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Biol. Lett. 2008 **4**, 476-478
doi: 10.1098/rsbl.2008.0282

References

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Female bushcrickets fuel their metabolism with male nuptial gifts

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In many arthropods, such as bushcrickets, males donate protein-rich nuptial gifts—so-called spermatophores—to females, which females ingest while the sperm enter the female's reproductive tract. Previously, it was shown that females route spermatophore nutrients over the course of hours and days to egg production or body synthesis. We investigated whether female bushcrickets fuel their metabolism with spermatophores immediately after consumption. We fed two male groups diets that were either enriched or depleted in ¹³C, and then tracked the isotopic changes in exhaled breath in female bushcrickets after spermatophore consumption. Within 3 hours, the stable carbon isotope ratio ($\delta^{13}\text{C}$) of female breath converged on the ratio of the male donor of the nuptial gift. This supports the idea that females quickly routed nutrients to metabolism, receiving immediate benefits from spermatophore feeding.

Keywords: female choice; homeostasis; nutrient routing

1. INTRODUCTION

Nuptial feeding—the transfer of a gift from a male to a female in exchange for mating—is a common phenomenon among arthropods (reviewed in Vahed 1998) and represents an interesting evolutionary scenario of cooperation and conflict between the sexes (Parker & Simmons 1989; Simmons & Parker 1989). In bushcrickets, spermatophore production is widespread (Gwynne 2001) and imposes severe costs on males (Vahed 2007a). Female bushcrickets usually prefer larger males as mates, since they provide larger spermatophores (Gwynne 1982; Lehmann & Lehmann 2008). Several studies found positive effects of spermatophylax consumption on female reproductive output, e.g. an increase in fecundity or offspring survival (reviewed in Gwynne 2001). But such positive effects on female fitness have not been detected in other species (Wedell & Arak 1989; Vahed & Gilbert 1997; Vahed 2003), questioning the generality of direct benefits of male nuptial gifts for female fitness (Vahed 2007b).

Since spermatophore compounds are also incorporated into the somatic tissue of females (Bowen *et al.* 1984; Wedell 1993; Voigt *et al.* 2006), nuptial gifts could also be important nutritional sources for females (reviewed in Gwynne 2008). For example, in the European bushcricket *Isophya kraussi*, nuptial gifts may provide sufficient energy to cover 2 or 3 days of a female's metabolic requirements (Voigt *et al.* 2005). Fuelling the metabolism of females with spermatophore nutrients may be advantageous for females, since they would then need to feed less on plant matter (Heller 1996; see Boggs (1990) for a model using butterflies) and may therefore be less exposed to predation (Heller 1992). Accordingly, spermatophores may benefit a male's offspring indirectly by increasing the survival rate of the egg-producing female (Wickler 1994). Therefore, we asked whether female bushcrickets may gain immediate benefits from nuptial gift consumption by routing spermatophore nutrients to metabolism. We enriched male donors of nuptial gifts with different levels of ¹³C, and predicted that females feeding on nuptial gifts should exhibit stable carbon isotope ratios in their exhaled breath according to the ratio of the male donor, if they combust spermatophore nutrients.

2. MATERIAL AND METHODS

(a) Insect maintenance and diet

We collected 21 male and 22 female *Poecilimon ornatus* as nymphs in Slovenia (45.611 N; 13.936 O; 900 m NN). Females were maintained until the onset of the experiment on a diet of flowering *Taraxacum officinale* (Asteraceae), *Plantago major* (Plantaginaceae) and *Bellis perennis* (Asteraceae). Immediately after capture, the males were assigned to two groups with different feeding regimes. Group 1 ($n=12$) was maintained on a diet of *Lactuca sativa* (Asteraceae) and group 2 ($n=9$) on a mixed diet of *L. sativa* (Asteraceae) and seaweed (Sushi Nori, OKF Corporation, South Korea). We started the experiment approximately three weeks after mature moulting. From each individual, we took an initial breath sample prior to the experiment (see below for a description of breath collection). The females were introduced each to a single male of either group 1 or 2 in small observational cages (70 × 80 × 23 cm). Once nuptial gifts were transferred to females, males were removed from the cages. We noted the onset of nuptial feeding in females and collected breath samples from females at the following time intervals: 1, 3, 5, 7, 9, 11 and 24 hours after the female started to feed on the nuptial gift.

(b) Breath collection and isotopic analysis

For breath collection, bushcrickets were transferred singly into a 65-ml plastic container to which a needle was hermetically fused with the tip facing the outside and the base the inside of the container. After closing the lid of the container (except for the needle as an air outlet), the container air was washed of CO₂ by flushing ambient air through NaOH and subsequently through the container via a plastic tube (diameter 3 mm) for 5 min at a flow-through rate of ca 0.5 l min⁻¹. Then, we sealed the plastic container for 1 hour to let the exhaled CO₂ accumulate in the container. To extract the exhaled CO₂ from the container, we penetrated the Teflon membrane of an evacuated vacutainer (LabcoTM, Buckinghamshire, UK) with the needle tip attached to the plastic container. Thus, approximately 10 ml of air was sucked via the needle into the vacutainer. Afterwards, the experimental animals were transferred back to their cage, where they continued to feed on the nuptial gift.

Breath samples were automatically flushed from the vacuainers in a stream of chemically pure helium, after which a gas chromatograph separated the CO₂ gas from the other gases before admitting it into the isotope ratio mass spectrometer in a continuous flow. Breath samples, together with internal standards that had been previously characterized relative to an international ¹³C standard (IAEA-CO-1), were analysed in duplicates. All ¹³C/¹²C values were expressed relative to the international standard using the δ notation (‰) and the following equation:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000,$$

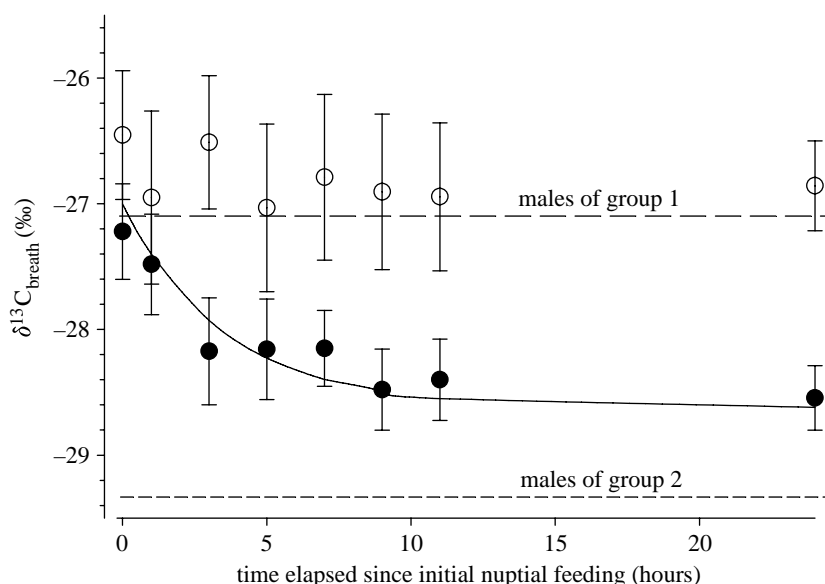


Figure 1. Mean $\delta^{13}\text{C}_{\text{breath}}$ (± 1 s.e.; ‰) of female *P. ornatus* during a 24 hour time period after they started to feed on nuptial gifts from males of groups 1 and 2, which differed in their stable carbon isotope signature. The mean $\delta^{13}\text{C}_{\text{breath}}$ of group 1 males is indicated by a long-dashed line and those of group 2 males by a short-dashed line. The $\delta^{13}\text{C}_{\text{breath}}$ of females feeding on nuptial gifts from males of group 1 (open circles) remained constant, whereas that of females feeding on nuptial gifts from males of group 2 (closed circles) decreased in ^{13}C enrichment over time.

where R_{sample} and R_{standard} represent the ratio $^{13}\text{C}/^{12}\text{C}$ of the sample and standard, respectively. Precision was better than ± 0.01 ‰ (1σ). All samples were analysed using a blind experimental protocol.

(c) Exponential model and statistics

We expected that changes in isotopic composition of the breath follow a single-pool exponential model after the females fed on nuptial gifts. Therefore, we calculated equations of the following type for females feeding on nuptial gifts contrasting in stable carbon isotope ratio to their previous diet,

$$\delta^{13}\text{C}(t) = \delta^{13}\text{C}(\infty) + (\delta^{13}\text{C}(0) - \delta^{13}\text{C}(\infty)) \times e^{-kt}$$

In this equation, $\delta^{13}\text{C}(t)$ is the stable carbon isotope ratio of exhaled CO_2 at time t , $\delta^{13}\text{C}(\infty)$ the asymptotic stable carbon isotope ratio of exhaled CO_2 when animals are equilibrated to the stable carbon isotope signature of their diet, $\delta^{13}\text{C}(0)$ the stable isotope ratio of exhaled CO_2 at time 0 of the experiment, and k the fractional incorporation rate of ingested carbon atoms into the pool of metabolized substrate. Estimation of k was performed on an iterative basis using SIGMAPLOT (SPSS v. 8.0). For each feeding group, we averaged regression coefficients for all individual values. We calculated the time at which 50% of carbon isotopes are exchanged in the animals' breath (t_{50} ; hours) according to the following equation: $t_{50} = -\ln(0.5)/k$, with \ln representing the natural logarithm and 0.5 the exchange of 50% isotopes in exhaled CO_2 . All values are given as mean ± 1 s.e. and all statistical tests were performed two-tailed at a significance level of 5%. We performed Mann-Whitney (MW) U -tests to test for differences in median $\delta^{13}\text{C}_{\text{breath}}(\infty)$ of females and $\delta^{13}\text{C}_{\text{breath}}$ of males and $\delta^{13}\text{C}$ of the plant diet.

3. RESULTS

Shortly before mating, the exhaled breath of the two male groups differed by *ca* 2.2 ‰ in ^{13}C enrichment (MW U -test: $n_1=9$, $n_2=12$, $U=17$, $U'=91$, $p=0.0073$). The stable carbon isotope ratio ($\delta^{13}\text{C}_{\text{breath}}$) averaged -27.11 ± 0.33 ‰ in males of group 1 and -29.34 ± 0.53 ‰ in males of group 2. The $\delta^{13}\text{C}_{\text{breath}}$ of females averaged -26.72 ± 0.32 ‰, which did not deviate significantly from the isotopic signature of their diet (MW U -test: $n_1=19$, $n_2=4$, $U=29$, $U'=47$, $p=0.51$). We then allowed females to copulate with males and monitored the change in $\delta^{13}\text{C}_{\text{breath}}$ of females for 24 hours following the

onset of nuptial feeding. Once females had started to feed on nuptial gifts, their $\delta^{13}\text{C}_{\text{breath}}$ converged quickly on the $\delta^{13}\text{C}_{\text{breath}}$ of the donor of the nuptial gift (figure 1). On average, females exchanged 50% of carbon atoms in exhaled breath with those from ingested nuptial gifts within 2.5 ± 0.6 hours. After 24 hours, females that had fed on nuptial gifts of males from groups 1 and 2 differed in $\delta^{13}\text{C}_{\text{breath}}$ by, on average, 1.9 ‰ (MW U -test: $n_1=9$, $n_2=8$, $U=8$, $U'=64$, $p=0.0055$), which was almost identical to the isotopic difference in exhaled CO_2 between the two groups of males. Exhaled CO_2 of females feeding on nuptial gifts of group 2 males was depleted in ^{13}C by 2.3 ‰ in relation to the stable carbon isotope signature of the plant food (MW U -test: $n_1=9$, $n_2=4$, $U=0$, $U'=36$, $p=0.0028$), which was available for all females throughout.

4. DISCUSSION

In our experiment, $\delta^{13}\text{C}_{\text{breath}}$ of females that had fed on nuptial gifts levelled off at values close to the $\delta^{13}\text{C}_{\text{breath}}$ of male donors. Since the $\delta^{13}\text{C}_{\text{breath}}$ of orthopteran insects closely matches the dietary $\delta^{13}\text{C}$ (DeNiro & Epstein 1978; this study), we suggest that female *P. ornatus* routed some of the spermatophore nutrients to metabolism. The nutritional benefit of ingesting and combusting a large nuptial gift may be substantial for reproducing female bushcrickets, since males donate up to 30% of their body mass to females (Wedell 1993; Vahed & Gilbert 1996) and dry matter of nuptial gifts mostly consists of energy-rich glycoproteins (Heller et al. 1998). Measurements of field metabolic rate in free-ranging bushcrickets indicated that females could sustain their metabolism for 2 or 3 days with the digestible energy included in a single nuptial gift (Voigt et al. 2005). This time period often elapses between two subsequent matings in similar-sized female bushcrickets (Heller & von Helversen 1991;

Vahed & Gilbert 1996; Vahed 2006). Females may then benefit from spermatophore consumption and combustion by not having to search for additional food (see Boggs 1990), and by being less likely to fall prey to predators (Heller 1992). Possibly, female bushcrickets follow a similar selective nutrient-routing strategy like egg-producing butterflies (O'Brien *et al.* 2002), allocating some essential amino acids from nuptial gifts selectively to egg production and some non-essential material to metabolism.

We conclude that females may route at least some of the spermatophore nutrients according to their immediate needs. The actual nuptial gift game in a species may then be the balanced result of cooperation and conflict between a male's interest to invest into his own fertilized eggs via nutrients essential for egg production and females metabolizing nutrients not essential for egg production (Vahed 2007b; Gwynne 2008).

We thank Arne Lehmann for comments on an earlier version of this manuscript.

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