels, which translates into strong selection for mated females to continue nectar foraging during or between oviposition bouts.

1. Introduction

Mutualistic interactions between flowering plants and insects often are based on the exchange of floral nectar (or other energetic rewards) for the effective transfer of pollen among conspecific plants. Animal-pollinated plants invest considerable amounts of carbon and water in the production of floral nectar, one of the primary resources used by plants to enlist animals as agents of accurate and efficient pollen transfer and outcrossing (Pacini and Nepi, 2007). Floral nectar has long attracted the attention of biologists seeking to determine whether its volume, secretion rate and chemical or caloric composition have been selected to match the physiological demands and digestive capabilities of specific animal pollinators (Martínez del Rio et al., 2001; Nicolson, 2002). One group of pollinators with very high energetic demands is the hawkmoth (Lepidoptera; Sphingidae), which has been shown to use cues revealing the presence of floral nectar (e.g. humidity gradients, CO_2 emissions) in order to optimize nectar foraging by reducing search and handling times (Thom et al., 2004; von Arx et al., 2012, 2013). During hovering flight, hawkmoths demonstrate one of the highest recorded metabolic rates among flower-visiting animals, and are able to increase oxygen consumption up to 148 times from rest to flight (Bartholomew and Casey, 1978). In adult hawkmoths, the metabolic resources utilized to support this energy-costly mode of flight are flexible, depending upon an individual's physiological history. Accordingly, hawkmoths have been shown to allocate either larval-acquired fat reserves or adult-acquired sugar as the primary flight fuel (Kammer and Heinrich, 1978; Ziegler and Schulz, 1986; Joos, 1987; O'Brien, 1999).

Besides its role in flight fuel metabolism, resource allocation also affects fecundity, through the provisioning of eggs in adult females. For example, the diurnal hawkmoth *Amphion floridensis* uses adult-acquired sugar meals to provide 50–60% of egg carbon after a few days of feeding (O'Brien et al., 2000). Adult nectar feeding is known to enhance fecundity in other Lepidoptera (e.g. Murphy et al., 1983; Hill, 1989; Hill and Pierce, 1989). In her review of butterfly reproductive ecology, Boggs (1997a) predicted that the degree to which larval- vs. adult-obtained nutrients are provisioned into eggs for a given species depends upon its access to specific nutrients during larval and adult stages. In the extreme case of *Heliconius* butterflies, which acquire carbohydrates (via nectar) and amino acids (via pollen) as adults, few if any eggs are provisioned at eclosion (Boggs, 1997b). Although clear fitness benefits are predicted for post-mating nectar meals, net fitness could decrease if nectar foraging comes at the expense of oviposition, e.g. by visiting plants that offer nectar but no oviposition substrate. Opportunity costs present additional constraints on lepidopteran fecundity, as many moths and butterflies have short adult life

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spans, and periods of activity could be limited by their flight performance under challenging abiotic conditions (Herrera, 1992) or high perceived predation pressures (Dukas, 2001). For example, in *Pararge aegeria*, fecundity is significantly decreased when climatic conditions force females to devote less time to oviposition (Gotthard et al., 2007). Thus, a mated female's allocation of time to oviposition vs. foraging is likely to directly impact her reproductive success (see Stanton, 1982).

Mated female moths or butterflies that forage for nectar face the problem of doing so at the cost of locating suitable oviposition sites, and the question arises of how important nectar meals become during this stage. Are the resources acquired during the larval- and the pre-mating stage sufficient for egg provisioning? Is the post-mating period best spent exclusively on oviposition or is continued nectar uptake throughout adult life more beneficial? In this paper we addressed these questions by measuring the effects of post-mating sucrose intake in the white lined sphinx moth (*Hyles lineata* Fab.). *H. lineata* is an excellent model species for such studies, because the feeding patterns of juvenile and adult individuals generate a mosaic of ecological interactions, ranging from strict herbivory (e.g. on *Portulaca*; Tuttle, 2007) or pollination (e.g. of *Aquilegia*; Brunet, 2009) to a complex pollinator-herbivore relationship (e.g. *Oenothera cespitosa* and related plants; Artz et al., 2010), across the Americas. Because *H. lineata* is such a highly generalized herbivore and flower visitor (Raguso et al., 1996), linking it to many plant species in the communities in which it is found, our field observations of female moths using *Oenothera* plants as both larval and adult hosts (Artz et al., 2010) struck us as unusual, and compelled the present study. Our results are discussed with reference to hawkmoth reproductive physiology and to community context, where we make predictions about the benefits of mated *H. lineata* females visiting host plants that offer nectar and oviposition substrate.

2. Materials and methods

2.1. Moth rearing

Eggs and larvae of *H. lineata* were collected on *Oenothera harringtonii* plants in Colorado Springs (CO) during the summer of 2009. Larvae were raised individually on an artificial diet adapted recently for *Manduca sexta* (Goyret et al., 2009). Wandering prepupae were placed in wooden pupation boxes as described by Yamamoto et al. (1969), then were removed after 10 days, identified to sex and stored in a walk-in incubator until they were ready to eclose. Eggs, larvae, and pupae were kept in a walk-in incubator at 24.0 °C, 50% humidity on a 16L:8D photoperiod.

Pupae near eclosion were placed in a flight cage ($0.6 \times 0.6 \times 0.6$ m) in a greenhouse (28.8 °C, 58.8% humidity, 16L:8D photoperiod). Emerging moths used to maintain the breeding colony are provided with sucrose solution (20%, by mass) for feeding as well as greenhouse-grown evening primrose plants (*O. cespitosa* ssp. *marginata*) for oviposition. Eggs were collected twice weekly to maintain the rearing colony.

2.2. Experimental protocol

2.2.1. Moth handling

Experiments took place during the summer of 2010. Pupae that were near eclosion were placed into a flight cage ($0.46 \times 0.46 \times 0.46$ m) in an incubator. Separate incubators were used for male and female moths. These pupae were stored at 24.0 °C, 34% humidity on a 16L:8D photoperiod. One-day-old moths were removed from the incubators daily at 15:00 h and were marked with permanent ink on the medial portion of the forewing to distinguish day of eclosion and sex.

After marking, male and female moths were added to a greenhouse flight cage ($0.6 \times 0.6 \times 0.6$ m) with an average daytime temperature of 28.8 °C and relative humidity of 58.8%. The photoperiod was based on the natural light cycle, and was approximately 15L:9D. Moths had *ad libitum* access to a 20% (by mass) sucrose solution in the cage. The sucrose solution was presented in a feeder (plastic cup with a yellow sponge), which the moths visited readily. This sucrose solution served as an adequate floral nectar surrogate, compared with standing crops of sucrose-dominated floral nectar from greenhouse grown *O. cespitosa* subsp. *navajoensis* (30.7 ± 2.2 mg/flower, 24.4 ± 0.9% sucrose-equivalents/flower; mean ± SEM, $N = 41$) and nectar collections in the field (*O. cespitosa*, mean volume: 35 µl, mean concentration: 32.5%; Stockhouse, 1975). Mating pairs were removed from the flight cage each morning. Female wing length was recorded and pairs were allowed to separate.

Earlier trials were performed under similar conditions in the walk-in incubator to determine the time of day when the moths mated, as well as average length of mating. This allowed for prompt removal of mating pairs from the greenhouse flight cage in later trials.

2.2.2. Post-mating feeding treatments

Once females were mated (average age of experimental *H. lineata* females at mating: 2.7 days; Fig. S2A) they were randomly assigned to three experimental groups (sucrose fed [20%, by mass], water fed, or unfed) and were placed singly into their corresponding flight cages ($0.46 \times 0.46 \times 0.46$ m), each with a potted evening primrose plant for oviposition (flower buds were removed), in the greenhouse. Feeding began the following morning. Moths were hand fed twice daily, once in the morning and once in the evening. The forewings were held by the costal margin in one hand, while a toothpick was used to manually extend the proboscis into a 1.5 ml Eppendorf feeding tube. The moths were allowed three rejections of the feeding tube before being returned to their cages. Intake was measured by calculating the difference in mass of the vial after vs. before feeding. A control feeding was done using dead moths to determine how much solution was absorbed by the toothpick or the moth's proboscis during feeding. The same method as described above was used, but the proboscis was immediately removed from the solution each time. This was performed 16 times, with the average fluid loss of 6.3 mg, which was then subtracted from all feeding data. Sucrose-fed females were required to drink a minimum of 175 mg within the first 3 days or they were excluded from the experiment. Water-fed females had no intake requirements, as females generally showed a low intake of water. Females that did not lay eggs within the first 3 days after mating also were excluded from the experiment.

2.2.3. Response variables

In order to observe the effects of post-mating sucrose feeding on fertility, eggs were collected once daily following morning feeding. The eggs were counted, sterilized in a 4% (by volume) bleach solution for 5 min, rinsed in dH_2O, and placed on artificial diet. After 5 days, eggs were observed to determine the proportion of fertilized versus unfertilized eggs (by then, unfertilized eggs had collapsed). Ovarian status of unmated females was assessed in newly eclosed, 3 day old and 6 day old moths. Euthanized moths were dissected in a 25% ethanol solution. Fat was removed from the ovaries to allow better visualization of the ovarioles. Eggs were counted and provisioning was determined based on size, color, and position within the ovaries, as described by Nijhout and Riddiford (1974).