Fed males increase oviposition in female hawkmoths via non-nutritive direct benefits

Eran Levin [a, *], Chandreyee Mitra [a, b, c], Goggy Davidowitz [a, b]

[a] Department of Entomology, University of Arizona, Tucson, AZ, U.S.A.
[b] Center for Insect Science, University of Arizona, Tucson, AZ, U.S.A.
[c] Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, U.S.A.

Direct benefits provided by males have large effects on the fitness of females and their offspring in many species. Here, we examined whether mating or feeding experience of male Carolina sphinx moths, *Manduca sexta* (Sphingidae), affects the quality of direct benefits that males provide to their mates. We mated virgin females with fed and unfed, virgin and previously mated males. Feeding experience affected male mating success, spermatophore size and flight muscle size. In addition, females mated to fed males laid more eggs than females mated to unfed males, and females mated to virgin males laid more eggs than females mated to previously mated males. Using ^{13}C-enriched glucose in the nectar of the fed males, we found that the second and third spermatophores of males were strongly labelled, but this labelled glucose was not present either in the female's fat body or in her eggs. Therefore, although fed males provided females direct benefits from the sugar in the nectar, the sugar was not used as a nutrient by females. We suggest that in short-lived species, where females mate singly and males mate multiply, and where nectar availability is highly variable, males may be selected to conserve their resources to maintain flight capability and to invest in sperm performance and, thus, provide only limited (non-nutritive) direct fitness benefits to their mates.

© 2015 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

In many species, a male's reproductive success is limited by his access to mates, while a female's reproductive success is limited by the number of offspring she can produce (Andersson, 1994). This pattern is thought to be prevalent because the male's investment per offspring, in the form of sperm, is generally considered to be low compared with females, which produce large and nutrient-rich oocytes. This has led to the general perception that male fitness in many species is increased by mating with as many females as possible (Lauwers & Van Dyck, 2006), whereas a female's fitness is increased by selecting high-quality mates who provide benefits that increase either her fitness or the fitness of her offspring (Burley, 1986; Eberhard, 1996; Wedell & Karlsson, 2003). These male-provided benefits may be indirect, where alleles from the preferred males increase offspring viability or attractiveness of male offspring (e.g. Fisher, 1930; Lande, 1981; Zahavi, 1975), or direct, where preferred males provide material resources that increase the fitness of the female or her offspring (e.g. Price, Schluter, & Heckman, 1993; South & Lewis, 2011). Despite direct benefits likely having larger effects on female fitness than indirect benefits, indirect benefits have received more attention in the literature (Wagner, 2011). As a result, there are still major gaps in our understanding of how male-provided direct benefits affect female and offspring fitness in different systems.

One of the difficulties with many past experimental studies of direct benefits is linking observed fitness increases to a benefit mechanism (Wagner, 2011). For example, to link these effectively, a study would need to not only identify a nutrient that males transfer to females, but also to show increased female/offspring fitness as a result of this male-provided nutrient. This link is most lacking in cases where the male-provided benefit cannot be easily observed, unlike, for example, male parental care (reviewed in Andersson, 1994). Where the male-provided benefit comes in the form of a nuptial gift, broadly defined as any materials other than gametes transferred by males to females during courtship and/or mating (Lewis & South, 2012), connecting fitness gains to a benefit mechanism has been exceedingly rare. Some studies have found that male transfer of materials to females increases female fitness (e.g. Boggs, 1990, 1995; Gwynne, 2008; South & Lewis, 2011; Vahed, 1998), but the specific materials involved were not identified.

* Correspondence: E. Levin, Department of Entomology, University of Arizona, Tucson, AZ 85721, U.S.A.
E-mail address: levineran1@gmail.com (E. Levin).

Similarly, multiple studies have identified specific materials that males transfer to females, but these studies found no evidence that these materials benefited females or their offspring (e.g. Arnqvist & Nilsson, 2000; Boggs, 1990; Eberhard, 1996; Lewis & South, 2012; Wolfner, 2009). Studies that have examined the positive effect of male-provided defensive compounds on offspring fitness are the exception (e.g. Dussourd, Harvis, Meinwald, & Eisner, 1989; Iyengar & Eisner, 1999). Therefore, studies that both examine fitness effects of male-provided benefits and explore the underlying benefit mechanisms are much needed, especially where the benefits are transferred to females via a nuptial gift.

The phenotypic quality of male insects is often reflected in their ejaculate, which can contain numerous substances other than gametes (Wolfner, 2009). These substances are produced by the accessory glands and are transferred to the female during mating in the spermatophore. In some species, multiple matings, or polyandry, can enhance a female's lifetime fecundity by nutrient acquisition from the seminal fluids of multiple males, in addition to ensuring high variation in quality and genetic make-up of the sperm they receive (reviewed in Arnqvist & Nilsson, 2000; South & Lewis, 2011). Secondly, in some female insects, larger or more spermatophores result in greater lifetime fecundity/fertility (South & Lewis, 2011; Torres-Vila & Jennions, 2005), providing further evidence that materials in male spermatophores are a form of male-provided direct benefit. Therefore, where females receive direct benefits from male seminal products, we might expect females to prefer to mate with multiple males and to mate with males that provide larger and higher-quality spermatophores.

Like many insects, males of most Lepidoptera species transfer spermatophores to females during mating that contain both sperm and other seminal materials. We know from previous work that females of many Lepidoptera species are polyandrous (Simmons, 2001). However, a careful inspection of available studies reveals that a positive correlation between polyandry and fecundity is not universal (reviewed by: Milonas, Farrell, & Andow, 2011; Torres-Vila, 2013; Torres-Vila & Jennions, 2005). Secondly, in many Lepidoptera, the ejaculate becomes smaller with repeated matings (Lauwers & Van Dyck, 2006; McNamara, Jones, & Elgar, 2007; Torres-Vila & Jennions, 2005). However, the effect of male mating history on mating success may vary among species (Milonas et al., 2011; Torres-Vila & Jennions, 2005; Velde, Damiens, & Van Dyck, 2011). Flight muscle can also be resorbed in Lepidoptera to reallocate resources to reproduction (Boggs, 2009; Stjernholm, Karlsson, & Boggs, 2005), and this resorption does not seem to impair flight ability (Stjernholm et al., 2005). However, it is unclear whether mating multiply results in resorption of flight muscle in males. Lastly, while it is well established that female feeding can increase female fecundity (Papaj, 2000; Wheeler, 1996), the effect of male feeding on female fecundity has been less studied (but see Cahenzli & Erhardt, 2012). Therefore, further study is needed to determine (1) whether females gain direct benefits from male spermatophores, (2) whether either the quality of these benefits decreases, or the resorption of the male's flight muscles increases with the number matings per male, (3) whether the feeding history of a male affects the fecundity of his mates and (4) the specific mechanism by which female mates receive direct benefits from males.

Here we examined the effects of mating experience and feeding history on male mating success and the quality of direct benefits provided by male Carolina sphinx moths, Manduca sexta (Sphingidae). For males, we examined whether feeding history influences mating success and whether feeding history and mating experience influence spermatophore size or flight muscle mass. To examine the benefit mechanism, we enriched the glucose fed to adult males with ^{13}C in order to examine whether this sugar was incorporated into male spermatophores and, if so, whether it was absorbed by females postmating. For females, we examined whether their fitness is affected by male feeding history, spermatophore size or mating experience. In addition, we tested whether female M. sexta are monandrous or polyandrous, as this has not been determined previously.

METHODS

Study Organism

In this study we used the Carolina sphinx moth, M. sexta (Lepidoptera: Sphingidae), from a colony maintained at the University of Arizona (Davidowitz & Nijhout, 2004; Davidowitz, Nijhout, & Roff, 2012). Manduca sexta is a large hawkmoth distributed across the Americas and is common in the Sonoran Desert in the southwestern United States (Tuttle, 2007). In the southwest, M. sexta has an innate preference for high-concentration, sucrose-based nectars (Datura wrightii, Solanaceae) but readily learns to use a low-concentration hexose flower (Agave palmeri, Agavaceae) as an alternate source of nectar (Alarcon, Davidowitz, & Bronstein, 2008; Riffell et al., 2008). Flight arena experiments showed that naïve males and females prefer D. wrightii over A. palmeri (Alarcon, Riffell, Davidowitz, Hildebrand, & Bronstein, 2010). In the same study, a no-choice experiment showed that females fed longer on A. palmeri whereas males spent more time foraging on D wrightii. Examination of pollen loads collected from the proboscis of field-collected M. sexta corroborated this preference: males carried significantly more D. wrightii pollen and females carried significantly more A. palmeri pollen (Alarcon et al., 2010). Based on these experiments, we fed male moths sugar concentrations similar to those found in D. wrightii (25%; see below).

Rainfall in the southwestern United States can be highly variable (Davidowitz, 2002), resulting in periods of limited nectar availability. An analysis of pollen grains on the proboscis of 200 M. sexta ranged from 0 to 3870 grains (mean = 278). Of these, 73% of the moths had 0–10 grains of their preferred host, D. wrightii, 34% had 0–10 grains of their main alternative host, A. palmeri, and 15% had fewer than 10 pollen grains of any plant species (Davidowitz & Levin, 2015). If we assume that these low numbers are indicative of fleeting flower probing due to lack of nectar, a high proportion of moths in nature may not feed as adults. Based on this, males in the experimental 'fed' group received 25% nectar and those in the 'unfed' control group received no nectar (see below).

Longevity of adult M. sexta increases with humidity and the amount of nectar consumed (Contreras et al., 2013). In the benign conditions of our laboratory colony, moths usually live 6–7 days but can live as long as 30 days under careful and attentive treatment conditions. The natural life span of M. sexta in the wild is not known. The mean ± SD longevity of 25 wild-caught moths brought back to a large mating cage for another study (Davidowitz & Levin, 2013) was 3.04 ± 1.4 days. Although we do not have data to support this, we expect that moths in the wild probably live only 4–5 days.

Virgin female M. sexta start calling immediately after eclosion from the pupae. The switch from virgin 'calling' behaviour to mated ovipositional behaviour is mediated by the presence of sperm and/or associated seminal fluids in the bursa copulatrix (Sasaki & Riddiford, 1984). Nothing is known of the mating frequency of individual female M. sexta in natural conditions.

Larvae were raised under a 16:8 h light:dark cycle in an environmentally controlled room set at 27 °C and 50% RH. Larvae were fed ad libitum with artificial diet (modified from Bell & Joachim, 1976) until pupation. Diet ingredients are given in Davidowitz, D'Amico, and Nijhout (2003).