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Free amino acids as phagostimulants in cricket nuptial gifts: support for the 'Candymaker' hypothesis

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Nuptial gifts that are manufactured by the male are found in numerous insect species and some spiders, but there have been very few studies of the composition of such gifts. If, as has been proposed recently, nuptial gifts represent sensory traps, males will be selected to produce gifts that are attractive to females but such gifts will not necessarily provide the female with nutritional benefits (the 'Candymaker' hypothesis). We examined the free amino acid content of the spermatophylax of the cricket *Gryllodes sigillatus* (Orthoptera: Gryllidae) using high performance liquid chromatography (HPLC). The spermatophylax (dry weight) consisted of approximately 7 per cent free amino acids. The free amino acid composition was highly imbalanced, with a low proportion of essential amino acids (18.7%) and a high proportion of proline and glycine. The main free amino acids found in the spermatophylax appeared to act as phagostimulants: the duration of feeding on artificial gels by females was positively related to the free amino acid content of the gels. The results therefore suggest that males use free amino acids to 'sweeten' a relatively low-value food item. A possible function of glycine in inhibiting female movement is also proposed.

Keywords: free amino acids; nuptial gifts; phagostimulants; sensory trap; sexual conflict

1. INTRODUCTION

Nuptial feeding occurs in numerous different forms in various insect taxa and some spiders (reviewed in Vahed 1998, 2007; Gwynne 2008). Recently, nuptial feeding has been re-examined from the perspective of sexual conflict (reviewed in Vahed 2007; Gwynne 2008; Wedell *et al.* 2008). Sakaluk (2000) and Sakaluk *et al.* (2006) proposed that nuptial gifts may act as 'sensory traps' (Christy 1995). By 'exploiting' the female's normal gustatory responses, a male may be able to entice a female to accept superfluous matings, transfer a greater volume of ejaculate than is in the female's interests and/or transfer substances that manipulate the remating or egg-laying behaviour of the female to serve the male's interests (reviewed in Arnqvist & Rowe 2005; Vahed 2007). The existence of costs to females associated with nuptial gifts and the extent to which the nutritional benefits of nuptial feeding might compensate the female for such costs is the subject of ongoing debate (Vahed 2007; Gwynne 2008; Wedell *et al.* 2008).

If nuptial gifts do act as sensory traps, they will be selected to be attractive to the female, but need not necessarily be selected to provide her with nutritional benefits (Sakaluk 2000; Vahed 2007). This has been termed the 'Candymaker' hypothesis (Warwick 1999). Relatively few previous studies have examined the chemical composition of nuptial gifts (reviewed in Vahed 2007). One way of increasing the attractiveness of orally ingested gifts might be to incorporate phagostimulants within them (for an example, see Kugimiya *et al.* 2003, reviewed in Vahed 2007). One group of phagostimulants in insects are free amino acids (reviewed in Calatayud *et al.* 2002), although the free amino acid content of the nuptial gift has only been quantified previously in the German cockroach, *Blatella germanica* (Kugimiya *et al.* 2003).

We examined the free amino acid content of the nuptial gift (spermatophylax) in the cricket *Gryllodes sigillatus*. The spermatophylax, a product of the male's accessory glands, is detached from the sperm-containing part of the spermatophore (the ampulla) and consumed by the female during sperm transfer. Having consumed the spermatophylax, a process that takes approximately 40 min, the female terminates sperm transfer by eating the ampulla (Sakaluk 1984). We found that while the proportion of 'essential' amino acids in the spermatophylax was low, there was a high concentration of other free amino acids, particularly glycine and proline. We show, using artificial gels, that these act as phagostimulants and probably serve to increase the attractiveness of a relatively low-value food item.

2. MATERIAL AND METHODS

(a) *Experimental stock*

The *G. sigillatus* used were taken from the continuous culture maintained at the University of Oxford, which was founded in 1997 from cultures at the University of Nottingham and the University of Derby. For details of rearing methods, see Warwick (1999). Sexes were separated prior to the final moult.

(b) *Spermatophylax composition*

Adult virgin males ($n=44$) that were at least 5 days old were each placed in a Perspex observation box (14.5×10×7.5 cm) with a virgin female and were observed for up to 30 min. The spermatophylax was removed immediately from the mated female using watchmaker's forceps and weighed to the nearest 0.01 mg using an electrobalance. The spermatophylaces were then freeze-dried and re-weighed. The carbon and nitrogen content of 10 spermatophylaces was analysed using mass spectrometry. To measure the free amino acid content, each spermatophylax was powdered and reconstituted in 1 ml of water, heated to 100°C for 5 min, passed through an anion exchange resin column (Dowex 1X8-200) and washed with 2 ml of water and 1 ml of 1 M ammonia solution, dried down and reconstituted in 1 ml of water. The resulting solution was analysed for free amino acids using HPLC. The molar concentration of free amino acids was calculated as moles per mass of water in the spermatophylax. Standard solutions containing known amounts of individual amino acids were included.

(c) *Free amino acids as feeding stimulants*

To assess whether free amino acids represent a feeding stimulus, a concentration series of amino acids was produced in gelatin-based gels. The gels were composed of 10 per cent gelatin wet weight, made in a physiological saline solution (Mordue & Goldsworthy 1969). A standard gel consisted of the following (values in mg per 20 ml water): gelatin (2222.2 mg); NaCl (39.3 mg); KCl (1.9 mg); $MgCl_2$ (2.9 mg); $CaCl_2$ (1.9 mg); NaH_2PO_4 (3.8 mg); $NaHCO_3$ (0.7 mg); and glucose (12.0 mg).

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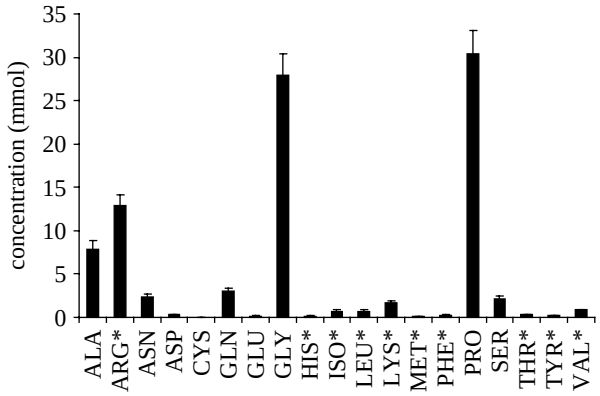

Figure 1. Millimolar concentrations of free amino acids in the spermatophylax (mean with 95% confidence interval). Essential amino acids (Dadd 1985) are marked with an asterisk.

A mixture of proline (40.9% of the amino acid mixture), glycine (24.5%), arginine (26.5%) and alanine (8.1%) was incorporated in the gels. These four amino acids accounted for 86 per cent of the total molar quantity of free amino acids found in the spermatophylax. Gels were made with varying concentrations of these amino acids: 0, 0.5, 2, 5, 8, 14 and 30% of the dry mass of gelatin. The constituent ingredients were mixed, heated to 50°C, poured onto a Petri dish, sealed and kept at 5°C overnight. Ten-milligram cubes of gel were cut from the Petri dish immediately before presentation to the insect. The gel cube was placed in a Perspex box measuring 20×30×20 cm, containing a virgin female *G. sigillatus*. The time the insect spent feeding on the gel was recorded for a period of 30 min. A total of 162 different females were used in the experiment. Only females that contacted the gel during the observation period were included in the analysis (36 females were excluded).

3. RESULTS

(a) *Spermatophylax composition*

The spermatophylax consisted of 83.6±13.9 per cent (mean±s.d.) water. The mean carbon : nitrogen ratio of the spermatophylax was 3.54 : 1. Such a low ratio confirms the expectation that protein, peptides and/or amino acids form over 90 per cent of the dry mass (Heller *et al.* 1998).

The concentrations of individual free amino acids in the spermatophylax are presented in figure 1. Free amino acids represented 6.79±0.06 per cent of the dry mass of the spermatophylax. Essential amino acids (Dadd 1985) comprised only 18.7 per cent of the total free amino acids in the spermatophylax, whereas proline and glycine were found in the highest concentrations.

(b) *Free amino acids as feeding stimulants*

The addition of the free amino acid mixture to the gelatin gels increased the time the insects spent feeding (feeding duration $(\log s) = 1.033 + 0.112aa + 0.0031aa^2$, where aa is the free amino acid concentration, $F_{1,125} = 12.1$, $p < 0.001$, $r^2 = 0.21$; figure 2). The time spent feeding increased to a peak at an intermediate amino acid concentration of approximately 14 per cent gelatin dry mass. The mean feeding duration increased by a factor of 12, from 19 s without free amino acids to 219 s with the addition of 14 per cent amino acids.

4. DISCUSSION

The spermatophylax of *G. sigillatus* was found to contain approximately 7 per cent (dry mass) free amino acids, particularly the non-essential amino acids proline and glycine, with lower concentrations of

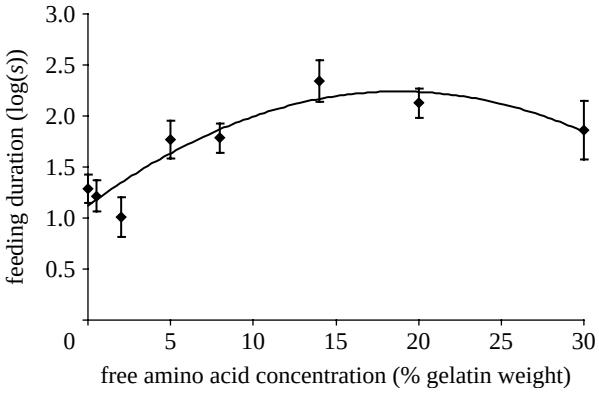

Figure 2. The effect of free amino acid concentration (percentage of gelatin dry mass) on the time spent feeding on artificial gels by female *G. sigillatus*. Time spent feeding during the 1800 s observation period was log transformed for homogeneity of variance. Points represent mean±s.e.

arginine and alanine. As such, the free amino acid complement is extremely unbalanced, although it should be noted that we did not analyse the amino acid composition of the spermatophylax proteins. Heller *et al.* (1998), however, in an analysis of the amino acid profile of spermatophylax nitrogenous compounds in five species of bushcricket (Orthoptera: Tettigoniidae), found a similar imbalance in amino acids, with glycine occurring in the highest proportions. Because the amino acid profile of egg proteins differed distinctly from that of the spermatophylax, Heller *et al.* (1998) concluded that spermatophylax amino acids are probably of limited use to the female in egg production. In support of this, the number of spermatophylaces consumed by female *G. sigillatus* has been found to have no effect on the number of eggs produced (Will & Sakaluk 1994; Kusaya & Sato 1998), although Kusayo & Sato (1998) did find that the rate of oviposition appeared to be increased by spermatophylax consumption in this species. Spermatophylax amino acids might, however, be used by the female as an energy source (Voigt *et al.* 2008) and the water content of the spermatophylax may provide benefits to the female at times of extreme water stress (Ivy *et al.* 1999).

Heller *et al.* (1998) proposed that the high level of glycine present in the spermatophylax could indicate the presence of elastin-like structural proteins that increase the gummy consistency of the spermatophylax. In support of this, the rate of ingestion of females feeding on the spermatophylax was an order of magnitude less than that of females feeding on artificial gels in the present study (2 μg s^{-1} compared with 20 μg s^{-1}; Warwick 1999).

The results of the experiments using synthetic gels indicate that the main free amino acids found in the spermatophylax act as phagostimulants in *G. sigillatus*. Males therefore appear to use free amino acids to 'sweeten' a relatively low-value, and difficult to ingest, food item (Warwick 1999). The benefit to the male *G. sigillatus* of ensuring that the female feeds on the spermatophylax and of prolonging feeding time is clear (reviewed in Vahed 1998, 2007). The spermatophylax has been viewed as a product of sexual conflict over the duration of ejaculate transfer, allowing males to deter

the female from removing the externally attached spermatophore ampulla prematurely and thus from exercising cryptic female choice (reviewed in Simmons 2001 and Vahed 2007). The spermatophylax therefore has the effect of increasing the volume of ejaculate transferred per mating (Sakaluk 1984) and the fertilization success of the male in the face of sperm competition (Calos & Sakaluk 1998). We predict that phagostimulants, including free amino acids, are likely to be found in nuptial gifts that are manufactured by the male in other insect taxa, such as the large spermatophylax in certain bushcrickets (reviewed in Vahed 1998, 2007).

Evidence that sensory 'exploitation' may have been involved in the evolutionary origin of the spermatophylax in *G. sigillatus* was provided by Sakaluk (2000) and Sakaluk *et al.* (2006). Sakaluk (2000) demonstrated that even in three cricket species that do not produce a spermatophylax, females readily accepted and consumed spermatophylaces obtained from *G. sigillatus* and accepted more sperm from their mates as a result of spermatophylax consumption. The presence of phagostimulants within the nuptial gifts of *G. sigillatus*, as documented in the present study, provides a proximate mechanism underlying this result. Sakaluk *et al.* (2006) found that in one of the species of non-nuptial feeding crickets used in Sakaluk's (2000) experiments, the consumption of the spermatophylax appeared to delay the female from remating. It is possible that the high level of free glycine in the spermatophylax, demonstrated here, could account for this delay in remating. Gwynne (2008) suggested that free amino acids in the nuptial gift could signal to the female that they have recently fed, thus inducing a delay in remating related to digestion. Elevated haemolymph titres of free amino acids are known to inhibit locomotion and delay feeding in other orthopterans (Simpson & Raubenheimer 2000). Furthermore, were it to cross the blood–brain barrier, it is well documented that glycine can inhibit neuronal activity in both vertebrates and invertebrates, including orthopterans (Heinrich *et al.* 1998).

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