Running title: Sakaluk et al. • Conflict and chemistry of nuptial food gifts

The troublesome gift: the spermatophylax as a purveyor of sexual conflict and coercion in crickets

S.K. Sakaluk[*1], K.R. Duffield[*], J.Rapkin[§], B.M. Sadd[*], J. Hunt[¶]

[*]Illinois State University, Normal, IL, United States

[§]University of Exeter, Penryn, Cornwall, UK

[¶]Western Sydney University, Sydney, Australia

[1]Corresponding author: E-mail: sksakal@ilstu.edu

ABSTRACT

Nuptial food gifts are an integral feature of the mating systems of a wide variety of insects. A pervasive feature of the majority of these gifts is that they afford the male direct access to the female's physiology. Although in some cases such gifts may represent a form of nutritional investment in females, an alternative hypothesis posits that nuptial food gifts may allow males to manipulate female physiology in favor of male interests. This hypothesis predicts that the chemical composition of nuptial food gifts should be driven by a sexual conflict over offspring paternity.

We evaluate this hypothesis by reviewing our accumulated studies on decorated crickets, *Gryllodes sigillatus*, a species that has illuminated key elements of this evolutionary arms race. Here, nuptial food gifts take the form of a spermatophylax, a gelatinous mass forming part of the male's spermatophore and consumed by the female after mating. We first review the evidence for the two principal sexual conflicts arising from the provision of the spermatophylax: i) a sexual conflict over female acceptance of gifts, and ii) a conflict over female remating that appears to be mediated by refractory-inducing substances contained in gifts. We then consider the mechanisms by which these conflicts are mediated, focusing specifically on: i) multivariate selection analyses of the free amino acid composition of the spermatophylax; ii) proteomic studies identifying candidate proteins that alter female behavior; and iii) comparative transcriptomic studies showing how gene expression is altered through nuptial feeding.

KEY WORDS: cricket, cryptic female choice, gene expression, mating behavior, nuptial food gift, proteomics, sexual conflict, sexual selection, spermatophore, sperm competition

1. WHAT ARE NUPTIAL FOOD GIFTS?

Nuptial food gifts comprise materials other than sperm that are offered by males to females at copulation, and are an integral feature of the mating systems of a wide variety of insects and spiders (Vahed, 1998, 2007a; Lewis & South, 2012; Lewis, Vahed, Koene, Engqvist, Bussiére, Perry, et al., 2014). Gifts come in an astonishing range of forms. Male hangingflies, *Hylobitticus apicalis*, offer up insect prey that they have captured on the wing or stolen from other males, before emitting a pheromone advertising their gifts to receptive females (Thornhill 1976). Male dance flies (Diptera: Empidinae) also proffer insect prey to females, but in some species the prey is encased in a silken balloon (Cumming, 1994). In a kind of evolutionary sleight-of-hand, this silken 'gift-wrapping' has enabled males of other species to deceive females by offering minute or shriveled prey, or no prey at all, within an inedible balloon (Cumming, 1994). During copulation in Madeira cockroaches, *Leucophaea maderae*, females clamber up on the backs of males to feed on the copious secretions produced by specialized tergal glands (Mondet, Abed-Vieillard, Gautier & Farine, 2008). Male ornate moths, *Utetheisa ornatrix*, transfer pyrrolizidine alkaloids, anti-predator defensive compounds sequestered from the plants upon which they feed as larvae, in their spermatophores; these beneficial chemicals augment the female's own supply, affording them increased protection and allowing them the luxury of transferring these protective compounds to their own eggs (Conner, Roach, Benedict, Meinwald & Eisner, 1990; Dussourd, Harvis, Meinwald & Eisner, 1991). Female sagebrush crickets, *Cyphoderris strepitans,* use their mandibles to tear through the integument of the male's fleshy forewings during copulation, consuming both tissue and hemolymph leaking from the wounds they inflict (Eggert & Sakaluk, 1994; Sakaluk, Campbell, Clark, Johnson & Keorpes, 2004). Male redback spiders, *Latrodectus hasselti,* participate in this kind of somatic sacrifice to an even greater degree: during copulation, the much smaller male somersaults backwards so that his abdomen is placed in easy reach of the female's chelicerae, whereupon the female consumes the male in his entirety (Andrade, 1996).

Notwithstanding their incredible variety, nuptial food gifts can be neatly and conveniently classified along two dimensions (Lewis & South, 2012; Lewis et al., 2014). The *source* of the gifts describes the manner in which they are obtained: endogenous gifts represent those that are manufactured *de novo* by males (e.g., glandular secretions), whereas exogenous gifts are gift items captured or collected by males (e.g., insect prey). The *mode of receipt* describes the manner by which gifts are taken in by females: oral gifts are orally ingested by females, seminal gifts are absorbed through the female's reproductive tract, and transdermal gifts are injected directly through the body wall of the female (Lewis & South, 2012; Lewis et al., 2014). It is not our intent to review the diversity of nuptial gifts, their various functions, and their distribution across the major insect taxa. Boggs (1995), Vahed (1998, 2007a), and Lewis & South (2012) offer exceptionally lucid and comprehensive reviews of these issues. We focus instead on the role of sexual conflict in the evolutionary origin and adaptive significance of nuptial food gifts, specifically with respect to the divergence in reproductive interests of males and females that arises with respect to the utilization of these gifts.

We begin with the recognition that a pervasive feature of the majority of these gifts is that they afford the male direct access to the female's physiology. Although in some cases such gifts may represent a form of nutritional investment in females (Gwynne, 2008; Lewis et al., 2014), an alternative hypothesis focuses on a more sinister purpose: nuptial food "gifts" may represent a vehicle by which males manipulate female physiology in a manner commensurate with males' fitness interests, even at a cost to females (Arnqvist & Nilsson, 2000; Sakaluk, Avery & Weddle, 2006; Vahed, 2007a). This hypothesis predicts that the chemical composition of nuptial food gifts should be driven by a fundamental sexual conflict over the paternity of a female's offspring. In this chapter, we will address the veracity of this hypothesis by reviewing our accumulated studies on decorated crickets, *Gryllodes sigillatus*, a species that has illuminated a number of key elements of this evolutionary arms race.

2. THE CRICKET SPERMATOPHYLAX

In decorated crickets, *Gryllodes sigillatus*, nuptial food gifts take the form of a spermatophylax, a gelatinous mass forming part of the male's spermatophore and consumed by the female after mating (Alexander & Otte, 1967). Males attract females for mating by producing a loud, stereotypic calling song, which they produce by stridulation of their forewings (Fig. 1a); the song comprises a series of regularly-spaced, three-pulse chirps produced at a carrier frequency of 6.6 kHz (Sakaluk & Belwood, 1984). Upon coming into antennal contact with a prospective mate, the male switches from calling song to courtship behavior, which entails a combination of acoustic, visual and, possibly, vibratory cues (Subramaniam & Subramoniam, 1990; Ryan & Sakaluk, 2009). In addition, chemical cues, in the form of cuticular hydrocarbons, influence the propensity of the female to mount the male (Ryan & Sakaluk, 2009; Weddle, Steiger, Hamaker, Ower, Mitchell, Sakaluk, et al., 2013; Capodeanu-Nägler, Rapkin, Sakaluk, Hunt & Steiger, 2014). During courtship, the male visibly tremulates via rapid lateral movements of his body, while producing a much softer, distinctive courtship song (Loher & Dambach, 1989; Zuk & Simmons, 1997). When courting, the male flattens his body against the substrate and makes repeated attempts to back under the female. If the female is sexually responsive, she dorsally mounts the male (Fig. 1b) at which point the male attempts to secure her subgenital plate with his epiphallus, a necessary prelude to spermatophore transfer (Sakai, Taoda, Mori, Fujino & Ohta, 1991). Copulation is completed with the successful transfer of the spermatophore, which, in most gryllid crickets, normally consists of a small, sperm-containing ampulla that remains secured outside the female’s body at the base of her ovipositor by a narrow spermatophore tube threaded into her genital opening (Zuk & Simmons, 1997).

In *G. sigillatus*, the ampulla is enveloped by a much larger, gelatinous spermatophylax, devoid of sperm (Alexander & Otte, 1967). Immediately upon dismounting the male after spermatophore transfer, the female detaches the spermatophylax from the ampulla with her mandibles and begins to consume it (Fig. 1c), hence its designation as a nuptial food gift. During the time she feeds on the food gift (Fig. 1d), sperm and other ejaculatory material are forcefully expelled into the female’s reproductive tract through osmotic pressure built up within the ampulla (Khalifa, 1949). The female requires about 40 minutes on average to fully consume the spermatophylax, and normally within a few minutes of doing so, she removes and eats the sperm ampulla (Fig. 2a). Females consume smaller spermatophylaxes more quickly than larger ones, and consequently, males providing such gifts experience premature ampulla removal and reduced sperm transfer (Sakaluk, 1984, 1985, 1987). The amount of sperm a male transfers is critical to his reproductive success because it is the principal determinant of his fertilization success, particularly when his sperm must compete with sperm of the female’s other mating partners (Sakaluk, 1986a; Sakaluk & Eggert, 1996; Calos & Sakaluk, 1998; Eggert, Reinhardt &

Sakaluk, 2003). The success of a male in sperm competition depends, in part, on the number of sperm that he transfers to the female (Fig. 3), because the sperm of a female's various mating partners are recruited for fertilizations in direct proportion to their relative abundance in the female's spermatheca (Sakaluk, 1986a; Simmons, 1987; Sakaluk & Eggert, 1996; Bussière, Hunt, Jennions & Brooks, 2006). Thus, the spermatophylax functions to entice females into relinquishing at least some of their control of the insemination process, thereby furthering the male's own reproductive interests.

3. SEXUAL CONFLICT OVER FEMALE ACCEPTANCE OF THE GIFT

It follows from the sequence of events leading to successful spermatophore transfer that males benefit most when their nuptial food gifts are fully consumed, because it is under these circumstances that sperm transfer is maximized. Females, in contrast, may benefit by prematurely discarding nuptial gifts if, upon mating with certain males, they find such males undesirable. Such behavior would enable some degree of female mate choice even after mating has occurred because females would thus be free to remove the sperm ampulla, thereby terminating sperm transfer. Indeed, it is not widely appreciated that females discard the spermatophylax by simply dropping it prior to its complete consumption in about a quarter of all matings (Sakaluk, 1984, 1987). Figure 2b shows the time after mating at which the female removed the sperm ampulla as a function of the time at which she discarded the spermatophylax. Two interesting patterns emerge from this plot. First, if the female opts to discard the spermatophylax, she typically does so within about 15 min of spermatophore transfer, long before complete sperm transfer is likely to have occurred. Second, as was true of the ‘rule of thumb’ that leads females to remove the sperm ampulla shortly after consuming the spermatophylax (Fig. 2a), many females removed the sperm ampulla shortly after discarding the spermatophylax (i.e., the data arrayed in a straight line along the right arm of the V-shaped cluster of points in Fig. 2b).

But why should females discard males’ nuptial food gifts? One possibility is that it is simply a non-adaptive consequence of satiation or some other factor intrinsic to females (i.e., age, previous mating experience). An alternative possibility, however, is that it is an adaptive mechanism by which females discriminate against certain males after mating (Sakaluk, 1997; Gershman & Sakaluk, 2010). We know, for example, that female decorated crickets derive a variety of genetic benefits by mating with different partners (Sakaluk, Schaus, Eggert, Snedden & Brady, 2002; Ivy & Sakaluk, 2005), including paternal genes that enhance offspring survival and benefits arising from the interaction of maternal and paternal genotypes (Ivy, 2007). It seems likely, therefore, that females could derive similar benefits by differentially discarding gifts based on some assessment of the benefits males have to offer, although this proposition has not been formally tested.

Given that females do discard males’ gifts and that this behavior can dramatically influence male fertilization success, it would be instructive to know which features females use in discriminating against males in this fashion. Although there a number of traits by which females might assess males, one obvious possibility is that females discard gifts based on their assessment of the gift itself, its taste or its texture. Initially, we chose to focus our attention on the concentration of free amino acids in the spermatophylax. Free amino acids represent essential nutrients for insects and a number of studies have revealed that free amino acids are phagostimulatory (reviewed in Chapman, 2003). As importantly, Stuart Warwick and his colleagues had earlier discovered that a large portion of the solid fraction of the spermatophylax consists of a variety of amino acids, and proposed that the free amino acids in the spermatophylax might influence its gustatory appeal to females (Warwick, 1999; Warwick, Vahed, Raubenheimer & Simpson, 2009). As proof of principle, Warwick et al. (2009) constructed artificial 'spermatophylaxes' composed of gelatin and the four most abundant free amino acids found in the spermatophylax: proline, glycine, arginine, and alanine. Female *G. sigillatus* offered these gels fed on them significantly longer than females fed control gels lacking these amino acids. The increased abundance of free amino acids of little nutritional value in the spermatophylax relative to the scarcity of essential amino acids, coupled with the highly phagostimulatory properties of the most abundant free amino acids, speaks to the attempted manipulation by males and is consistent with the hypothesis of sexual conflict (Warwick et al., 2009).

If free amino acids in the spermatophylax influence its gustatory appeal to females, we might expect a difference in the amino-acid profile of those gifts discarded by females after mating and those that are fully consumed. Thus, a critical test of this prediction would entail a direct comparison of these two classes of gifts. But this raises an obvious problem: how do you measure the concentration of amino acids in a gift that has already been consumed? To circumvent this problem, we used a screening process that leveraged earlier work documenting the time course over which female discard the spermatophylax (Sakaluk, 1984). As noted earlier, whenever a female decides to discard the spermatophylax, she typically does so within 15 min of mating (Fig. 2b). This means that whenever a female feeds on a spermatophylax for at least 15 min, she is likely to fully consume it, and in such cases, the female can be considered to have “accepted” the nuptial food gift. This enabled us to compare the amino acid profiles of two classes of spermatophylaxes, those that were discarded by females less than 15 min after mating (and which we were able to recover), and those that were fed on for at least 15 min (i.e. destined to be fully consumed), but which we forcibly removed from the female with forceps 15 min after mating (Gershman, Mitchell, Sakaluk & Hunt, 2012). The free amino acids in the gifts were subsequently quantified using gas chromatography-mass spectrometry, which revealed a veritable alphabet soup of different amino acids (Table 1). We used multivariate selection analysis (Lande & Arnold, 1983) on these data to estimate the strength and form of linear and nonlinear sexual selection acting on the amino acid composition of the male spermatophylax via their effect on the female’s acceptance of the gift (Gershman et al., 2012). This analysis revealed a complex pattern of selection that produced a fitness surface with two local peaks (Fig. 4). Notwithstanding this complexity, it is clear that the free amino acid composition of the spermatophylax in *G. sigillatus* profoundly influences it gustatory appeal, and hence, the probability that the female will discard it before she has completely eaten it. This, in turn, has important fitness consequences for males: the rule-of-thumb that directs a female to remove the male’s sperm ampulla shortly after discarding the spermatophylax greatly reduces the number of sperm he transfers and his share of paternity should the female mate with other males (Sakaluk & Eggert, 1996; Calos & Sakaluk, 1998; Eggert et al., 2003). Thus, the gustatory response of females to the free amino acids in the spermatophylax represents arguably one of the most well-documented mechanisms underlying cryptic female mate choice, a preference exerted after copulation has occurred that influences a male’s fertilization success (Thornhill, 1983; Eberhard, 1996).

The sexual conflict over the female’s acceptance of the spermatophylax was further reinforced in subsequent work capitalizing on nine highly inbred lines developed as part of an earlier study designed to measure the genetic benefits of polyandry in decorated crickets (Ivy, 2007). One particular model of sexual conflict, the chase-away model, proposes that males evolve increasingly enticing display traits to induce females to mate, whereas females evolve resistance to these inducements by decreasing their responsiveness to these traits (Holland & Rice, 1998). In the context of the evolution of food gifts, this suggests that sexual conflict should favor males that produce increasingly alluring food gifts, and females that resist this manipulation. This model assumes that both the gustatory appeal of food gifts and females’ propensity to consume them are significantly heritable. To address these issues, we first analyzed the amino acid composition of spermatophylaxes synthesized by males of the nine inbred lines. We then offered spermatophylaxes to females in a diallel experimental design in which females in each line received food gifts from males in each line in all possible combinations, measuring the time spent feeding on spermatophylax as an objective measure of a female’s acceptance of, or conversely, resistance to, the particular amino acid composition represented by a gift (Gershman, Hunt & Sakaluk, 2013). Both the amino acid profile of the spermatophylax and the time females spend feeding on it were significantly heritable, supporting the basic premise that the gustatory appeal of the spermatophylax and females’ propensity to consume them can respond to selection. More interestingly, perhaps, was the positive genetic correlation between spermatophylax attractiveness and female feeding duration that emerged in the study. This suggests that genes expressed in males that synthesize spermatophylaxes with more appealing amino acid compositions (i.e. those that stimulate females to feed for longer periods) are positively linked to genes expressed in females that make them more susceptible to these inducements (Gershman et al., 2013). Although such a positive correlation is consistent with sexual conflict (Gay, Brown, Tregenza, Pincheira-Donoso, Eady, Vasudev, et al., 2011), it is also a key prediction of traditional models of preference evolution (Lande, 1981). However, the fact that females of a non-gift-giving species, *Acheta domesticus*, when offered a *Gryllodes* spermatophylax, fed on it longer than female *Gryllodes* (Gershman et al., 2013), would appear to favor sexual conflict as the more parsimonious hypothesis in this case.

We end this section by noting that the high concentration of amino acids found in the *Gryllodes* spermatophylax is not unique, as amino acids comprise a major component of the spermatophylaxes of bushcrickets in the related family Tettigoniidae (Heller, Faltin, Fleischmann & von Helversen, 1998; Jarrige, Body, Giron, Greenfield & Goubault, 2015). Here too, amino acids have been viewed as functioning to extend the time females spend feeding on the spermatophylax, either through their effect on its texture, taste, or both. If true, this would represent an intriguing example of convergent evolution, as the bushcricket spermatophylax and the spermatophylax in *Gryllodes* almost certainly represent independent evolutionary origins (Gwynne, 1995). However, a major difference between the bushcricket spermatophylax and the *Gryllodes* spermatophylax is that the vast majority of the amino acids in the former are protein bound, and of potentially significant nutritional value to the female through their effect on egg production (Jarrige et al., 2015); indeed, consumption of the bushcricket spermatophylax has been shown to have a positive influence on female longevity and reproduction, in accordance with the paternal investment hypothesis (Gwynne, 2008).

4. SEXUAL CONFLICT OVER FEMALE REMATING

Females in an array of crickets exhibit high levels of polyandry (Rost & Honegger, 1987; Souroukis & Murray, 1995; Bretman & Tregenza, 2005), and this is true also in *G. sigillatus*, which continue to seek out new mating partners up until they die (Sakaluk et al., 2002; Ivy & Sakaluk, 2005; Ivy, 2007). Female *G. sigillatus* can store the sperm of multiple mates over extended periods which, coupled with their propensity for polyandrous mating, fosters high levels of sperm competition (Sakaluk, 1986a). This, in turn, generates an intense sexual conflict over female remating because, while polyandry might confer important indirect genetic benefits to females (Sakaluk et al., 2002; Ivy & Sakaluk, 2005; Ivy, 2007), it does so at the expense of the reproductive success of their various partners.

These details on the polyandrous mating system of *G. sigillatus* suggest that even a modest delay in female remating would reduce the level of sperm competition experienced by a female's most recent mate, enhancing his overall reproductive success. It also raises the interesting possibility that, when plying females with substances that enhance the gustatory appeal of the spermatophylax, the male might also benefit by including compounds that decrease her receptivity to future mating attempts. The idea that male-derived ejaculatory substances might influence female receptivity is not new. In perhaps the most well-documented example, male *Drosophila* transfer accessory-gland proteins in their ejaculates that reduce female receptivity to mating (Wolfner, 1997, 2002). In fact, the existence of receptivity-inhibiting substances in male ejaculates is fairly ubiquitous in insects (Gillott, 2003). What makes the possibility intriguing in *Gryllodes* is the possibility that these substances are orally ingested as opposed to being absorbed in the genital tract as in female *Drosophila.* We cannot, of course, discount the possibility that seminal fluid proteins contained in the cricket ampulla, the function of which remains largely unknown (Simmons, Beveridge, Li, Tan & Millar, 2014), might similarly affect female remating.

Based on a meta-analysis of the fitness benefits accruing to multiple mating in female insects, Arnqvist & Nilsson (2000) were the first to propose that receptivity-inhibiting substances are transferred in the nuptial food gifts of males. A particularly intriguing finding was that although female reproductive success increased markedly with mating rate in gift-giving taxa, female mating rate in these taxa appeared to be far lower than optimal, a pattern consistent with the incorporation of refractory-inducing substances in males' gifts. The first clue that the spermatophylax of *G. sigillatus* might contains such substances came from a study in which Sakaluk (2000) offered spermatophylaxes taken from male *Gryllodes* to mated females of several related, but non-gift-giving species of crickets, as a means of testing the universal gustatory appeal of these gifts. Unexpectedly, females of one non-gift-giving species, *Acheta domesticus*, exhibited a significant decrease in sexual receptivity following the consumption of the *Gryllodes* spermatophylax, as evidenced by longer refractory periods compared with mated females who received no such gifts. However, because female *Acheta* receiving food gifts also retained the sperm ampulla for longer periods, and thus received a greater amount of sperm and male ejaculatory substances, the observed decrease in receptivity could be attributed as much to accessory gland proteins or other substances in the male's ejaculate as to substances contained in the ingested spermatophylax. To remedy this deficiency, and to incorporate a parallel manipulation in *G. sigillatus* in which females were experimentally prevented from consuming the spermatophylax, Sakaluk, Avery & Weddle (2006) repeated the experiment holding ampulla attachment duration (and the concomitant transfer of any ejaculatory compounds) constant across treatments. Again, female *A. domesticus* allowed to consume food gifts of male *Gryllodes* took significantly longer to remate than females given no such opportunity (Fig. 5a), but surprisingly, the consumption of food gifts had no comparable effect on the propensity to remate in female *Gryllodes* (Fig. 5b). Based on these results, Sakaluk et al. (2006) proposed that nuptial food gifts transferred by male *G. sigillatus* contains receptivity-inhibiting substances, but that female *G. sigillatus* have evolved reduced responsiveness (i.e., resistance) to these substances to retain control of their mating rate. They further contended that the reason why females of the non-gift-giving species showed a reduction in sexual receptivity is that, having had no evolutionary experience with the spermatophylax, they have not been under selection to evolve a comparable resistance to refractory-inducing substances contained in the spermatophylax.

Gryllodes is not the only species in which the incorporation of refractory-inducing substances in nuptial food gifts has been implicated. For example, a comparative analysis of bushcricket spermatophores using independent contrasts revealed that larger spermatophylaxes are associated with longer female refractory periods (Vahed, 2007b); however, such gifts are also associated with larger ejaculates, such that the increase in female refractory periods could be due as much, or more, to receptivity-inhibiting substances contained in seminal fluid. In male scorpionflies, *Panorpa cognate*, males secrete a salivary mass that the female consumes during copulation. Engqvist (2007) demonstrated that the refractory period of females is positively correlated with the amount of saliva consumed by the female during mating. This result suggests that the salivary mass contains receptivity-inhibiting substances, but it could also represent an adaptive female response if direct benefits received from the consumption of the saliva reduces the need for future matings (Engqvist, 2007). The manipulation of female physiology via nuptial gifts is not limited to effects on sexual receptivity, as substances in the gifts may also promote increased sperm storage or transitory increases in the rate of egg laying, effects that benefit males, but which could be detrimental to females (Vahed, 2007a).

If male *Gryllodes* do, in fact, incorporate receptivity-inhibiting substances in their nuptial food gifts, what might these compounds be? Given their ubiquity in the ejaculates of other insects, accessory gland proteins would appear to be a likely candidate, especially considering that a major portion of the solid fraction of the spermatophylax comprises proteins (Warwick et al., 2009). Indeed, our recent proteomics analysis of the spermatophylax detected 30 different proteins, of which, 18 were encoded by genes expressed in the male accessory glands (Pauchet, Wielsch, Wilkinson, Sakaluk, Svatoš, ffrench-Constant, et al., 2015); the number of proteins contained in the bushcricket spermatophylax is about an order of magnitude larger, but these remain entirely unidentified (Lehmann, Lehmann, Neumann, Lehmann, Scheler & Jungblut, 2018). The majority of the spermatophylax proteins we identified in *Gryllodes* show no similarity to proteins of known biological function. However, we identified two proteins of special interest, SPX4, a serine protease inhibitor that may inhibit *G. sigillatus*' own digestive proteinases, and SPX6, a protein that shows a high degree of similarity to known polypeptide growth factors described in other insects (Fig. 6).

Given its high degree of similarity to imaginal disc growth factors in *Drosophila* and the noctuid moth, *Mamestra brassicae* (Kawamura, Shibata, Saget, Peel & Bryant, 1999; Zhang, Iwai, Tsugehara & Takeda, 2006), it seems likely that SPX6 may promote cell growth and development in its target tissue within the female's body or influence female reproduction (Pauchet et al., 2015). The presence of such a protein in the spermatophylax could have important consequences with respect to female reproductive output, depending on the location of its target tissue within the female body. If SPX6 was able to reach the ovaries intact after being ingested, and exert its potential ability to promote cell growth and development in immature eggs, this too could represent another avenue by which males manipulate females. Although consumption of a spermatophylax by female *G. sigillatus* has been found to have no effect on the number of eggs produced (Will & Sakaluk, 1994; Kasuya & Sato, 1998; Ivy & Sakaluk, 2005), it has been found to increase the rate of oviposition (Kasuya & Sato, 1998). Such an effect would be highly advantageous to the male because female *G. sigillatus* are highly polyandrous (Sakaluk et al., 2002), a behavior that promotes a high degree of sperm competition leading to a dilution of male paternity (Sakaluk & Eggert, 1996). Thus, even a transitory increase in oviposition rate could result in a greater number of eggs fertilized by a male before the female remates with another male.

Gwynne (2008) suggested that nuptial food gifts that are orally consumed would be unlikely to harbor manipulative compounds, because complex compounds such as proteins would be digested in the gut of the female. However, if SPX4 was able to protect other spermatophylax proteins from proteolysis after the ingestion of the spermatophylax by the female, then other potentially manipulative proteins present in the spermatophylax could reach their intended target(s) in the female body with limited risk of being degraded as they pass through the female's digestive tract. Moreover, manipulative substances contained in the spermatophylax need not pass through the gut to effect a change in female behavior and physiology. They could instead activate gustatory receptors leading to neural pathways that terminate in the brain, thereby modulating female locomotor activity or sexual receptivity directly (Ignell, Anton, & Hansson, 2000; Rogers & Newland, 2003). In support of this possibility, Gordon, Gershman & Sakaluk (2012) probed the receptivity-inhibiting potential of two of the most abundant free amino acids found within the *Gryllodes* spermatophylax, glycine and proline, by injecting them directly into the haemocoel of female house crickets, *Acheta domesticus* (the species known to be susceptible to the refractory-inducing effects of spermatophylax consumption), or by feeding females experimental pectin-based 'gels' containing these amino acids and designed to simulate the experience of consuming a spermatophylax. Females fed gels containing glycine took longer to both remount and remate a previous mating partner following consumption of the gel compared with females fed control gels, but surprisingly, glycine injected directly into the haemocoel of females had no effect on their receptivity. The fact that ingestion, but not injection, of glycine induces a refractory period in females led Gordon et al. (2012) to suggest that glycine may be stimulating taste neurons that have downstream neurological effects on female behavior. Although female *Gryllodes* may not always be susceptible to such receptivity-reducing effects of individual components, by using a non-gift giving species, it is possible to investigate potential steps in the sexually antagonistic coevolutionary process in the way that might be achieved in an experimental evolution experiment where evolved males are tested against ancestral females (details on one such experiment are described later in this chapter).

5. THE COST OF PRODUCING A SPERMATOPHYLAX

With respect to its nutritional value to females, the spermatophylax appears to be more of a sham than a true “gift”. Will & Sakaluk (1994) experimentally manipulated the number of spermatophylaxes that female *Gryllodes* were permitted to consume each day while simultaneously varying the total amount of food available to experimental subjects. There was no effect of spermatophylax consumption on female survival, egg size, or lifetime reproduction, even when females were completely deprived of food, a result that has since been replicated in subsequent studies (Kasuya & Sato, 1998; Warwick, 1999; Ivy & Sakaluk, 2005). The absence of detectable nutritional benefits to courtship feeding is not unique to *G. sigillatus* but appears to be widespread across a variety of gift-giving taxa (reviews in Vahed, 1998, 2007a). However, in this respect, the spermatophylax of *Gryllodes* is fundamentally different from the spermatophylax of many bushcrickets: whereas the *Gryllodes* spermatophylax is small (2-3% of a male’s body mass; Sakaluk 1985, 1997), composed primarily of water, and nutritionally depauperate, bushcricket spermatophylaxes can constitute up to 40% of a male’s body mass, are often loaded with proteins that represent a significant source of nutrition to females, and whose consumption has been shown to contribute significantly to female fitness in a number of species (reviewed in Gwynne, 2008). Female *Gryllodes* can, however, secure important hydration benefits from spermatophylax consumption during periods of water stress (Ivy, Johnson & Sakaluk, 1999).

The absence of nutritional benefits to spermatophylax consumption might lead one to erroneously conclude that it is relatively cheap to produce. Indeed, Warwick et al. (2009) likened the spermatophylax to "candy", a low-quality food gift that is "sweetened" with readily available, free amino acids. However, the fact that male refractory periods in *G. sigillatus* are an order of magnitude or longer compared to non-gift-giving gryllid species (Sakaluk, 1985) suggests that the spermatophylax incurs significant costs with respect to its synthesis. An emerging body of evidence further suggests that males face serious constraints with respect to the size and composition of the spermatophylax, and that these constraints arise, in part, because of important life-history trade-offs with respect to other components of reproductive effort and, in some cases, male survival. For example, Kerr, Gershman & Sakaluk (2010) examined the potential life-history trade-off between male spermatophore investment and male immunity by inducing an immune response in some males by injecting them with lipopolysaccharides, thereby simulating an infection without the attendant costs of a real disease, and, in a reciprocal experiment, forced males to synthesize additional food gifts to determine if this results in decreased immunity. Immune-challenged males produced smaller food gifts than control males, and males that synthesized more food gifts exhibited lower immunity, revealing a fundamental trade-off between immunity and reproduction. A companion study similarly revealed a trade-off between the lytic activity of male hemolymph and spermatophylax mass in outbred, but not inbred crickets (Gershman, Barnett, Pettinger, Weddle, Hunt & Sakaluk, 2010).

More recent work has shown that life-history trade-offs can also inform the chemical composition and gustatory appeal of the spermatophylax offered by males to females. Duffield, Hunt, Rapkin, Sadd & Sakaluk (2015) injected male *G. sigillatus* with heat-killed bacteria in a repeated-measures design in which they assessed the amino acid profile of the spermatophylax produced by a male both before and after the immune challenge. By mapping amino acid profiles onto the fitness surface describing the likelihood that a spermatophylax would be consumed and thus lead to maximal insemination of the female (Gershman et al., 2012), Duffield et al. (2015) showed that immune-challenged males actually synthesize gifts of greater gustatory appeal than control males. They concluded that immune-challenged male *Gryllodes* terminally invest with respect to the composition of the spermatophylax, increasing their investment in the quality of the food gift at a cost to their future reproduction or survival.

The costs of producing a spermatophylax has, perhaps, been most convincingly demonstrated by assessing how males regulate their dietary intake of protein and carbohydrate in optimizing the size and gustatory appeal of the spermatophylax. Rapkin, Jensen, Lane, House Sakaluk & Hunt (2016) conducted experiments in which they varied the ratio of protein (P) and carbohydrate (C) among a series of artificial diets to determine how the availability of these nutrients influence the mass and gustatory appeal of the gift, and to ascertain how males regulate their intake when permitted to choose among diets of varying P:C ratios that differentially influence the size and quality of the gift. They showed that the mass and gustatory appeal of the gift increased with overall nutrient intake, attesting to the cost of producing a spermatophylax, but, as importantly, that the mass and amino acid composition of the spermatophylax was optimized at a P:C ratio of 1:1.3. It may seem surprising, then, that when given a choice, males exhibited a higher intake of carbohydrate relative to protein than is optimal. This seemingly suboptimal intake of nutrients may be reflective of trade-offs with other fitness-related traits whose optimization demands different P:C ratios, among them, the cuticular hydrocarbon profile of the male (Rapkin, Jensen, House, Sakaluk, Sakaluk & Hunt, 2017), which can greatly influence a female's decision to mate (Weddle et al., 2013; Capodeanu-Nägler et al., 2014), and the production of calling song, a major component of male reproductive effort (Rapkin, Jensen, Archer, House, Sakaluk, del Castillo, et al., 2018).

6. ORIGIN OF NUPTIAL FOOD GIFTS

The lively discussion surrounding the function and selective factors contributing to the maintenance of nuptial food gifts (Vahed, 1998, 2007a; Gwynne, 2008; Lewis & South, 2012; Lewis et al., 2014) has overshadowed an equally important, but somewhat overlooked aspect of their evolution: how do nuptial food gifts arise in the first place? The answer to this question needs to accommodate several pervasive features of nuptial food gifts: 1) they invariably occur in taxa in which females largely are in control of the extent to which they are inseminated, 2) they almost always promote increased insemination of the female, and 3) their remarkable diversity in form and distribution across the insects means that they must have independently arisen on multiple occasions in different taxa (Vahed, 1998, 2007a). How did this happen with such frequency? Sakaluk (2000) proposed that nuptial gifts arise as a form of sensory trap (Christy, 1995), specifically, that such gifts emulate properties of food items that females normally find appealing, and that by engaging the innate gustatory response of the female during copulation, the male overrides any impulse she might have to interfere with sperm transfer or to prematurely terminate the mating. However, this proposition rests on two key assumptions, that: (1) the absence of nuptial feeding is the ancestral condition in groups in which nuptial gifts have arisen, and (2) the gustatory response elicited by the food gift was present before the food gift actually evolved (see Basolo, 1995). Orthopteran nuptial food gifts meet the first criterion quite well, as a phylogenetic analysis of the suborder Ensifera has revealed that a simple, externally attached sperm ampulla and female consumption of the ampulla were the most likely ancestral character states, whereas the provision of a spermatophylax and other forms of nuptial food gifts likely arose only after female consumption of the ampulla had evolved (Gwynne, 1995). To address the second of these criteria, Sakaluk (2000) offered food gifts taken from male *Gryllodes* and offered them to mated females of three related cricket species that manifest the ancestral condition of a simple naked ampulla that females consume after mating. Not only did females of these non-gift-giving species eagerly accept and consume the proffered spermatophylax, the consumption of this 'foreign' food gift afforded the same degree of protection to the male's ampulla as it normally does in *Gryllodes*: females consuming a spermatophylax retained their mate's ampulla longer and experienced greater insemination than females that were offered no such inducement. Thus, this study provided an important proof-of-principle, at least, that nuptial food gifts could indeed arise by exploiting a preexisting gustatory bias in females.

This initial study was followed by subsequent investigations invoking the same kind of sensory exploitation to account for the origin of other kinds of nuptial food gifts, including a silk-wrapped gift in a spider, *Pisaura mirabilis*, that appears to mimic the female's egg sac (Stålhandske, 2002), and tergal secretions of male Madeira cockroaches, *Leucophaea maderae*, that are consumed by females at mating (Mondet et al., 2008). But, in what must be considered the most remarkable example of evolutionary convergence, Albo, Macías-Hernández, Bilde & Toft (2017) staged matings between males of a spider, *P. mirabilis*, that offers insect prey wrapped in silk as a nuptial food gift (Prokop & Maxwell, 2012), and females of a related species, *Cladycnis insignis*, in which males offer no such inducement. Not only did female *C. insignis* readily accept a gift from an interspecific mating partner, but the receipt of this gift resulted in significantly longer matings compared with normal intraspecific pairings. Because *C. insignis* is more basally derived in the clade containing the two species, Albo et al. (2017) concluded that, as in *Gryllodes*, these results are consistent with the hypothesis that gift-giving in *P. mirabilis* arose as a form of sensory exploitation of a female's motivation to feed.

One final example should suffice to underscore the potential of the sensory-trap hypothesis to account for the evolution of nuptial food gifts, and it involves the reverse of the experimental approach employed by Sakaluk (2000) and Albo et al. (2017). Instead of offering novel food gifts to females of non-gift-giving species, LeBas & Hockham (2005) substituted the normally valuable gifts offered to females of a gift-giving species with worthless gifts. In the dance fly, *Rhamphomyia sulcate*, males capture insect prey that they offer as nutritionally valuable, nuptial food gifts to females at mating. In a field experiment, LeBas & Hockham (2005) replaced the gift that a male was about to confer on the female in naturally mating pairs with either another insect prey, or a worthless gift, a small ball of cotton. The use of cotton as a worthless gift was meant to simulate the wind-blown seed tufts offered as inedible token gifts in other dance fly species. Although females receiving a large insect prey copulated for the longest durations, females receiving a token cotton gift copulated as long as those females receiving a small, but nutritious, insect prey. LeBas & Hockham (2005) concluded that worthless nuptial gifts can evolve via the invasion of cheating males that employ token gifts to exploit the sensory biases of females selected to respond to authentic gifts.

7. RESOLVING THE CONFLICT OVER SEXUAL CONFLICT

The idea that sexual conflict might have influenced the evolution of nuptial food gifts is not new. Nearly thirty years ago, Parker & Simmons (1989) developed theoretical models exploring how sexual conflict could influence the allocation of male-derived resources contained in food gifts and the temporal patterns of egg production and oviposition in females. They determined that when gifts are infrequently offered, males benefit most from a more immediate use of resources in egg production, whereas females often benefit from a delay; earlier reports of ejaculatory substances that affect vitellogeneis and oviposition led them to further suggest that this conflict might be resolved more in the direction of male interests. In a companion paper, Simmons & Parker (1989) also addressed a long-standing and occasionally acrimonious debate over the function of nuptial gifts (Gwynne, 1984; Wickler, 1985; Quinn & Sakaluk, 1986; Sakaluk, 1986b): do nuptial food gifts serve primarily to maximize the fertilization success of the male (*mating effort* hypothesis) or do they instead advance the fitness interests of the male through the nutritional benefits derived from the offspring they sire (*paternal investment* hypothesis)? Theoretical considerations led Simmons & Parker (1989) to propose that oral endogenous gifts most likely originated via their effects on the mating or fertilization success of the male, because, initially at least, the magnitude of any nutritional effects would have been relatively trivial; however, they did not discount the possibility that paternal investment might later contribute to the maintenance of such gifts.

Simmons & Parker's (1989) suggestion proved to be prescient. In a subsequent and wide-ranging review of the adaptive significance of nuptial food gifts in insects, Vahed (1998) showed that, in the majority of species, oral food gifts actually do promote increased mating success or lead to increased sperm transfer, observations consistent with the hypothesis that nuptial food gifts function as male mating effort. In an update to this review, Vahed (2007a) considered how gift composition might subsequently be tailored to enhance the gustatory appeal of nuptial gifts to females or to maximize their handling time, but also their potential to induce female sexual refractory periods. He concluded that nuptial food gifts likely impose costs on females in many cases, through their effects on female lifespan, rate of oviposition, and female sexual receptivity, effects best understood within the context of sexual conflict.

In highlighting potential costs to females of nuptial feeding, Vahed (2007a) suggested that, by way of contrast, the fecundity-enhancing benefits of nuptial food gifts had been exaggerated in the literature. This conclusion was subsequently challenged by Gwynne (2008), who reviewed a number of studies showing that females often experience direct nutritional benefits from consuming gifts, and called into question both the empirical and comparative evidence that oral gifts contain substances that manipulate female sexual receptivity. Although not directly stated, Gwynne's (2008) assessment seems to imply that the existence of a nutritional benefit to the consumption of nuptial food gifts necessarily precludes a role for sexual conflict in shaping these gifts as a vehicle by which males manipulate female physiology or behavior. This inference is probably misguided on at least two counts. Nuptial gifts that function to promote male reproductive interests through their manipulation of females may confer a nutritional benefit merely as an incidental and unavoidable consequence of an inability to produce gifts entirely free of compounds useful to females (e.g., proteins); Quinn & Sakaluk (1986) elaborate on the importance of disentangling incidental effects from the evolved function of male prezygotic investments. Moreover, potential nutritional benefits of gifts and the occurrence of manipulative compounds are not mutually exclusive, as a recent proteomic analysis of butterfly ejaculates would attest. Meslin, Cherwin, Plakke, Hill, Small, Goetz, et al., (2017) explored the molecular basis and structural complexity of the spermatophore of the cabbage white butterfly, *Pieris rapae*, which, following its transfer at copulation, is slowly digested within the bursa copulatrix, a chamber forming part of the female reproductive tract. Their analysis revealed that the hard outer envelope of the spermatophore and the more soluble softer inner matrix are characterized by vastly different suites of proteins. Meslin et al. (2017) speculated that whereas the inner matrix might serve as a source of female nutrition, the indigestible outer envelope might hinder digestion thereby allowing males to delay female remating. More surprisingly still, they discovered that females contribute a large portion of protein in spermatophores in the form of proteases that could contribute to the more rapid digestion of the spermatophores. Collectively, these observations are suggestive of a history of sexually antagonistic coevolution with respect to the biochemistry of butterfly spermatophores.

We conclude by encouraging investigators to recognize that sexual conflict does not represent an alternative hypothesis to the mating effort and paternal investment hypotheses, but rather, a selective milieu shaping the chemical composition of nuptial gifts regardless of whether they function to increase male fertilization success or as a form of parental investment, or both. Indeed, we suggest that sexual conflict is likely a pervasive force acting on the majority of gift-giving taxa because in most, if not all of these species, females mate polyandrously, leading to inevitable sexual conflicts over the use of sperm, female remating behavior, and the allocation of male-derived resources.

A major empirical obstacle to unraveling the evolutionary history of nuptial food gifts is that we might expect coevolutionary interactions surrounding gift-giving to be dynamic: gifts that initially are nutritionally beneficial to females might evolve to become more harmful, and vice versa (Lewis & South, 2012; Lewis et al., 2014). Comparative phylogenetic analysis might profitably be used to assess competing evolutionary scenarios for the evolution of nuptial gifts (for an especially instructive example concerning the evolution of nuptial food gifts in harvestmen, see Kahn, Cao, Burns & Boyer, 2018). However, such an approach requires not only well-supported phylogenies, but detailed information on the mating system, life history, and reproductive behavior of the species included in any such analysis (Lewis et al., 2014). An experimental evolution approach offers a powerful alternative means of probing the influence of sexual conflict on the evolution of nuptial gifts because the intensity of sexual selection and conflict is easily altered through manipulation of the operational sex ratio; no study has, to our knowledge, successfully applied this methodology to examine the coevolutionary dynamics surrounding the evolution of male nuptial gifts and female responses to them.

We have, accordingly, recently embarked on just such a study to explore the role of sexual conflict in driving the evolution of the chemical composition of males’ nuptial gifts and females’ responses to them in *G. sigillatus*. In addition to monitoring evolutionary modifications in the free amino acid profile of the spermatophylax, and targeted gene expression to quantify changes in key constituent proteins, we are employing a comparative transcriptomics approach to characterize differential gene expression in brain and gut tissue of females in lines subject to intensified sexual conflict (i.e., male-biased) and those from which conflict have been largely eliminated (i.e., female-biased). Preliminary data (Hunt, J., Sharma, M.D., Rapkin, J., ffrench-Constant, R.H. & Sakaluk, S.K., unpublished) suggest that female gene expression patterns are altered following nuptial gift consumption. It seems likely that included among the differentially expressed genes are ones that underlie changes to female behavior and physiology that are beneficial to male fitness interests. Interestingly, gift consumption by females altered gene expression in the gut, where it might be expected, but also in the head (Fig. 7). Linking gene expression to the behavior and physiology of females following nuptial gift feeding will enable specific pathways to be uncovered, and is a promising avenue of research to increase our understanding of the proximate mechanisms underlying male-female interactions mediated by nuptial gifts. By coupling a powerful experimental evolution approach with the tools of modern genomics (Wilkinson, Breden, Mank, Ritchie, Higginson, Radwan, et al., 2015), we aim to provide one of the most comprehensive examinations of how sexual selection and conflict contribute to the evolution of a widespread behavioral trait that mediates sexual interactions across a diverse array of insect taxa.

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Table 1. Identification of 22 free amino acids contained in the spermatophylax of *Gryllodes sigillatus* and their relative contribution, expressed as the mean percentage (±SE) of the total abundance of all compounds. *Excerpted from Gershman, S.N., Mitchell C., Sakaluk, S.K. & Hunt, J. (2012). Biting off more than you can chew: sexual selection on the free amino acid composition of the spermatophylax in decorated crickets. Proceedings of the Royal Society B, 279, 2531-2538.*

Amino acid	**Abbrev**	**Mean % (±SE)**
Alanine	ALA	15.00 ± 0.20
Aspargine	ASN	0.45 ± 0.01
Aspartic acid	ASP	1.27 ± 0.02
Glutamic acid	GLU	7.71 ± 0.14
Glutamine	GLN	4.49 ± 0.12
Glycine	GLY	33.98 ± 0.28
Glycyl-proline	GPR	0.02 ± 0.00
Histidine	HIS	1.43 ± 0.05
4-Hydroxyproline	HYP	0.10 ± 0.01
Isoleucine	ILE	0.43 ± 0.01
Leucine	LEU	0.41 ± 0.01
Lysine	LYS	0.96 ± 0.02
Methionine	MET	0.10 ± 0.00
Orthinine	ORN	0.12 ± 0.02
Phenylalanine	PHE	0.14 ± 0.00
Proline	PRO	26.88 ± 0.28
Serine	SER	4.17 ± 0.07
Threonine	THR	0.83 ± 0.02
Tryptophan	TRP	0.02 ± 0.00
Tyrosine	TYR	0.09 ± 0.00
Valine	VAL	1.34 ± 0.17
α-aminobutyric acid	AAA	0.07 ± 0.01

FIGURE LEGENDS

Figure 1 Mating sequence in decorated crickets, *Gryllodes sigillatus.* (A) Male stridulating to attract sexually receptive females. (B) Copulation. The female is mounted dorsally on the male, and the spermatophore that he transfers to her can be seen extruding from his spermatophoric pouch. (C) Immediately upon dismounting the male, the female reaches back to detach the spermatophylax (the translucent portion of the spermatophore) from the sperm-containing ampulla. (D) Female feeding on the spermatophylax (nuptial food gift) while sperm are evacuated from the sperm ampulla (the small white ball attached at to her genital opening). All photos by David Funk.

Figure 2 (A) The time after mating at which the female removes the sperm ampulla as a function of the time it takes her to fully consume the spermatophylax. *Redrawn from Sakaluk, S.K. (1984). Male crickets feed females to ensure complete sperm transfer. Science, 223, 609-610.* (B) The time after mating at which the female removes the sperm ampulla as a function of the time at which she discards the spermatophylax. The red line in each panel denotes the amount of time required for the ampulla to be completely emptied of sperm based on sperm trajectory studies. Points below the red line in each panel represent males that did not transfer their full complement of sperm because their ampulla was prematurely removed. The inset shows a sample of *G. sigillatus* sperm stained with Hoechst 33528, a DNA-specific stain, and visualized using fluorescence microscopy (Sakaluk & O'Day, 1984).

Figure 3 The effect of the number of sperm transferred by competing males on the paternity of offspring produced by doubly mated female *G. sigillatus*. As the proportion of sperm transferred by the second male increases, so too does the proportion of offspring sired by the second male. *Redrawn from Sakaluk, S.K. & Eggert, A.-K. (1996). Female control of sperm transfer and intraspecific variation in sperm precedence: antecedents to the evolution of a courtship food gift. Evolution, 50, 694-703.*

Figure 4 Contour-map visualization of the two major axes of nonlinear selection (m1 and m3) operating on the amino acid composition of the spermatophylax produced by *Gryllodes sigillatus*. Scale on the right shows fitness values associated with regions of different color; regions of more intense red represent spermatophylaxes more likely to be consumed, whereas blue regions represent spermatophylaxes more likely to be discarded. The amino acid profile of the actual spermatophylaxes of individual males are mapped onto the selection surface (open circles). *Redrawn from Gershman, S.N., Mitchell C., Sakaluk, S.K. & Hunt, J. (2012). Biting off more than you can chew: sexual selection on the free amino acid composition of the spermatophylax in decorated crickets. Proceedings of the Royal Society B, 279, 2531-2538.*

Figure 5 Proportion of females remaining unmated as a function of whether they consumed a spermatophylax. Females *Acheta domesticus* that consumed a spermatophylax of a male *G. sigillatus* after an initial mating took significantly longer to remate compared to control females (P = 0.029), whereas spermatophylax consumption had no influence on the latency to remating in female *G. sigillatus* (P = 0.83). *Redrawn from Sakaluk, S.K., Avery, R.L. & Weddle, C.B. (2006).*

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Figure 6 Separation of proteins from the spermatophylax of male *G. sigillatus*. Proteins were separated by anion exchange chromatography. Positively identified proteins are labelled SPX1 to SPX15. *From Pauchet, Y., Wielsch, N., Wilkinson, P.A., Sakaluk, S.K., Svatoš, A., ffrench-Constant, R.H., et al. (2015). What's in the gift? Towards a molecular dissection of nuptial feeding in a cricket. PLoS ONE, 10, e0140191.*

Figure 7 Smear plots showing differential gene expression in the (A) head and (B) gut of female *G. sigillatus* that were fed a spermatophylax or not. logCPM is the log2 value of read counts per million, and logFC is the log fold-change, the log difference between groups. Gray points represent non-significant transcripts, whereas red points are transcripts that are significantly differentially expressed between fed and unfed females (adjusted P value <0.1). Red points at the top of each panel show much higher expression in females consuming a spermatophylax than females that did not, and vice versa for points at the bottom of each panel. *Data from unpublished study by Hunt, J., Sharma, M.D., Rapkin, J., ffrench-Constant, R.H. & Sakaluk, S.K.*

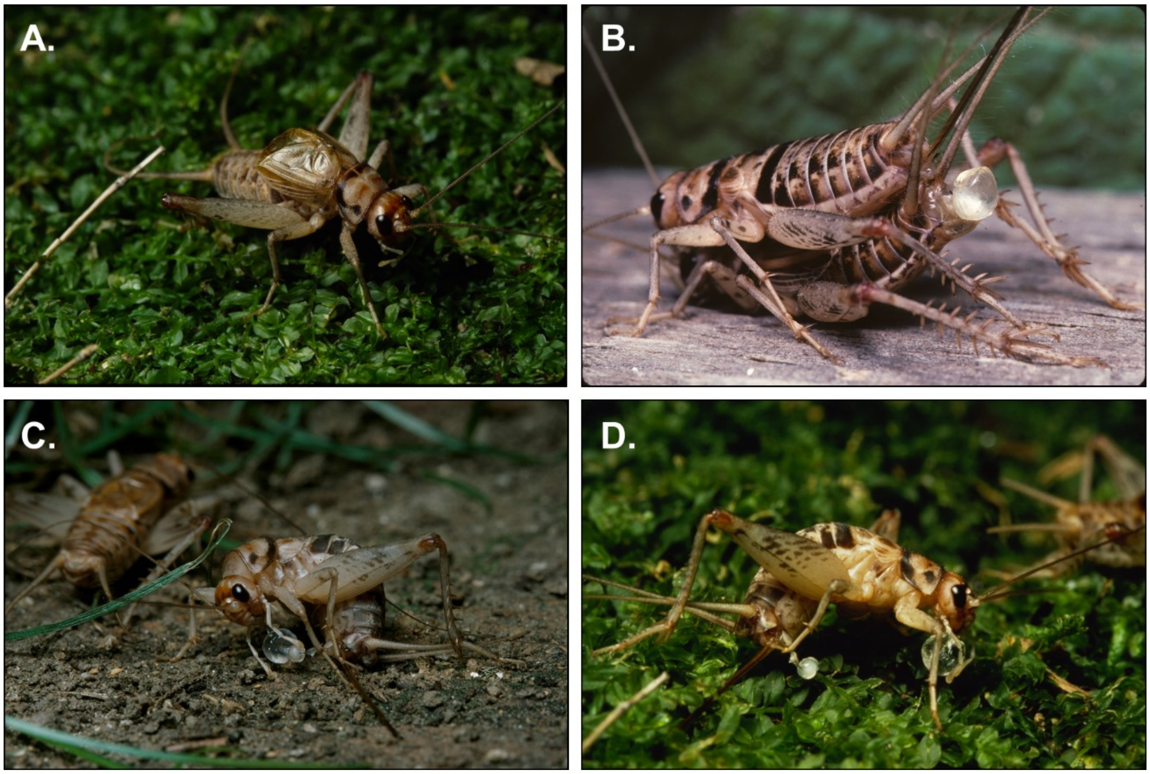
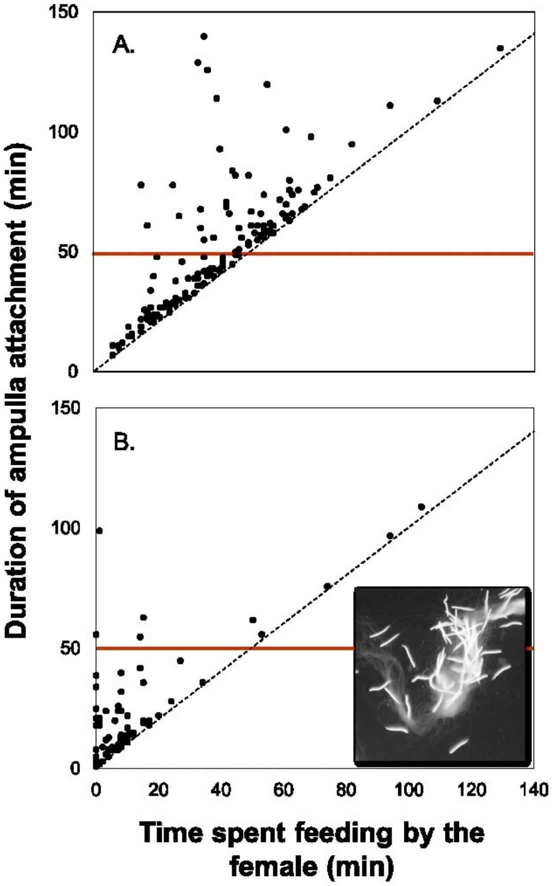


Figure 1. Sakaluk et al.



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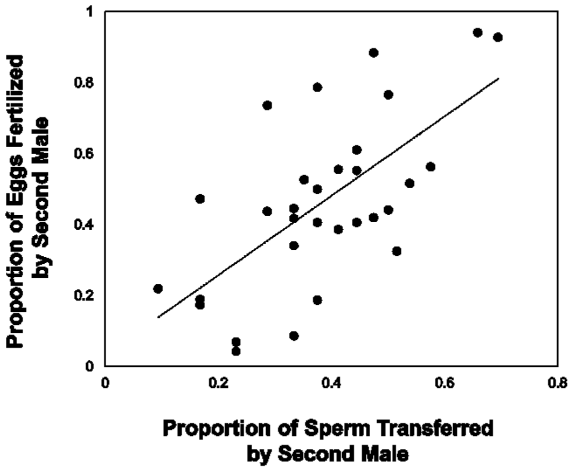

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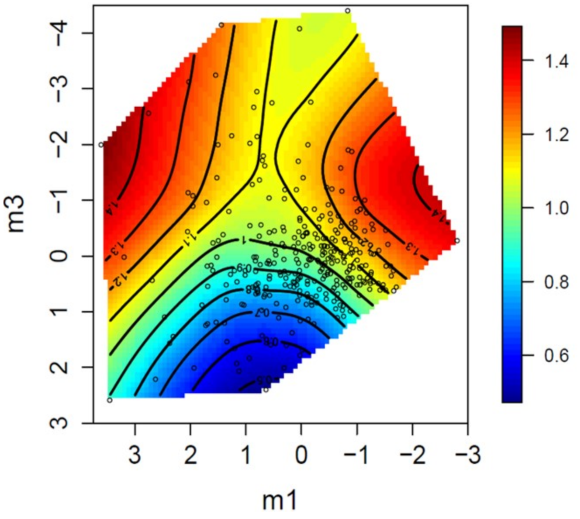
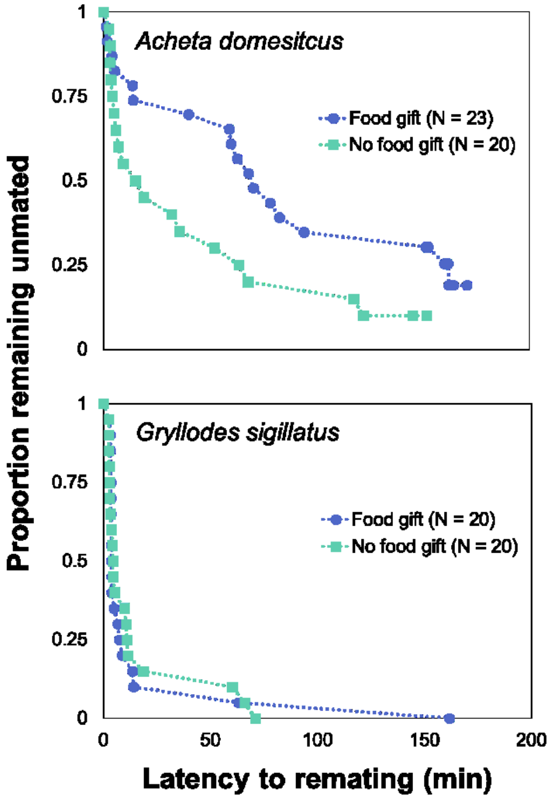


Figure 4. Sakaluk et al.



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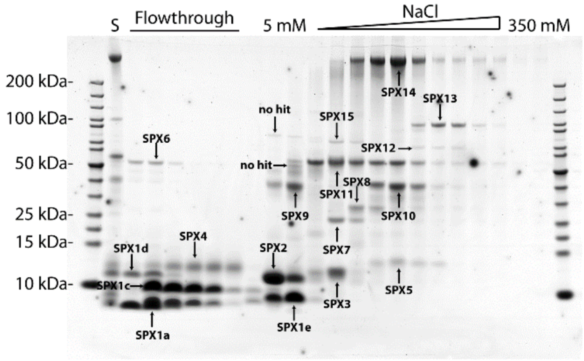

Figure 6. Sakaluk et al.

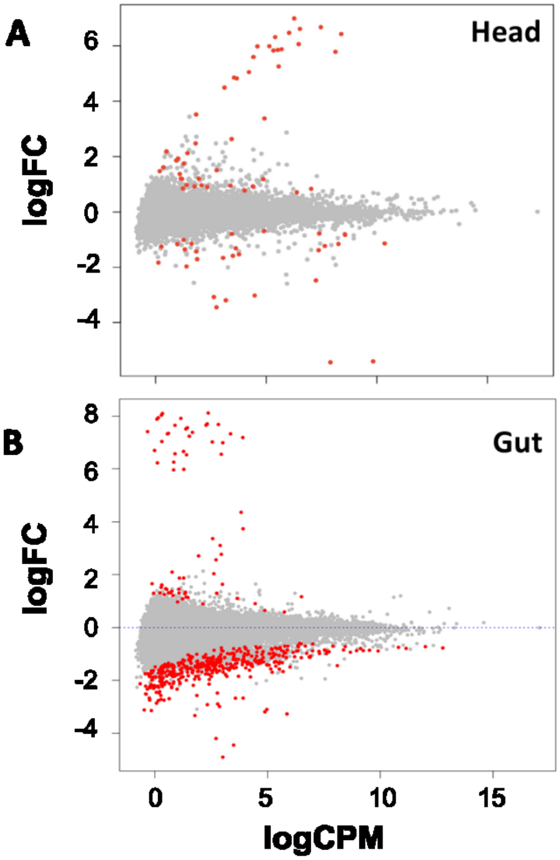

Figure 7. Sakaluk et al.