Physiology & Behavior 151 (2015) 463–468

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Amino acid composition of the bushcricket spermatophore and the function of courtship feeding: Variable composition suggests a dynamic role of the nuptial gift

Alicia Jarrige *, Mélanie Body [1], David Giron, Michael D. Greenfield, Marlène Goubault

Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261, Université François-Rabelais, Parc Grandmont, 37200 Tours, France

HIGHLIGHTS

- We investigated the amino acid composition of a bushcricket nuptial gift.
- We examined amino acids composition in regard to the receiving female's traits.
- Gift composition varied both in quality and quantity according to the female traits.
- Nuptial gift composition may represent a form of cryptic male mate choice.

ARTICLE INFO

Article history:
Received 7 May 2015
Received in revised form 3 August 2015
Accepted 4 August 2015
Available online 5 August 2015

Keywords:
Ephippiger diurnus
Free amino acids
Male mate choice
Protein bound amino acids
Strategic allocation

ABSTRACT

Nuptial gifts are packages of non-gametic material transferred by males to females at mating. These gifts are common in bushcrickets, where males produce a complex spermatophore consisting in a sperm-containing ampulla and an edible sperm-free spermatophylax. Two non-mutually exclusive hypotheses have been suggested to explain the function of the spermatophylax: the *paternal investment hypothesis* proposes that it represents a male nutritional investment in offspring; the *mating effort hypothesis* proposes that the spermatophylax maximizes the male's sperm transfer. Because gift production may represent significant energy expenditure, males are expected to adjust their investment relative to the perceived quality of the female. In this study, we first examined the free amino acid composition and protein-bound amino acid composition of the nuptial gift in the bushcricket, *Ephippiger diurnus* (Orthoptera: Tettigoniidae). Second, we investigated whether this composition was altered according to female age and body weight. Our study represents the first investigation of both free and protein-bound amino acid fractions of a bushcricket spermatophylax. We found that composition of the nuptial gift varied both qualitatively and quantitatively with respect to traits of the receiving female: older females received larger amounts of protein-bound amino acids (both essential and non-essential), less water and less free glycine. This result suggests that gift composition is highly labile in *E. diurnus*, and we propose that gift allocation might represent a form of cryptic male mate choice, allowing males to maximize their chances of paternity according to the risk of sperm competition that is associated with mate quality.

1. Introduction

Nuptial feeding, i.e. material donations transferred to the opposite sex at mating, is widespread among insects and encompasses a wide variety of forms, including prey, body parts and glandular secretions [15,23]. One of the most intensively studied examples of courtship feeding can be found in bushcrickets (Orthoptera: Tettigoniidae), where males transfer the product of their accessory glands as an edible sperm-free spermatophylax attached to a sperm-containing ampulla, together forming the spermatophore [6]. Following copulation, the female consumes the spermatophylax while the sperms migrate to her genital tract [6].

The function of nuptial feeding has been the focus of considerable debate among evolutionary biologists for several decades and two main, non-mutually exclusive, hypotheses have been proposed [15,23,24]. According to the *paternal investment hypothesis*, donations would consist of substances, potentially nutritive, that enhance female 'condition' (e.g. higher egg load or survival and larger eggs), and ultimately increase the number or quality of the male's offspring. Alternatively, the *mating effort hypothesis* proposes that donations 'protect' the donor's

* Corresponding author at: Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261, Université François-Rabelais, Parc Grandmont, 37200 Tours, France.
E-mail address: alicia.jarrige@gmail.com (A. Jarrige).
[1] Present address: Division of Plant Sciences, 312 Christopher S. Bond Life Sciences Center, 1201 Rollins Street, University of Missouri, Columbia, MO 65211, USA.

http://dx.doi.org/10.1016/j.physbeh.2015.08.009
0031-9384/

sperm by prolonging the female remating interval, hence reducing the risk of sperm competition. Current studies show that spermatophore function probably differs among bushcricket species [7,23,27].

Bushcricket spermatophylaxes mainly consist of water, protein (4–27% of the wet mass), and a small amount of lipids [10,11]. Female condition is thought to be predominantly determined by the amount of free and protein-bound amino acids obtained via the spermatophylax. In line with the parental effort hypothesis, protein-bound amino acids, especially essential ones, may strongly affect female fecundity, because vitellogenesis is protein-limited [14,27,28,32]. In support of this hypothesis, ingested protein-bound amino acids have been found to be incorporated into female soma and eggs in bushcrickets [18,29,30], and experimental evidence showed that gift consumption positively affects female longevity and reproductive output [6,7,23]. In contrast, free amino acids are phagostimulants in many insects and may instead improve the gift's gustatory appeal and/or texture [2,4,19,21,26]. Besides, the low concentration of free amino acids in the spermatophylax implies that these substances are unlikely to represent a significant contribution to the female's diet. Consequently, this fraction may be more indicative of the male's mating effort than his parental effort.

Despite the considerable variation in spermatophylax size between species (from 2 to 40% of male body mass), gift-giving behaviors are costly for males and strongly limit their reproductive rate [6,15]. Consistent with this premise, males from various bushcricket species strategically allocate their resources by selectively mating with particular females, or by adjusting the size of their donation, and thereby protein content, according to i) female quality [13,29], ii) the risk of sperm competition [20] and iii) their own physiological condition [13,31]. In contrast to other types of nuptial gifts transferred prior to mating [23], bushcricket spermatophylaxes are manufactured by male reproductive glands during copulation [6]. This opens the opportunity for cryptic male mate choice through the manipulation of the biochemical composition of the gift according to female quality. However, to our knowledge, no study has yet jointly investigated the amino-acid composition of nuptial gifts and their variation with regard to female traits. In this study we focused on determining the free amino acid composition and protein-bound amino acid composition of spermatophylaxes produced by males in *Ephippiger diurnus* (Orthoptera: Tettigoniidae) and measuring the extent to which males modify spermatophylax composition with respect to traits (age and body weight) of the receiving female.

In this species, males produce an unusually large spermatophore (20–30% of their body mass) that appears to be particularly costly: its transfer results in a 4–5 day refractory period [31].

Moreover, the nitrogen content of the spermatophylax decreases with successive matings [13,31], suggesting that at least part of these resources cannot be replenished via feeding. Consequently, males allocate their scarce resources strategically by modifying the size, and thereby the total protein content, of their donation according to mate quality [13].

The present study provides a detailed analysis of the amino acid content in the *E. diurnus* spermatophylax. Our results revealed quantitative, as well as qualitative, variations in the gift's amino acid composition according to female body mass and age. Together, these results indicate that gift production represents a plastic process by which males adjust their reproductive expenditure according to the potential fitness returns associated with mate quality.

2. Methods

2.1. Rearing and maintenance

E. diurnus (Dufour, 1841) (Orthoptera: Tettigoniidae) used in experiments were offspring of individuals collected in the field at Col de Mantet (42°29′N, 2°3′E, Pyrénées Orientales, France) in July 2008. Eggs were cultured following the standard methods for this species [8] which consisted of two diapauses of 60 days at 4 ± 2 °C, separated by an interval of 120 days during which eggs were kept at 20 ± 2 °C. Eggs were placed on cotton covered by filter paper in Petri dishes and regularly sprayed with a 1% methyl-4-hydroxybenzoate solution to prevent desiccation and mold development. Nymphs were reared individually in 5 cm diameter × 8 cm height plastic containers and fed ad libitum with cabbage bee pollen, and flaked goldfish food. After the final molt, adults were individually transferred to larger plastic cages (10 cm diameter × 15 cm height) and provided with the same diet as nymphs. Rearing and experiments took place in environmental chambers maintained at 25 ± 2 °C on a L:D 16 h:8 h cycle.

2.2. Mating and spermatophores

2.2.1. Mating

To obtain spermatophores, 28 males aged from 25 to 35 days after final ecdysis were randomly paired with virgin females ranging from 16 to 63 days old; 24 females were 24-to-49 days old, 1 female was 16 days old and 3 females were between 56 and 63 days old. *E. diurnus*, have a long lifespan, and females within this age range are able to reproduce and produce viable eggs. Mating took place between 8 am and 15 pm, the peak period for singing and mating in *E. diurnus* (Busnel 1955). Males and females were weighed on a microbalance (±1 mg; Mettler-Toledo, Greifensee, Switzerland) prior to mating sessions, and female age was recorded.

2.2.2. Spermatophore collection and analysis

Immediately after mating, spermatophores were carefully removed from the females' genitalia. Fresh ampullae and spermatophylaxes (SPFx) were weighed separately on a microbalance (±1 mg; Mettler-Toledo, Greifensee, Switzerland). We estimated the water content of a spermatophylax by comparing its fresh and dry weights. Desiccation was achieved by freeze-drying (primary drying: 1 h at −10 °C, 25 mbar, secondary drying: −76 °C, 0.001 mbar overnight; Bioblock Scientific Alpha1-4LDplus lyophilizator). The dried subsamples were ground to powder with a mortar and stored at −80 °C until subsequent analysis.

2.3. Free and protein-bound amino acid analysis

From a subset of 5 mg of powdered spermatophylax, free amino acids were extracted with 1.2 mL acetonitrile 25% in HCl 0.01 N (1:3, v:v). From another subset of 5 mg of powdered spermatophylax, proteins were hydrolyzed into their protein-bound amino acids in a sealed glass tube at 150 °C for 2 h with 500 μL of 4 M methanesulfonic acid after flushing out air with a gentle stream of nitrogen gas. Following hydrolysis, the hydrolysates were partially neutralized with 1 mL sodium carbonate 1 M. Prior to analysis, samples were transferred to a 1.5 mL Eppendorf tube, and pH was checked to confirm that it was between 1.5 and 5.0. Free and hydrolyzed protein-bound amino acids were extracted and derivatized as described in the EZ:faast amino acid analysis kit (Phenomenex Ltd, Aschaffenburg, Germany). Subsequent samples were then concentrated under a stream of nitrogen gas and immediately injected into the GC–MS system composed of an AutoSystem XL gas chromatograph (ZB-AAA column (10 m × 0.25 mm), Phenomenex Ltd) coupled to a TurboMass mass spectrometer (Perkin-Elmer, Courtabœuf, France). Helium served as the carrier gas and its flow was held constant at 1.1 mL/min. The oven temperature program was a 30 °C/min ramp from 110 °C to 320 °C, with the temperature of the injection port maintained at 250 °C. The MS ion source (electronic impact) and inlet line temperatures were 200 °C and 310 °C, respectively. The scan range was 3.5 scans/s and atomic masses between 45 and 450 Da were detected. Under these conditions, a 2 μL sample was injected in splitless mode during 30 s. We used Norvaline at 200 $nmol \cdot mL^{-1}$ as an internal standard. Calibration curves for each of the standard physiological amino acids were produced using an original concentration of