The Evolution of Animal Nuptial Gifts

SARA LEWIS and ADAM SOUTH

DEPARTMENT OF BIOLOGY, TUFTS UNIVERSITY, MEDFORD, MASSACHUSETTS, USA

Rich gifts wax poor when givers prove unkind
William Shakespeare, *Hamlet, Prince of Denmark* (3.1.101)

I. INTRODUCTION

A. WHAT ARE NUPTIAL GIFTS?

Nuptial arrangements in many human cultures include gift-giving traditions (Cronk and Dunham, 2007; Mehdi, 2003), and this behavior plays an important role in the mating systems of other creatures as well (Boggs, 1995; Fabre, 1918; Gwynne, 2008; Lack, 1940; Thornhill, 1976; Vahed, 1998, 2007; Zeh and Smith, 1985). In species widely distributed across the animal kingdom, males transfer many different non-gametic materials to females during courtship and mating. Such materials can include lipids, carbohydrates, proteins, peptides, amino acids, uric acid, minerals, water, antipredator defensive compounds, anti-aphrodisiac pheromones, and neuroendocrine modulators of recipient physiology. These nuptial gifts are an important aspect of reproductive behavior and animal mating systems (Andersson, 1994; Thornhill and Alcock, 1983). However, when compared to more conspicuous sexually selected traits such as male weaponry or ornamentation, such gifts have received relatively little attention from behavioral, ecological, and evolutionary research. Nuptial gifts heighten male reproductive investment, thus limiting male mating rates and altering courtship sex roles and sexual size dimorphism (Boggs, 1995; Gwynne and Simmons, 1990; Leimar et al., 1994). Selection acts on both gift-givers and receivers to shape nuptial gift structure and biochemical composition, as well as gift-giving behaviors. Not only do nuptial gifts form the basis for dynamic coevolutionary interactions between the sexes, but they also link

together male and female resource budgets (Boggs, 1990). Because they are thus strategically poised at the intersection of nutritional ecology, sexual selection, and life-history evolution (Boggs, 2009), understanding the evolutionary origins and maintenance of nuptial gifts is of fundamental importance.

Animal nuptial gifts come in multitudinous forms (Fig. 1), including food offerings, various male body parts, hemolymph, salivary gland secretions, seminal fluid, spermatophores (sperm-containing packages manufactured by male reproductive glands), and love darts. Many birds engage in courtship feeding, during which males provide prey to their own pair-bond partner or to extra-pair females (Lack, 1940; Mougeot et al., 2006). Scorpionfly males offer females dead insects or secretions from their
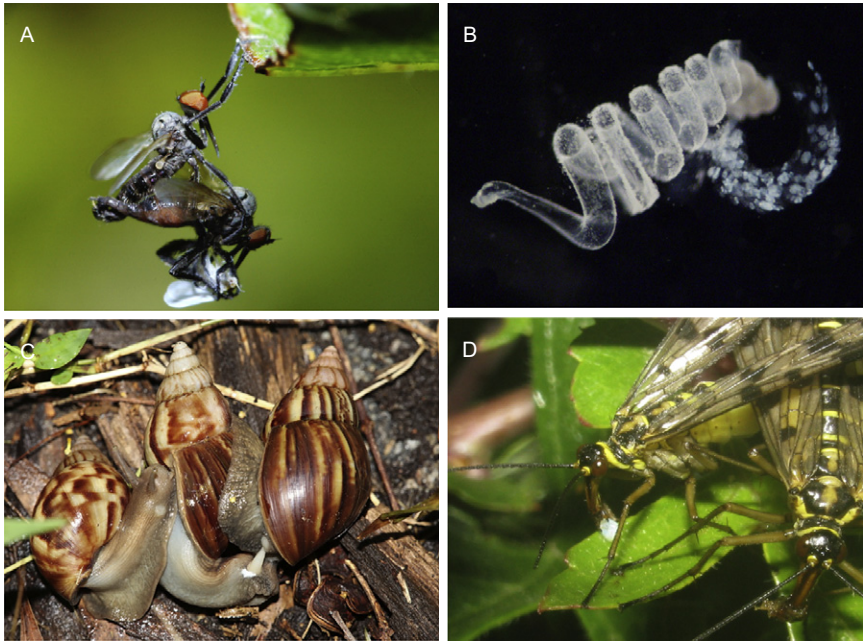

Fig. 1. A sampler illustrating the extraordinary diversity of animal nuptial gifts: (A) During mating, a female dance fly (Diptera: Empididae) feeds upon a dead insect provided by her mate (Photo by Rob Knell). (B) Sperm rings are released from a spermatophore manufactured by male accessory glands in *Photinus* fireflies (Coleoptera: Lampyridae). (C) During mating, hermaphroditic land snails (Gastropoda: Achatinidae) shoot their partner with a love dart that delivers mucus gland secretions (Photo by James Koh). (D) A male scorpionfly (Mecoptera: Panorpodidae) secretes a white salivary mass that will be consumed by a female during mating (Photo by Arp Kruithof).

enlarged, sexually dimorphic salivary glands (Liu and Hua, 2010; Thornhill, 1981). In some ground crickets, females imbibe hemolymph from a specialized spur located on their mate's hindleg (Gwynne, 1997; Piascik et al., 2010). In numerous animals (including salamanders, molluscs, crustaceans, annelids, leeches, and most insects), males transfer biochemically diverse spermatophores to females during mating (Mann, 1984). Nuptial gifts are not limited to animals with separate sexes, as during copulation many hermaphrodites inject chemicals that induce a physiological response in their partner (Koene and Schulenburg, 2005; Koene and Ter Maat, 2001; Michiels and Koene, 2006; Schilthuizen, 2005). Neither is gift-giving an exclusively male behavior: in heteropteran Zeus bugs, males feed upon glandular secretions provided by the female (Arnqvist et al., 2003).

Clearly, if we intend to move beyond merely describing these traits to begin formulating and answering questions about how animal nuptial gifts have evolved, we will need to start with a carefully articulated, coherent definition that encompasses this remarkable diversity. In this review, we begin by proposing such a definition, and then offer a conceptual framework for systematically classifying nuptial gifts. We go on to discuss some ecological conditions and life-history traits that might favor the evolution of nutritive nuptial gifts, that is, those that contribute to female resource budgets. From the male perspective, gift-giving behavior will usually (but not always) provide a net benefit. We analyze these potential benefits by describing how nuptial gifts can increase male reproductive fitness over multiple selection episodes that take place before, during, and after copulation. As a case study, we describe previous work on the katydid *Requena verticalis* that has elucidated how gift-giving males benefit from this behavior. Rigorously testing the many scenarios that have been proposed about nuptial gift evolution requires a comparative phylogenetic approach, and we discuss results from three insect groups where such an approach has been applied: crickets and katydids, fireflies, and *Drosophila* fruitflies. We also summarize work on rates of evolutionary change in an important constituent of *Drosophila* nuptial gifts known as seminal fluid proteins. Finally, we suggest several directions for future research that promise to deepen our understanding of nuptial gift evolution.

B. TOWARD A BROADER VIEW OF NUPTIAL GIFTS

Before considering how nuptial gifts might have evolved, it is essential to clarify some relevant terminology. Previous studies have most often relied on *ad hoc* definitions of nuptial gifts, an approach reminiscent of the infamous "I know it when I see it" definition of hard-core pornography that Justice Potter Stewart used in his written opinion on the US Supreme

Court case Jacobellis v. Ohio (1964). The *Oxford English Dictionary* (1989) provides a legal definition of gift as ''the transference of property or a thing by one person to another, voluntarily.'' Further, in colloquial English, the term ''gift'' generally implies some benefit for the recipient. However, because coevolutionary interactions between the sexes can continually alter costs and benefits for both givers and receivers, we contend that a broader view is essential for understanding the evolution of animal nuptial gifts. Within the scientific community, some researchers have limited the scope of nuptial gifts to encompass only ''nutritive'' gifts, that is, those that contain male-derived substances used by females to sustain metabolic activities (e.g., Andersson, 1994; Boggs, 1995; Gwynne, 2008; Thornhill, 1976). Others have excluded from consideration any gifts that are not contained within a consolidated package (e.g., Thornhill and Alcock, 1983). Again, we suggest that such restrictions may hinder progress toward the ultimate goal of understanding nuptial gift evolution.

As an alternative to this disjointed approach, we advocate the following definition (modified from South et al., 2011b; Lewis et al., 2011): *Nuptial gifts are materials beyond the obligatory gametes that are transferred from one sex to another during courtship or mating*. Importantly, this definition makes no assumptions concerning either: (1) how the gift currently affects fitness; thus, at certain times during its evolutionary trajectory, a gift might be beneficial, neutral, or detrimental to either sex, or (2) the presence or absence of gift-wrapping: thus, we include soluble proteins and other materials that are transmitted in seminal fluid or mucus as gifts, albeit unpackaged. In articulating this broad definition, we hope to unify what have previously been disconnected lines of investigation. For example, the protein content of insect spermatophores is often used as a measure of gift quality (Bissoondath and Wiklund, 1996; Cratsley et al., 2003; Wedell, 1994). This reasonable inference is based on female vitellogenesis being protein-limited (Wheeler, 1996), coupled with evidence that male-derived amino acids are incorporated into female eggs and soma (e.g., Boggs and Gilbert, 1979; Rooney and Lewis, 1999). However, male seminal fluid in *Drosophila melanogaster* contains soluble proteins secreted by reproductive accessory glands and the male ejaculatory duct (Chapman, 2008). Many of these reproductive proteins have been identified and demonstrated to alter female reproduction by stimulating ovulation and oviposition, increasing sperm storage, and lengthening females' latency to remate (Avila et al., 2011; Wolfner, 2009). However, because these proteins are transmitted in seminal fluid and are not encapsulated within a discrete package, traditionally they have not been considered as nuptial gifts (but see Markow, 2002; Simmons and Parker, 1989; Vahed, 1998). Yet it is becoming clear that male

spermatophores contain many of the same protein classes (Andres et al., 2006, 2008; Braswell et al., 2006; Sonenshine et al., 2011; South et al., 2011a) and these components may produce similar effects on females. It could perhaps be argued that including seminal fluid makes our nuptial gift definition overly broad. However, because various constituents of seminal fluid have been shown to exert diverse effects on both male and female fitness (Gillott, 2003; Leopold, 1976; Poiani, 2006), such inclusion seems appropriate. Thus, we argue that drawing an arbitrary distinction between seminal products encased within a discrete package versus unpackaged seminal products transferred in a liquid ejaculate may have inadvertently obscured basic similarities in gift composition and function, as well as similarities in the evolutionary origin and maintenance of male reproductive accessory glands, the main gift-producing structures.

A key point is that this broad perspective on nuptial gifts allows for possible changes over evolutionary time in how gifts will affect the recipient's net fitness. While some degree of cooperation is required for sexual reproduction to occur, males and females have distinct reproductive interests (Arnqvist and Rowe, 2005; Parker, 1979; Trivers, 1972). As a result, coevolutionary interactions between the sexes will cause nuptial gifts to evolve dynamically in a manner that alters the cost/benefit ratio of nuptial gifts for each sex. Thus, a nuptial gift that originates because it provides mutual fitness benefits to both sexes may evolve into a gift that reduces the recipient's net fitness, and *vice versa*.

In summary, even though some may find fault with our definition, there is an undeniable need for a more systematic approach to defining what exactly constitutes a nuptial gift. Furthermore, a broader definition such as the one we propose here will allow us to better track the changes in nuptial gift costs and benefits that are certain to occur over evolutionary time.

C. Classifying Nuptial Gift Diversity

In any contest, insects would surely emerge as the undisputed champions of gift diversity. For comprehensive insight into this fascinating diversity, readers are referred to excellent reviews by Boggs (1995) and Vahed (1998). Here, we highlight just a few notable patterns observed among insects before proposing a classification scheme that will encompass animal nuptial gifts.

First, gifts are conspicuously diverse, not only between different insect groups, but also within particular clades. For example, spermatophores are ubiquitous within the insect order Lepidoptera, yet they are absent in the Diptera and occur only sporadically within the Coleoptera (Davey,

1960; Mann, 1984). Within the beetle family Lampyridae (fireflies), some males pass elaborate spermatophores while firefly males of other species transfer free (unpackaged) ejaculates (Lewis and Cratsley, 2008; South et al., 2011b). Beyond spermatophores, orthopteran nuptial gifts have flowered into an especially impressive display of diversity (described in Section IV.A).

A second notable pattern is that some groups show surprising plasticity in their gift-giving behavior. For example, male *Panorpa* scorpionflies (Mecoptera: Panorpidae) pursue alternative mating tactics using different gift types (Sauer et al., 1998; Thornhill, 1981). In *P. cognata*, gift-giving behavior depends on a male's nutritional state: well-fed males secrete salivary masses that females consume during copulation, while low-nutrition males instead offer females a dead arthropod (Engqvist, 2007b). Similarly, in several empidid dance flies (Diptera: Empididae), males optionally offer females either a dead prey insect or inedible tokens such as silk balloons or seed tufts (Preston-Mafham, 1999; Vahed, 2007).

Here we propose a taxonomy for animal gifts that we hope will facilitate mapping the landscape of nuptial gift evolution (for other classification schemes see Gwynne, 2008; Simmons and Parker, 1989; Vahed, 1998). Table I presents four nuptial gift categories, with examples of relevant structures and behaviors from various taxa. One key distinction is based on the method of gift production. Thus, we distinguish between *endogenous gifts* that are manufactured by males themselves and *exogenous gifts* that consist of externally procured food items such as seeds or prey that males gather and then transfer to females. Another important distinction is based on how gifts are absorbed by the recipient. Gwynne (2008) distinguished *oral gifts* that are taken in through the female digestive system (e.g., food items, spermatophylaces, hindwing secretions), from gifts we term *genital gifts* that are absorbed through the female reproductive tract: this includes both unpackaged secretions from male reproductive glands (conveyed in liquid seminal fluid) as well as those encased in discrete packages (spermatophores). We propose here another category consisting of *transdermal gifts* that are injected through the skin into the partner's body (e.g., snail love darts, intradermally implanted squid spermatophores, hypodermic insemination in leeches and bedbugs). Although nuptial gifts are often commingled together into a single category (e.g., Arnqvist and Nilsson, 2000), we believe the distinctions drawn here will prove useful as a basis for future studies of the evolution of nuptial gift structure and composition. The primary reason for proposing this classification scheme is because, as discussed below, very different predictions can be made about how various gift types might affect fitness of both sexes (see also Simmons and Parker, 1989 and Section III).

TABLE I
A CLASSIFICATION SCHEME FOR NUPTIAL GIFTS

Gift production	Gift absorption	Nuptial gift examples	Taxonomic group and references
Endogenous	Oral	Hemolymph from tibial spurs	Ground crickets (Piascik et al., 2010)
		Spermatophylax	Katydids and crickets (Gwynne, 1997)
		Salivary secretions	*Panorpa* scorpionflies (Engqvist, 2007a)
		Anal secretions	*Drosophila nebulosa* (Steele, 1986)
		Metanotal secretions	Tree crickets (Brown, 1997; Bussiére et al., 2005)
		Male body (sexual cannibalism)	Red-backed spider, mantids (Elgar and Schneider, 2004)
Endogenous	Genital	Spermatophores	Salamanders, lepidopterans, molluscs, copepods, crabs, spiders (Mann, 1984)
		Seminal fluid proteins	*Drosophila* spp. (Chapman, 2008; Markow, 2002; Wolfner, 2007)
Endogenous	Transdermal	Love darts	Land snails (Koene and Schulenburg, 2005)
		Setal gland injection	Earthworms (Koene et al., 2005)
		Intradermal spermatophore implantation	Squid (Hoving and Laptikhovsky, 2007), leeches (Mann, 1984)
		Hemocoelic injection of seminal fluid	Bedbugs (Stutt and Siva-Jothy, 2001)
Exogenous	Oral	Courtship feeding	Birds: kestrels, shrikes (Lack, 1940; Mougeot et al., 2006)
		Seeds	Lygaeid bugs (Carayon, 1964)
		Insect prey	Hangingflies, scorpionflies (Thornhill, 1981), empidid flies (Cumming, 1994), *Pisaura* spiders (Austad and Thornhill, 1986)

1. Exogenous Oral Gifts

These consist of food items that males capture or collect, so these are most likely to contain nutritive materials (defined as substances that contribute to female metabolic reserves). Thus, most exogenous oral gifts are predicted to deliver net fitness benefits to females, measured as increased lifetime fecundity. From the male perspective, these gifts are generally predicted to increase male fitness across several selection episodes (reviewed in Gwynne, 2008; Vahed, 1998, 2007). First, because they can be assessed (visually or by gustation) prior to mating, exogenous oral gifts should affect a male's ability to attract and successfully mate with females. Second, because females remain stationary while feeding, food gifts may make it easier for males to initiate copulation. Third, because females feed on these gifts while copulating, such gifts are expected to increase both copulation duration and the quantity of sperm transferred.

2. Endogenous Oral Gifts

This category includes diverse materials that are secreted by male salivary, reproductive, and other glands, as well as parts or the whole of the male's body; these materials are then consumed by females before, during, or after copulation (reviewed by Boggs, 1995; Elgar and Schneider, 2004; Vahed, 1998). Thus, in *Oecanthus nigricans* tree crickets (Orthoptera), females feed upon proteinaceous secretions produced by dorsally located male glands, while females of some true flies (Diptera) and scorpionflies (Mecoptera) consume male salivary secretions, and female *Allonemobius* ground crickets drink hemolymph from male hindleg spurs (Bidochka and Snedden, 1985). Females in many katydids and crickets (Orthoptera) consume a spermatophylax, a gelatinous portion of the spermatophore produced by male reproductive glands. Many mantids and orb-weaving spiders engage in sexual cannibalism, where females kill and consume males either before or after insemination (Elgar and Schneider, 2004); in both cases, the male body represents an endogenous oral gift under our definition, even when it is given involuntarily (i.e., gifts can have a negative effect on male fitness).

Since they derive from such diverse sources, endogenous oral gifts are likely to have quite varied effects on females. Some endogenous oral gifts, such as hemolymph or male body parts, may closely resemble exogenous gifts of prey or other food items in contributing to females' nutrient budgets (Boggs, 1995; Gwynne, 2008). Rather than replicating whatever nutritional mixtures are available in the diet, however, glandular gifts have the potential to provide more targeted dietary supplements. These specialized glandular gifts might supply nutrients which are otherwise absent or limited in

female diets, such as macronutrients (proteins, lipids, carbohydrates), micro-nutrients (sodium, zinc), or defensive compounds (cantharidin, pyrrolizidine alkaloids, cyanogenic glycosides). In cockroaches (Dictyoptera: Blattidae), males provide endogenous oral gifts that constitute an important nitrogen source for females and their eggs (reviewed by Boggs, 1995; Vahed, 1998). In many cockroaches, males accumulate uric acid in their accessory glands before packaging it into their spermatophore; after mating, females expel and eat the spermatophore. In other roaches, females feed directly on uric acid as it is secreted from male glands.

On the other hand, sexual conflict theory predicts the evolution of male glandular gifts that benefit males even though they may adversely affect female net fitness (Arnqvist and Nilsson, 2000; Arnqvist and Rowe, 2005; Rice, 1998). Through reciprocal sexual coevolution, an escalating arms race might then ensue in which females evolve the ability to metabolize or otherwise counteract manipulative male substances (Arnqvist and Nilsson, 2000; Eberhard, 1996). However, it has been pointed out (Gwynne, 2008) that such oral gifts might be less likely to contain manip-ulative substances because those would be subject to degradation while passing through the digestive tract. Thus, the category of endogenous oral gifts is diverse and includes nuptial gifts that may have positive, negative, or no effects on female fitness.

From the male perspective, when endogenous oral gifts (such as secreted salivary masses) can be inspected by females, they could resemble exogen-ous gifts that increase male mating success, copulation duration, and possi-bly sperm quantity transferred during copulation. For example in spiders, sexual cannibalism that takes place after insemination can benefit the sacrificed male by prolonging copulation duration, thus increasing sperm transfer and male paternity share, in addition to increasing female fecundity and offspring survival (Andrade, 1996; Elgar and Schneider, 2004; Herberstein et al., 2011; Welke and Schneider, 2012). For orally ingested glandular gifts, such as the orthopteran spermatophylax, males may be selected to incorporate phagostimulants that increase their gifts' gustatory appeal for females (Sakaluk, 2000; Vahed, 2007). Selection may also alter male gift composition to slow female consumption rates if this allows more time for males to transfer sperm. For example, in many crickets and katy-dids (Orthoptera: Ensifera), the male spermatophylax has a sticky, gelati-nous consistency that prevents rapid ingestion by females (Vahed, 2007).

3. Endogenous Genital Gifts

This category includes materials that are produced by secretory tissue in the male reproductive tract, transferred in seminal fluid or spermatophores, and absorbed through the female genital tract. Although spermatophores

may have originated to prevent sperm loss or desiccation (Davey, 1960; Khalifa, 1949), in many animals these structures have become vastly elaborated (Mann, 1984; Thornhill, 1976). Many ideas have been proposed about the evolutionary origin of elaborated male ejaculates such as spermatophores. Wickler (1985) proposed that spermatophores originated as a way for males to prevent females from digesting sperm, as an adaptation secondary to intrasexual selection for greater sperm quantity. It has also been suggested that female choice, based on the quality or quantity of nonsperm ejaculate components, might have favored the elaboration of male ejaculates (Cordero, 1996). Arnqvist and Nilsson (2000) proposed that elaborated male ejaculates represent ''manipulative and sinister superstimuli'' that evolved through sexual conflict over female remating rates. However, given the wide taxonomic distribution and diversity of endogenous genital gifts, it is unrealistic to expect a single explanation for their evolution. Rather, even a brief overview of gift constituents indicates that endogenous genital gifts have probably had multiple evolutionary origins and diverse trajectories.

Like orally ingested glandular gifts, the products of male reproductive glands can also supply nutrients that are absent or limited within female diets. The geometric framework in nutritional ecology may provide a useful perspective for thinking about the evolution of nutritive nuptial gifts. This framework is based on locating an organism's nutritional requirements and dietary choices within a multidimensional resource space (Raubenheimer, 2011; Raubenheimer et al., 2009). Importantly, rather than replicating nutritional mixtures that are available in the female diet, nuptial gifts could provide a vector that targets the female-specific requirements for vitellogenesis (Boggs, 1990). Thus, selection may shape male glandular products to augment females' resources by providing them with entirely different nutritional mixtures compared to those gained through feeding. Empirical studies of numerous Orthoptera, Lepidoptera, and Coleoptera have demonstrated that diverse substances derived from endogenous genital gifts are incorporated into female somatic tissue and eggs; these substances include amino acids, zinc, phosphorus, and sodium transferred in male spermatophores (reviewed by Boggs, 1995; Vahed, 1998). For example, many lepidopteran males engage in puddling behavior on damp soil, dung, or carrion where they obtain sodium, which is a scarce nutrient for most folivores (Molleman, 2010). Males accumulate this element in their reproductive glands and transfer sodium-rich spermatophores during mating; in the moth *Gluphisia septentrionis*, a single spermatophore contains >50% of the male's total body sodium content (Smedley and Eisner, 1996). Females pass sodium along to their eggs, and in the skipper, *Thymelicus lineola*, such gifts enhance larval survivorship (Pivnick and McNeil, 1987, but see

Molleman et al., 2004). In addition, reproductive glands can serve as a reservoir for defensive compounds that males derive from dietary sources, and these compounds are later transferred to females within spermatophores or seminal fluid (reviewed by Vahed, 1998). Thus, endogenous genital gifts can contain defensive compounds that protect the female or her eggs against predators or microbial attack; such gifts include cantharidin in *Neopyrochroa flabellata* beetles (Eisner et al., 1996), pyrrolizidine alkaloids in *Utetheisa ornatrix* moths (Eisner and Meinwald, 1995), cyanogenic glycosides in several *Heliconius* butterflies (Cardoso and Gilbert, 2007), and vicilin-derived peptides in *Callosobruchus maculatus* cowpea beetles (Alexandre et al., 2011).

On the other hand, some components of endogenous genital gifts may reduce female fitness. In some male insects, reproductive accessory glands manufacture compounds that have diverse effects on female reproductive physiology and behavior (Eberhard, 1996; Gillott, 2003). In *D. melanogaster*, for example, seminal fluid proteins have been shown to heighten female oogenesis and oviposition, increase sperm storage and utilization, and to reduce female remating rates and life span (reviewed by Chapman, 2008; Chapman and Davies, 2004; Ravi Ram and Wolfner, 2007a,b; Wolfner, 2007). For most taxa, little is known concerning the nature of these secretions, although recent work has elucidated seminal fluid composition in *Aedes* mosquitoes (Sirot et al., 2008), *Gryllus* and *Allonemobius* crickets (Andres et al., 2006; Braswell et al., 2006), *Heliconius* butterflies (Walters and Harrison, 2010), *Tribolium* flour beetles (South et al., 2011a), and honeybees (Baer et al., 2009; Collins et al., 2006). In many species, male gifts contain anti-aphrodisiacs that reduce a female's likelihood of remating (*Tenebrio* beetles, Happ, 1969; *Pieris napi* butterflies, Andersson et al., 2004; *Heliconius* butterflies, Estrada et al., 2011). Selection on males to reduce sperm competition risk favors inclusion of such substances, yet anti-aphrodisiacs can lower female net fitness if they depress remating rates below some female optimum.

Thus, endogenous genital gifts are complex mixtures that have likely been shaped by multiple selective forces. While it has been argued that male ejaculate composition will be selected primarily to manipulate female reproductive physiology and should carry a net fitness cost borne by gift recipients (Arnqvist and Nilsson, 2000; Arnqvist and Rowe, 2005), it is clear that understanding the complex effects that male ejaculates have on females will require a broad and balanced perspective.

4. Endogenous Transdermal Gifts

These nuptial gifts include male seminal and glandular products that are transferred and absorbed outside the female's digestive or reproductive systems. This happens during extragenital insemination in bedbugs (Stutt

and Siva-Jothy, 2001) and intradermal spermatophore implantation in deep-sea squid (Hoving and Laptikhovsky, 2007). In the bedbug, *Cimex lectularius*, male ejaculates include (in addition to sperm) antioxidants, micronutrients, and antibacterial compounds (Reinhardt et al., 2009). Hypodermic injection of seminal products is particularly widespread among simultaneous hermaphrodites such as leeches, sea slugs, and poly-clad flatworms (Michiels and Koene, 2006). Another type of transdermal gift consists of allohormones, substances that induce a direct physiological response in the recipient (Koene and Ter Maat, 2001). These can be injected through the skin of a mating partner during copulation while sperm are being passed to the reproductive organs. This mode of delivery allows male products to bypass both digestive and reproductive tracts, where various gift components might get broken down. During copulation, hermaphroditic earthworms *Lumbricus terrestris* use their ventral copula-tory setae to inject their partner with setal gland products that induce sperm uptake and storage (Koene et al., 2005). A similar benefit for male function occurs in *Helix aspersa* land snails, which penetrate their partners with a calcareous dart coated with allohormones produced by a mucus gland; these substances inhibit sperm digestion and enhance sperm storage by the recipient (Koene and Schulenburg, 2005; Schilthuizen, 2005). As in other endogenous gifts, selection on transdermal gift production may favor the inclusion of compounds that benefit males yet are detrimental to female fitness.

II. Effects on Recipient Fitness

Empirical studies in numerous taxa have documented how male gifts affect several different female fitness components (including egg and clutch size, rate and timing of offspring production, longevity), as well as female net fitness (lifetime fecundity measured as the total number of eggs or offspring produced). Many studies have found that nuptial gifts can provide females with direct material benefits measured as an increase in the reci-pient's net fitness. Such evidence has been compiled and summarized by previous literature reviews for arthropod nuptial gifts (Boggs, 1995; Gwynne, 2008; Rooney and Lewis, 1999; Vahed, 1998, 2007) and for sexual cannibalism (Elgar and Schneider, 2004), as well as by some meta-analyses (Arnqvist and Nilsson, 2000; South and Lewis, 2011).

Rather than recapitulating these synopses here, we simply advocate that the term nuptial gift be used in its broadest sense, that is, independently of whether such materials currently exert a positive, a negative, or no effect on recipient net fitness (Fig. 2). Others have used narrower terminology, using

Effect on female fitness

FIG. 2. Our nuptial gift definition encompasses a range of possibilities for how nuptial gifts might influence male and female net fitness (benefit minus cost). Gifts that provide a net fitness benefit to males can have negative, positive, or no effects on female fitness (top row). When gifts provide a net fitness benefit for females, they can be maintained whether or not males derive a fitness benefit; thus, sexual cannibalism would fall into the rightmost column (e.g., cases when the male is consumed before insemination would fall into the bottom right cell). The position of any gift is likely to shift over evolutionary time, as sexual interactions modify costs and benefits for each sex. (Gray areas indicate that evolutionary maintenance is unlikely as these gifts carry net fitness costs for one or both sexes.)

nuptial gifts to mean only nutritive gifts or those that increase female fitness (i.e., those falling within the upper-right cell of Fig. 2). On the other hand, because male and female reproductive interests are not perfectly aligned, sexual conflict may drive the evolution of nuptial gifts that provide fitness benefits to males while reducing female net fitness. Arnqvist and Nilsson (2000) and Arnqvist and Rowe (2005) suggested that the term ''Medea gift'' (named after a mythological Greek sorceress who used a beautifully embroidered, poisonous robe to murder a rival) should be used for any gifts that reduce female net fitness (i.e., those falling within the upper-left cell of Fig. 2). However, because coevolutionary interactions are expected to create dynamic changes over time in gifts' cost/benefit ratios for their recipients, we believe such restrictive terminology is counterproductive to the goal of understanding nuptial gift evolution. One example of this shifting balance of costs and benefits is seen in the bedbug *C. lectularius*. Although traumatic insemination through the abdominal wall causes wounding that reduces female life span (Stutt and Siva-Jothy, 2001), male ejaculates contain compounds that increase female net fitness via increased lifetime fecundity and oviposition rate, and delayed reproductive senescence (Reinhardt et al., 2009). One evolutionary scenario proposed by these authors is that male ejaculates were originally detrimental and that subsequent female counteradaptations evolved to neutralize, and eventually reverse, these harmful effects. Alternatively, they suggest, male ejaculates may have positively affected female net fitness when they originated. While distinguishing between these evolutionary trajectories must

await future phylogenetic studies coupled with ancestral trait reconstruction, it is clear that a more holistic framework will be required to understand the evolution of nuptial gifts.

Possible evolutionary trajectories leading to manipulative male gifts have been presented in detail by others (e.g., Arnqvist and Rowe, 2005; Eberhard, 1996; Rice, 1998; Sakaluk, 2000). Here we focus on circumstances that might lead to the evolution of nutritive nuptial gifts, that is, those providing material benefits that directly increase fitness for the gift recipient.

Among the ways that nuptial gifts might increase a male's fitness relative to other males in the same population is by enhancing female fecundity relative to other females in the population. The enhanced fecundity hypothesis for paternal investment was proposed by Tallamy (1994), who suggested that male investment will evolve whenever males can provide materials whose availability constrains female reproductive output. While he mainly focused on postzygotic male investment (e.g., paternal brood care), Tallamy pointed out that this hypothesis should also apply to the evolution of nuptial gifts (i.e., prezygotic male investment). In addition, recent theoretical work has shown that depending on the degree of fecundity enhancement, male nuptial gifts can alter intersexual coevolutionary dynamics and lead to a stable evolutionary equilibrium with mutual fitness benefits for both sexes (Alonzo and Pizzari, 2010).

In general, female reproduction will be resource-constrained because of the higher gametic investment by this sex (Trivers, 1972); in oviparous organisms, all the nutritional resources required for embryogenesis must be contained within each egg. Female egg production is most often limited by protein availability (Wheeler, 1996). In insects, as in most oviparous animals, oocyte development is fueled mainly by vitellogenin, a female-specific glycolipoprotein; insect eggs also contain lipids and some carbohydrates in the form of glycogen (Klowden, 2007). Females need to obtain these macronutrients from larval feeding, from adult feeding, or from male nuptial gifts (Boggs, 1990). Thus, the enhanced fecundity hypothesis predicts that selection for nuptial gifts will be influenced by the availability and quality of specific nutritional resources needed for female reproduction. Resource availability will in turn depend on organismal life-history traits, as well as on temporal and habitat variation within a particular species. Below we consider some combinations of ecological conditions and life-history traits that are expected to favor the evolution of nutritive gifts that enhance female fecundity through male contributions to female resource budgets (Boggs, 1990, 1995). We do not discuss mating systems; while several studies have explored the relationship between nuptial gifts and polyandry (e.g., Karlsson, 1995; Karlsson et al., 1997), it is difficult to determine causal relationships between these two highly correlated features.

First we discuss some life-history features that are expected to lead to female-specific resource limitation. These include location of a species along the continuum between income and capital breeding, temporal dynamics of female oogenesis, and requirements for female dispersal and flight. Income breeders are those that fuel reproduction using current energetic income, while capital breeders support their reproduction with energy stores accumulated at an earlier life stage (Houston et al., 2007; Stearns, 1992). In purely capital breeders, male nuptial gifts could provide resources to supplement reserves that otherwise would be depleted over a female's reproductive life span. One such example is *Photinus ignitus* firefly beetles (reviewed by Lewis and Cratsley, 2008; Lewis et al., 2004), which are capital breeders that entirely lack adult feeding. Both sexes mate repeatedly over their 2-week adult life span. Males manufacture a complex spermatophore from several reproductive glands, and spermatophore-derived proteins are allocated to females' developing oocytes. Females that receive multiple spermatophores gain increased lifetime fecundity. In addition, a seasonal reversal in courtship roles occurs: late in the mating season when both sexes face depleted resource stores, females compete for access to gift-providing males and males selectively mate with more fecund females (Cratsley and Lewis, 2005).

Nuptial gift evolution may also depend on interspecific life-history differences in the temporal dynamics of female oogenesis (Boggs, 1990, 1995, 2009). In some insect taxa, adult females emerge with their entire complement of eggs already matured (e.g., mayflies), while in others (e.g., *P. ignitus* fireflies) females will continue to mature eggs throughout their reproductive lives (Jervis and Ferns, 2004; Jervis et al., 2005 compiled relevant data for many parasitoid wasps). When egg maturation is distributed over time, selection should favor male nuptial gifts that could enhance female reproductive output by replenishing resources.

A final life-history trait that may alter selection for nuptial gifts relates to female mobility. If females must fly in order to locate food, mates or suitable oviposition sites, to disperse, or to escape predators, then wing-loading constraints may restrict how many mature eggs a female can carry at any point in time. In addition, females face a trade-off between allocating resources to flight or to oogenesis (Boggs, 2009; Wheeler, 1996). Lewis and Cratsley (2008) presented a conceptual model proposing that because flightless (wingless) females can devote all their resources to egg production, selection for nuptial gifts will be relaxed due to limited scope for any further increases in females fecundity. A recent evolutionary trait analysis in fireflies supported this predicted intersexual correlation between female flight ability and male nuptial gifts (South et al., 2011b; see Section V.B below). Females of ancestral fireflies most likely were fully winged and

received male nuptial gifts in the form of spermatophores. In several lineages, after females lost their flight ability (possibly driven by fecundity selection), males subsequently lost the ability to produce these nuptial gifts.

Selection for nuptial gifts should also be influenced by within-species variation in the availability and quality of specific resources required for female reproduction. When such resources are limited, females may increase their mating activity to gain access to nutritive nuptial gifts (Boggs, 1990; Gwynne, 1990). For example, in the pollen katydid *Kawanaphila nartee* (Orthoptera: Tettigonidae), scarcity of pollen (a protein-rich food source for both sexes) generates intersexual competition among females for access to endogenous oral gifts in the form of the male spermatophylax (Simmons and Bailey, 1990). In the pollen-feeding butterfly *Heliconius cydno*, pollen load varies among females and is negatively correlated with number of matings, and thus nuptial gifts, that females acquire (Boggs, 1990). In addition, many experimental studies have found that nuptial gifts provide larger fecundity increases when females are food-limited (Gwynne and Simmons, 1990; reviewed by Boggs, 1990; Gwynne, 1991, 2008). Finally, Leimar et al. (1994) provided comparative data from butterflies suggesting that variation in available resources (rather than average) will increase selection for nuptial gifts. Similarly, another comparative study across butterfly species found increased polyandry with greater variation in female body size, again indirectly suggesting that species with more variable larval food resources might experience increased selection for nuptial gifts (Karlsson, 1995).

Thus, several ecological conditions and life-history traits linked to female resource allocation are predicted to favor the evolution of fecundity-enhancing male gifts. Indeed, an entire suite of correlated life-history traits seems likely to select for fecundity-enhancing nuptial gifts. Based on the connections outlined here between nuptial gifts, life-history traits, and nutritional ecology, testing hypotheses about trait combinations that favor the evolution of nutritive nuptial gifts seems like an important and relatively unexplored research area. As Boggs (1995) pointed out nearly 20 years ago, we still need rigorous comparative phylogenetic studies focused on testing for evolutionary associations between nuptial gift presence (and type) and interspecific variation in resource conditions and life-history traits.

III. Potential Gift Benefits for Males

Considerable evidence indicates that the collection and manufacture of nuptial gifts is costly for males (reviewed by Boggs, 1995). In addition, males have been shown to strategically allocate their gifts depending on female reproductive status or age (e.g., Simmons et al., 1993; Sirot et al.,

2011; Wedell, 1992). While colloquial usage views gifts as something given voluntarily, in the case of sexual cannibalism there may also be involuntary gift-giving that carries a net fitness cost for males (Elgar and Schneider, 2004, lower-right cell of Fig. 2). Yet as mentioned above, in some species cannibalized males gain posthumous benefits through increased paternity share and decreased likelihood of the female remating (Andrade, 1996; Herberstein et al., 2011: upper-right cell of Fig. 2). In most cases, however, the male structures that produce nuptial gifts and the various behaviors associated with gift-giving will only be maintained if they confer a net fitness benefit on males; that is, the gift-giving males must be able to sire more offspring compared to other males in the same population. These fitness advantages could accrue across different reproductive episodes, including through higher mating success, increased paternity share relative to other males mating with the same female, and/or enhanced female fecundity compared to other females in the population.

In determining what specific benefits a male might derive from his gift-giving behavior, much previous work has been caught up in a largely unproductive semantic debate. For several decades many attempts were made to distinguish between two particular hypotheses for the origin and maintenance of male gifts (Alexander and Borgia, 1979; Gwynne, 1984; Sakaluk, 1986; Simmons and Parker, 1989; Vahed, 1998). The mating effort hypothesis suggested that gifts function to ensure mating and sperm transfer, while the paternal investment hypothesis suggested that gifts function to increase the number or quality of the gift-giver's own offspring. However, attempts to sort nuptial gifts neatly into these two categories were unsuccessful, and a fatal terminological quagmire gradually developed (see also Gwynne, 2008; Simmons, 1995; Simmons and Parker, 1989; Vahed, 1998; Wickler, 1985). Among the reasons for this failure was that these two hypotheses represent two nonindependent gift functions (i.e., the latter depends on the former), and also that the paternity data necessary for empirical tests of the paternal investment hypothesis were lacking.

Moving forward, we suggest that a more constructive approach will be to think about nuptial gifts as selection targets during several sequential episodes that occur *before*, *during*, and *after* mating (Fig. 3). A similar approach was suggested by Gwynne (1997; his Table 6-1). For example, nuptial gifts may enhance a male's mating success by increasing his ability to attract females (episode 1) and to successfully copulate with them (episode 2). During copulation, nuptial gifts may improve a male's insemination success (episode 3, measured as whether or not any sperm transfer occurs), or increase the number of sperm transferred (episode 4). After mating, nuptial gifts may increase the viability and storage of male sperm within the female reproductive tract (episode 5). In competitive mating situations

Episodes of selection for male gifts

1. Mate attraction

2. Copulation

3. Insemination

4. # Sperm tranferred

5. Sperm storage

6. Paternity share
(cryptic female choice,
sperm defense,
sperm offense)

7. Female fecundity

8. Egg and offspring survival

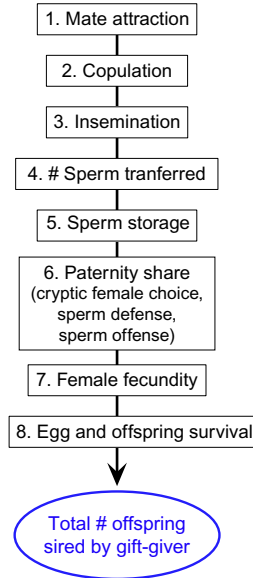Total # offspring
sired by gift-giver

Fig. 3. Potential fitness benefits gained by males from nuptial gifts across sequential selection episodes. Nuptial gifts may increase mate attraction, copulation, or insemination success, quantity of sperm transferred or stored, and paternity share in competitive mating situations (gained through cryptic female choice, protecting paternity share when the female remates, and/or when the female has previously mated). Nuptial gifts may also provide fitness benefits to males by increasing overall female fecundity, egg or offspring survival, if on average such gifts increase the production and survival of the gift-giving male's own offspring.

(polyandry or polygamy), providing a nuptial gift may increase male paternity share (proportion of offspring sired by the gift-giving male) relative to other males mating with the same female (episode 6); this may occur through cryptic female choice favoring certain male or gift traits, or by increased sperm defense or offense. As discussed in Section II, nuptial gifts may also increase a male's fitness by enhancing overall female fecundity (episode 7), as well as egg and offspring survival (episode 8), relative to that of other females in the population. Numerous experimental or observational studies of nuptial gift function have demonstrated that larger or more nuptial gifts lead to higher male fitness during one or more of these sequential selection episodes; we provide just a few examples below.

Many oral nuptial gifts (both exogenous and endogenous) provide a benefit to males by attracting females and also by increasing the likelihood that females will copulate once they have been attracted. Among such gifts are

the edible and inedible gifts offered by male empidid dance flies (Lebas and Hockham, 2005; Preston-Mafham, 1999), prey and salivary secretions provided by *Panorpa* scorpionflies (Engqvist, 2007a), and food regurgitated by *Drosophila subobscura* males (Steele, 1986). Some oral gifts, such as male hindwings and hemolymph in *Cyphoderris* hump-winged crickets (Eggert and Sakaluk, 1994), also facilitate successful insemination, as males can more readily accomplish sperm transfer when females hold still while feeding.

Oral nuptial gifts (both exogenous and endogenous) also function during mating to increase the quantity of sperm transferred to the female reproductive tract; these include the spermatophylax in several crickets and bushcrickets (Sakaluk, 1984; reviewed by Gwynne, 1997; Vahed, 1998, 2007), prey gifts in *Bittacus* hangingflies (Thornhill, 1976), and salivary secretions in *Panorpa* scorpionflies (Engqvist, 2007a).

Other nuptial gifts benefit males during postcopulatory episodes of selection, including enhancing female storage of sperm that will later fertilize eggs. Acp36DE is a seminal fluid protein (endogenous genital) transferred by *D. melanogaster* males that causes an increase in sperm numbers stored within the female reproductive tract (Qazi and Wolfner, 2003). Similarly, hermaphroditic *H. aspersa* garden snails penetrate their partner with a mucus-coated dart (endogenous transdermal gift) that increases sperm storage (Rogers and Chase, 2001).

Many endogenous gifts (both oral and genital) also include materials that inhibit the female's mating receptivity (thus reducing the risk of sperm competition) and/or increase her latency to remate (thus increasing sperm defense). Such receptivity-inhibiting materials include *Drosophila* sex peptide, unknown ejaculate components in *R. verticalis* katydids (Gwynne, 1986), nonfertilizing apyrene sperm in Lepidoptera (Wedell, 2005), and salivary secretions of *Panorpa* scorpionflies (Engqvist, 2007a). In addition, many spermatophores (endogenous genital) contain anti-aphrodisiacs that deter other males from approaching a mated female (e.g., Estrada et al., 2011; Happ, 1969).

Although we lack information on male paternity share for most gift-giving taxa, nuptial gifts can also influence what proportion of offspring produced by a multiply-mated female gets sired by the gift-giving male. For example, larger salivary secretions (endogenous oral gifts) offered by male *Panorpa* scorpionflies increase male paternity share by increasing copulation duration (Engqvist et al., 2007; Sauer et al., 1998). Also, larger spermatophores (endogenous genital gifts) increase the paternity share of male *Photinus greeni* fireflies (South and Lewis, 2012a). Many nuptial gifts (including exogenous oral, endogenous oral, and endogenous genital gifts) have been demonstrated to play a role in increasing female fecundity, either through nutritive contributions or allohormones that stimulate female

ovulation or oviposition (see Arnqvist and Nilsson, 2000; Boggs, 1995; Eberhard, 1996; Gwynne, 2008; South and Lewis, 2011). A central concern is that these gifts will provide a fitness benefit to males only if they increase the total number of offspring sired by the gift-giving male, yet the requisite information on offspring paternity is often not gathered.

As described in Section I.C.3, endogenous genital gifts can contain chemical defenses that protect a female and/or her eggs against predators, thus increasing offspring survival (e.g., cantharidin in spermatophore of male *Neopyrochroa* beetles [Eisner et al., 1996], pyrrolizidine alkaloids in *U. ornatrix* moths [reviewed by Eisner and Meinwald, 1995]).

Thus, male costs incurred in manufacturing or procuring nuptial gifts are apparently outweighed by fitness benefits that can accrue during multiple selection episodes before, during, and after mating. Endogenous nuptial gifts are especially likely to contain complex mixtures that will operate across multiple selection episodes to increase male fitness. Also, nuptial gift composition and the associated male fitness benefits will shift dynamically over time due to coevolutionary interactions between the sexes.

IV.   A Case Study of Male Benefits: *Requena verticalis*

A.   Orthopteran Nuptial Gifts

Orthopteran insects (grasshoppers, crickets, and katydids) display a dazzling array of endogenously produced nuptial gifts (Fig. 4); these include male body parts and glandular secretions that females absorb orally, genitally, or in some cases, both. Female *Pteronemobius* and *Allonemobius* (Gryllidae: Nemobiinae) ground crickets receive an endogenous oral gift by chewing on a modified hindleg spur and drinking the male's hemolymph (Fedorka and Mousseau, 2003; Mays, 1971). Similarly, female *Cyphoderris* hump-winged crickets (Tettigonioidea: Haglidae) drink hemolymph after feeding on the male's fleshy hindwings (Dodson et al., 1983; Morris, 1979). In *Oecanthus* tree crickets (Gryllidae: Oecanthinae), females consume secretions produced by dorsal glands on the male's thorax (Brown, 1997); such metanotal gland feeding also occurs in many other orthopterans (see Vahed, 1998). Many species with endogenous oral nuptial gifts also transfer a genital gift during mating in the form of a spermatophore (Gwynne, 2001; Vahed, 1998). In most species within the suborder Ensifera (katydids, crickets, and wetas), males produce spermatophores that can comprise between 2% and 40% of their total body weight. These two-part structures are produced by two distinct accessory glands: the smooth glands produce the small, sperm-containing ampulla, while the rough glands produce the
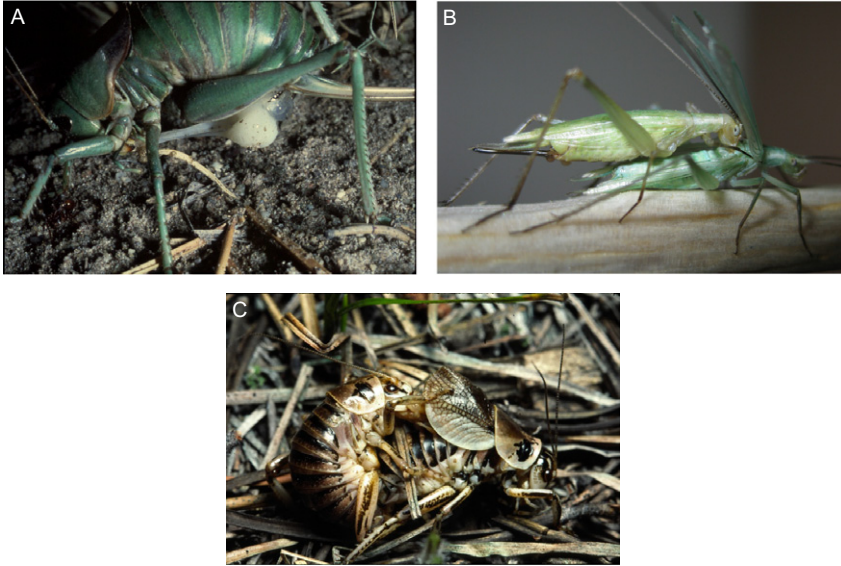
Fɪɢ. 4. Diverse endogenous oral gifts are produced by males within the insect order Orthoptera (crickets, katydids, grasshoppers and their allies): (A) After mating, a female Mormon cricket (*Anabrus simplex*) consumes the gelatinous spermatophylax portion of the male's spermatophore (photo by Darryl Gwynne). (B) A female tree cricket (*Oecanthus quadripunctatus*) feeds on the secretions from a male's metanotal gland (photo by Kevin Judge). (C) A female hump-winged cricket (*Cyphoderris*) feeds on a male's hindwings (photo by David Funk).

larger, gelatinous spermatophylax (Gwynne, 1997, 2001). During copulation, the ampulla tube is inserted into the female's genital opening, while the remainder of the gift is deposited externally. When the male departs after coupling, the female ingests the spermatophylax while sperm and associated seminal fluid drain from the ampulla into the female's reproductive system. Once the female finishes consuming the spermatophylax, she removes and consumes whatever remains of the ampulla (Gwynne, 1984; Gwynne et al., 1984; Sakaluk, 1984).

The Australian katydid *R. verticalis* (family Tettigoniidae) has been extensively studied as a model system for understanding the costs and benefits of nuptial gifts for both sexes. Thus, this species provides an excellent case study for illustrating the episodes of selection framework presented in Section III. Like other tettigonids, *R. verticalis* males produce a two-part spermatophore that is approximately 15–20% of total male body weight (Davies and Dadour, 1989). The spermatophylax

alone (without the sperm-containing ampulla) comprises 78% of total spermatophore weight (Bowen et al., 1984). The spermatophylax is composed of 13.5% protein (Bowen et al., 1984). In this species, both sexes mate multiple times and both courtship and mating are costly to males. The chirping acoustic signals that males use to attract females require an energetic investment that averages 3.2 kJ/h (Bailey et al., 1993) and spermatophore production requires 1.1 kJ (Simmons et al., 1992); together these two components make up approximately 70% of a male's daily energy budget (Simmons et al., 1992). Nuptial gift costs also limit male mating frequency. After mating, males require 2.5–5 days (depending on diet quality) to manufacture another spermatophore before they are able to mate again (Davies and Dadour, 1989; Gwynne, 1990). Furthermore, when male diet is restricted, males invest less energy into courtship signals but nuptial gift production remains constant (Simmons et al., 1992).

What fitness benefits might balance out these well-established costs of nuptial gifts for *R. verticalis* males? Below we expand on Gwynne's (1997) analysis of current gift function to examine the fitness benefits that males derive from spermatophylax production across multiple selection episodes that occur before, during, and after mating.

B. FITNESS BENEFITS TO *R. VERTICALIS* MALES

1. *Male Insemination Success and Number of Sperm Transferred*

When *R. verticalis* females are deprived of a spermatophylax they will remove and eat the ampulla, effectively halting sperm transfer (Gwynne et al., 1984). Gwynne et al. (1984) demonstrated that while sperm drainage from the ampulla is completed within 3 h, females take ∼5 h to eat the spermatophylax before moving on to consume the ampulla. In some other orthopteran species, in contrast, male spermatophylax size attains only the minimum necessary to ensure complete sperm drainage (e.g., the cricket *Gryllodes supplicans*; Sakaluk, 1984). Thus, the *R. verticalis* spermatophylax serves to protect male ejaculates by insuring insemination and maximizing the number of sperm transferred (Fig. 3, selection episodes 3 and 4).

2. *Paternity Share*

Male nuptial gifts in *R. verticalis* also affect male paternity share postmating (Fig. 3, episode 6). Laboratory studies indicate that *R. verticalis* generally show complete first-male sperm precedence; that is, the first male that mates with a virgin female will sire all of her offspring even when the female remates (Gwynne, 1988a; Simmons and Achmann, 2000). When females were given a longer intermating interval and allowed to oviposit between

matings, second males gained ∼20% paternity share (Gwynne and Snedden, 1995). In addition, first males that had greater spermatophore mass retained higher paternity share. Radiolabeling studies show that amino acids derived from second males become incorporated into eggs that were fertilized by the first male; thus, second male gifts are allocated to offspring sired by another male. However, such cuckoldry may happen infrequently under natural conditions, as field estimates of female polyandry suggest that females in nature remate less frequently than in the lab (Simmons et al., 2007).

The high degree of first-male paternity seen in *R. verticalis* suggests that males should be selected to preferentially mate with virgin females, but males appear incapable of discriminating females' mating status (Lynam et al., 1992; Simmons et al., 1993, 1994). This may represent sexual conflict, with selection acting on females to hide their mating status to obtain the benefits provided by additional spermatophores (Simmons et al., 1994). However, Simmons et al. (1994) did find that males are able to discriminate among potential mates based on female age. By preferentially mating with younger females, males may increase their chance of mating with virgins and may thus gain higher paternity share.

Sperm competition theory predicts that males should strategically allocate their ejaculates depending on female mating status (Simmons, 2001); when mating with a previously mated female, males should maximize sperm number in the ampulla (to increase their sperm offense ability), but minimize spermatophylax investment due to their the low probability of siring offspring. Instead, Simmons et al. (1993) found that when mating with young females, *R. verticalis* males transfer identical spermatophores regardless of female mating status; however, males transfer spermatophores with 50% more sperm and 25% less spermatophylax material when mating with older compared to younger females. Thus, *R. verticalis* males appear to strategically allocate their ejaculates when mating with older females to increase their sperm offense ability, and thus their potential paternity share.

Nuptial gifts produced by *R. verticalis* also affect male postcopulatory fitness by increasing sperm defense. Given the high cost of producing nuptial gifts, males should be selected to increase female latency to remate as a mechanism of reducing sperm competition. By experimentally manipulating ampulla attachment times, Gwynne (1986) demonstrated that the ampulla contains receptivity-reducing substances that act in a dose-dependent fashion, normally rendering females non-receptive for approximately 4 days. Substances in the ampulla also appear to negatively affect female longevity. Wedell et al. (2008) found that when females received the contents of three male ampullas (each without a spermatophylax), they had significantly shorter life spans, and this negative effect was not counteracted by spermatophylax consumption.

3. Female Fecundity and Egg/Offspring Survival

Finally, nuptial gifts can also increase male fitness through effects on female fecundity and the survival of offspring sired by the gift-giving male (Fig. 3, selection episodes 7 and 8). Radiolabeling experiments demonstrated that male protein derived from the *R. verticalis* spermatophore is incorporated into the female's eggs (Bowen et al., 1984; Gwynne, 1988a). Furthermore, females that consume more spermatophylaces produce more and heavier eggs (Gwynne, 1984), and offspring from larger eggs had greater overwintering survival (Gwynne, 1988b). If receiving spermatophylax nutrients directly benefits female fitness, nutrient-limited females would be expected to seek out matings to obtain additional nuptial gifts. Indeed, female *R. verticalis* females kept on a low-quality diet remate more often than females kept on a high-quality diet (Gwynne, 1990).

By applying this framework in *R. verticalis*, we see that nuptial gifts increase male fitness across several episodes of selection, ultimately increasing the number of offspring sired by the gift-giving male. A complete spermatophore (ampulla + spermatophylax) is necessary for insemination to occur (i.e., ejaculate protection), as otherwise the ampulla will be removed and eaten before sperm transfer. Presence of a spermatophylax increases the duration of ampulla attachment, and spermatophylax size exceeds that required for complete sperm transfer. Unidentified substances present in the male ampulla act to reduce female receptivity to additional matings, helping to ensure a male's paternity share relative to his rivals. Additionally, males discriminate against older females that have likely already mated as a mechanism to reduce incidence of cuckoldry. Spermatophylax consumption increases female fecundity and has the potential to enhance fitness by increasing the number of offspring sired by the gift-giving male. Paternity success of second mating males increases if they mate with a female after she has had an opportunity to oviposit. Finally, spermatophylax consumption increases egg size, which enhances survival of a male's offspring. Thus, this work on *R. verticalis* clearly illustrates how costly nuptial gifts might provide males with demonstrable fitness benefits measured across several sequential episodes of selection.

V. PHYLOGENETIC INSIGHTS INTO THE EVOLUTION OF NUPTIAL GIFTS

Despite the key role that nuptial gifts play in the reproductive ecology of so many animals, surprisingly few studies have rigorously examined the evolution of nuptial gifts using a comparative phylogenetic approach. To

thoroughly test the various evolutionary scenarios that have been proposed for nuptial gifts, it will be essential to map gifts and other relevant traits onto robust phylogenies developed for particular taxa. Using this approach will provide insight into the evolutionary sequence of gift trait transitions and will also allow tests of correlated evolution between nuptial gifts, life-history, and ecological traits. To date, however relatively few studies have applied these methods. Here we review work from three insect taxa where a comparative phylogenetic approach has provided insight into nuptial gift evolution: 1) endogenous oral gifts within katydids and crickets (Ensifera: Orthoptera), 2) correlated evolution of wingless females and male nuptial gifts in fireflies (Lampyridae: Coleoptera), and 3) patterns of male ejaculate incorporation, as well as rates of seminal fluid protein evolution, in the genus *Drosophila* (Drosophilidae: Diptera).

A. ENDOGENOUS ORAL GIFTS IN KATYDIDS AND CRICKETS

The first comparative phylogenetic study of nuptial gift evolution was presented by Gwynne (1995, 1997, 2001), who examined the origins and elaboration of edible glandular gifts within the orthopteran suborder Ensifera (katydids, crickets and their allies). Gwynne's (1995) phylogenetic reconstruction was based upon morphological characters and suggested that the ancestral trait in this group was an exposed spermatophore (essentially a naked sperm-containing ampulla) that was deposited externally on the female genitalia (Fig. 5). Female consumption of this unprotected spermatophore was hypothesized to be ancestral for all ensiferans, followed in the superfamily Tettigonioidea by the origin of the spermatophylax as an edible addition to the spermatophore. In the evolutionary branch leading to the family Gryllidae (true crickets), there were numerous origins of diverse glandular gifts consumed by females before and after mating, along with male spermatophylaces.

A more detailed analysis (Gwynne, 1995, 1997) showed a total of 11 origins of males producing endogenous oral gifts within the Ensifera; these included 3 origins of a spermatophylax, 4 origins of metanotal glands, 1 tibial gland, and 3 others instances where females feed on other male body parts. This work also indicated several independent spermatophylax losses or size reductions; these occurred in some wetas (Stenopelmatidae; loss in *Deinacrida*, size reduction in *Hemideina*) and katydids (Tettigoniidae; loss in *Tympanophora*, *Decticita*, size reduction in *Neoconocephalus*). Interestingly, such losses were often associated with origins of other endogenous gifts, such as secretions from metanotal or tibial glands. Within the Grylloidea, Gwynne's analysis reveals that there were also seven likely losses of nuptial gifts and three origins of postcopulatory mate guarding.
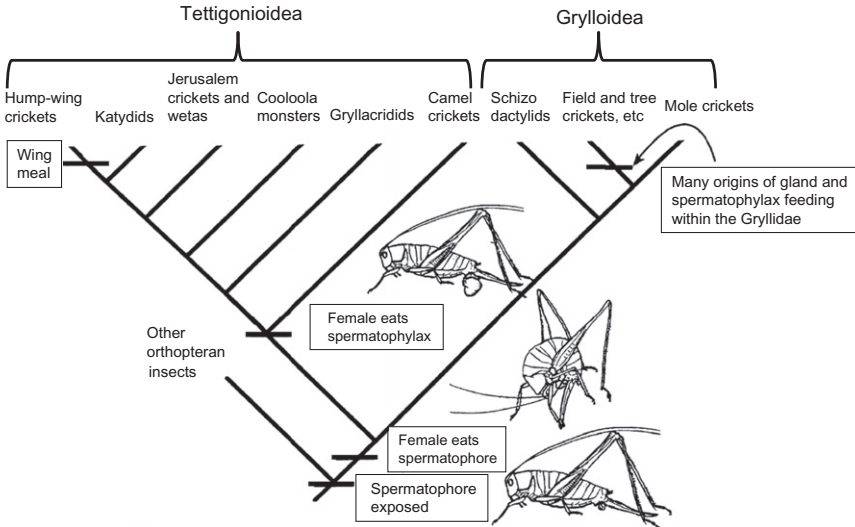
FIG. 5. Proposed evolution of spermatophylax nuptial gifts within the orthopteran suborder Ensifera (two superfamilies indicated), with gift-related traits mapped onto the most parisimonious tree based on morphological characters (figure modified from Gwynne, 2001).

This analysis supports an evolutionary scenario in which males first used a simple, externally attached ampulla to transfer their sperm (for review see Gwynne, 2001). Food limitation may have initially driven females to consume this proteinaceous package, leading to sexual conflict over ampulla attachment times. Selection on males to maximize sperm transfer could have lead to the origin of male reproductive glands that produced an additional spermatophore component, the edible spermatophylax. Thus, the spermatophylax likely originated as an ejaculate protection mechanism, prolonging ampulla attachment times and allowing sufficient time for sperm to fully drain from the ampulla into the female reproductive tract. Further elaboration of the spermatophylax might have occurred if male gifts increased the number of offspring sired by increasing female fecundity and/or offspring survival. As females increased their mating rates to obtain nutritional supplements from these oral gifts, male ejaculates (genital gifts) would have undergone selection to include compounds that suppress female receptivity to further matings, thus reducing sperm competition risk.

While this analysis provides considerable insight into the evolution of orthopteran nuptial gifts, additional work could increase taxon coverage and incorporate more detailed information on species' life-history and ecological traits. Further studies could also help elucidate what conditions

led to the spermatophylax loss seen across several ensiferan lineages, and what factors underlie the explosion of nuptial gift diversity seen among modern day Orthoptera.

B. Firefly Spermatophores: Coevolution with Female Flight

Recent work on fireflies (Coleoptera: Lampyridae) also shows the power of a comparative phylogenetic approach and offers new insights into how nuptial gift evolution is linked to other life-history traits (South et al., 2011b). This analysis also allowed reconstruction of ancestral character states as well as the sequence of evolutionary transitions, and demonstrated trait coevolution between the sexes.

Based upon Tallamy's (1994) enhanced fecundity hypothesis and Boggs' (1990) female allocation model, Lewis and Cratsley (2008) developed a conceptual model to explore the evolution of nuptial gifts in lampyrids as a function of female allocation trade-offs between flight and reproduction. Because nuptial gifts can link together male and female resource budgets, they have the potential to alter the allocation strategies used by both sexes. Thus, selection for nuptial gifts might depend on female reproductive allocation, which in turn depends on allocation to other activities, including flight. If females do not require flight, fecundity selection can act to maximize female reproductive allocation. In this case, because female reproductive output is already at its maximum (E_{max} in Boggs, 1990), male nuptial gifts will have limited scope to further enhance female fecundity. On the other hand, when female reproductive allocation is constrained by the energetic and biomechanical demands of flight, nuptial gifts could provide larger proportional fecundity increases for females. Therefore, this model predicts that nuptial gifts would not be selected in species with flightless females. Fireflies present an opportunity to test this relationship, as they demonstrate variation in not only nuptial gift-giving, but also in female flight ability.

As fireflies are capital breeders and both sexes mate multiply, nuptial gifts can have major fitness consequences for both sexes (Lewis and Cratsley, 2008). Firefly nuptial gifts consist of spermatophores (endogenous genital gifts) that are manufactured by several accessory glands and transferred to females during mating (Lewis et al., 2004). Some female fireflies possess a specialized reproductive sac to receive and break down the spermatophore after sperm are released into the female spermatheca (van der Reijden et al., 1997). Radiolabeling experiments in *Photinus* fireflies have shown that spermatophore-derived proteins are incorporated into the female's developing oocytes (Rooney and Lewis, 1999), and male gifts benefit females by increasing their lifetime fecundity

(Rooney and Lewis, 2002) and longevity (South and Lewis, 2012b). Gift production is costly for males, as spermatophore size declines across successive matings in *Photinus* (Cratsley et al., 2003). Among the 2000 extant species of firefly worldwide, spermatophores are present in some, yet absent in others (Hayashi and Suzuki, 2003; Lewis et al., 2004; South et al., 2008, 2011b; van der Reijden et al., 1997). Those species that lack spermatophores show reduced male accessory glands and females do not have a spermatophore-receiving sac (Demary and Lewis, 2007; South et al., 2011b). What accounts for such interspecific variation in nuptial gifts?

Fireflies also exhibit extensive interspecific variation in life-history traits. In some fireflies, females have greatly reduced wings and as a result are flightless, while in other species both sexes have normal wings and can fly (Jeng, 2008). Hayashi and Suzuki (2003) first proposed that female wing reduction might be negatively associated with male nuptial gifts in Japanese fireflies. Thus, the existing variation in both spermatophore production and female flight within the Lampyridae provided an opportunity to test Lewis and Cratsley's (2008) model and to examine whether this life-history trait could help explain how nuptial gifts are distributed across fireflies.

South et al. (2011b) performed a phylogenetic analysis of the relationship between spermatophore production and female flightlessness within the Lampyridae (Fig. 6). These two traits were measured in 32 taxa and mapped onto a lampyrid molecular phylogeny constructed by Stanger-Hall et al. (2007). Ancestral state reconstruction revealed it was highly likely that firefly males originally produced spermatophores, but these nuptial gifts were subsequently lost in four separate lineages (Fig. 6, right). This reconstruction also revealed that ancestral fireflies had flight-capable females, and females then lost their flight ability at least five times (Fig. 6, left). Furthermore, this work revealed a remarkably congruent pattern between male nuptial gifts and female flight, with the correlated loss of both female flight and male gifts occurring in many lineages. This congruence (statistically confirmed by Pagel's test of correlated evolution) demonstrated coevolution between two traits expressed in different sexes. Finally, transitional probability analysis demonstrated that first females lost their flight ability, subsequently followed by male spermatophore loss.

Thus, female flight ability provides a compelling explanation for observed patterns of nuptial gifts in fireflies, but what selected for female flight loss in the first place? Based upon considerable evidence demonstrating that flightless females can allocate more to reproduction, the most likely explanation for female-specific flight loss is selection for increased fecundity. Thus, these results strongly support the conclusion that male nuptial gifts are co-adapted with patterns of female reproductive allocation, at least

Female flight

○ No
● Yes

Male nuptial gifts

○ No
● Yes

Photuris lucicrescens
Pleotomus pallens
Lampyris noctiluca
Pyrocoelia discicollis
Pyrocoelia fumosa
Pyrocoelia m. matsumurai
Diaphanes formosus
Pyrocoelia rufa
Pyrocoelia miyako
Pyrocoelia atripennis
Microphotus angustus
Ellychnia corrusca
Photinus pyralis
Pyropyga nigricans
Pyractomena angulata
Lucidota atra
Lucidina biplagiata
Lamprohiza splendidula
Phosphaenus hemipterus
Phausis reticulata
Cyphonocerus ruficollis
Stenocladius shiraki
Rhagopthalmus ohbai
Hotaria parvula
Luciola yayeyamana
Luciola cruciata
Luciola lateralis
Luciola kuroiwae
Curtos okinawana
Curtos costipennis
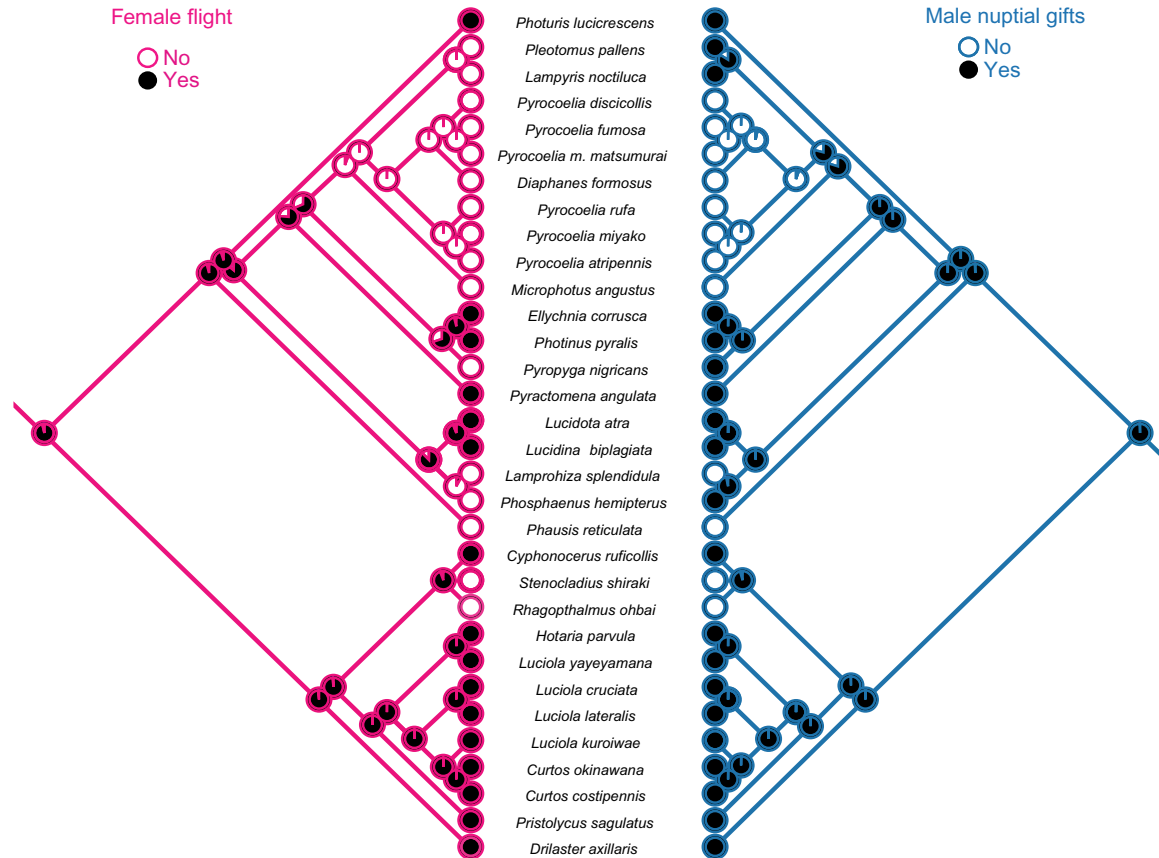*Pristolycus sagulatus*
Drilaster axillaris

FIG. 6. Firefly beetles (Coleoptera: Lampyridae) show correlated evolution (Pagel's test, $P < 0.001$) between male nuptial gifts (spermatophores) and female flight ability (based on presence of functional wings). For 32 worldwide firefly species, these two traits were mapped onto a lampyrid molecular phylogeny based on 18S, 16S, and *cox1* DNA sequences (Stanger-Hall et al., 2007). For each trait, pie charts at each node indicate the proportional likelihood support for ancestral states (figure modified from South et al., 2011b).

in fireflies. These results could be broadly applicable to other capital bree-
ders and could help explain patterns of nuptial gift evolution in other taxa.
Further studies are needed to see whether variation in other ecological and
life-history traits associated with resource allocation can provide additional
insights into nuptial gift evolution.

C. FEMALE INCORPORATION OF EJACULATE-DERIVED PROTEINS IN *DROSOPHILA* FRUITFLIES

In our taxonomy of nuptial gifts, the category of endogenous genital gifts
explicitly includes seminal products that are transferred in a liquid ejacu-
late; this occurs in many Diptera, including *Drosophila* fruitflies. *Droso-
phila* species vary widely in several aspects of their mating systems,
including female remating latency, male ejaculate composition, mating
behavior, and the degree to which substances from male ejaculates are
incorporated into female tissue (Markow, 2002; Markow and Ankney,
1984; Markow and O'Grady, 2005; Pitnick et al., 1997). Studies mapping
reproductive traits onto a *Drosophila* phylogeny provide insight into the
evolutionary history of these unpackaged nuptial gifts.

For 34 species of *Drosophila*, Pitnick et al. (1997) used radiolabeled
amino acids to determine how much protein transferred in male ejaculates
was incorporated into female ovarian or somatic tissue. *Drosophila* species
showed dramatic variation in the degree to which females incorporated
male-derived proteins (Fig. 7). Females in most species, including those in
the *melanogaster* group, showed no incorporation into their ovarian tissue
and about half showed no incorporation into somatic tissue. However,
Drosophila species within the *subpalustris* group showed substantial incor-
poration into somatic tissue, and those within the *mojavensis* cluster
showed substantial incorporation of male-derived protein into both somatic
tissue and oocytes.

By mapping these data onto a molecular phylogeny, Pitnick et al. (1997)
showed that incorporation of male-derived protein into female somatic
tissue has independently evolved multiple times (Fig. 7). In the *mojavensis*
cluster, high levels of ovarian incorporation were also seen to accompany
high incorporation into somatic tissue. This phylogeny also reveals some
degree of lability, as incorporation into both tissue types seems to have
been subject to both gains and losses. Thus, this work provides evidence
that multiple *Drosophila* groups have evolved male ejaculates that contri-
bute to female somatic maintenance or reproduction.

As pointed out by Pitnick et al. (1997), these patterns of female ejacu-
late incorporation might be related to differences among species in their
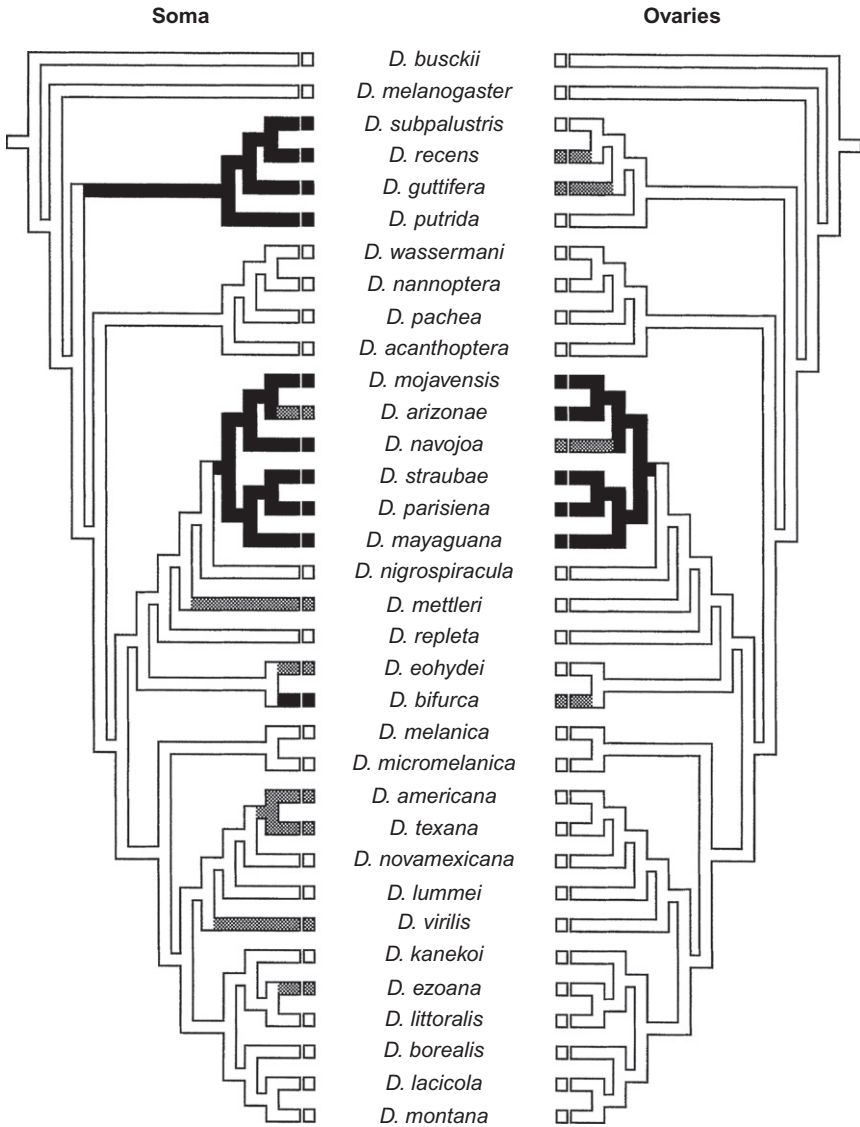nutritional ecology, as the host resources exploited by *Drosophila* vary

Soma **Ovaries**

- *D. busckii*
- *D. melanogaster*
- *D. subpalustris*
- *D. recens*
- *D. guttifera*
- *D. putrida*
- *D. wassermani*
- *D. nannoptera*
- *D. pachea*
- *D. acanthoptera*
- *D. mojavensis*
- *D. arizonae*
- *D. navojoa*
- *D. straubae*
- *D. parisiena*
- *D. mayaguana*
- *D. nigrospiracula*
- *D. mettleri*
- *D. repleta*
- *D. eohydei*
- *D. bifurca*
- *D. melanica*
- *D. micromelanica*
- *D. americana*
- *D. texana*
- *D. novamexicana*
- *D. lummei*
- *D. virilis*
- *D. kanekoi*
- *D. ezoana*
- *D. littoralis*
- *D. borealis*
- *D. lacicola*
- *D. montana*

FIG. 7. Phylogenetic distribution of nutritive male ejaculates in 34 species of *Drosophila* fruitflies (Diptera: Drosophilidae). Shading indicates the degree to which females have incorporated ^{14}C-labeled proteins derived from male ejaculates into their somatic tissue (shown on left) and ovaries (shown on right) 6–8 h after mating. White bars indicate no female incorporation (0–50 corrected DPM), gray bars indicate a small degree of female incorporation (51–100 DPM), and black bars indicate substantial incorporation of male-derived protein by females (>100). Figure from Pitnick et al. (1997).

widely in quality (Markow and O'Grady, 2008). Species in the *mojavensis* group (*D. mojavensis*, *D. navojoa*, *D. straubae*, *D. parisiena*, *D. mayaguana*, and *D. arizonae*) all breed and feed on necrotic cactus (see Markow and O'Grady, 2005, 2008), which contains lower levels of both nitrogen and phosphorus compared to the fruit hosts used by the *melanogaster* group (Markow et al., 1999). In addition, by manipulating nutritional content of the cactus host, Brazner et al. (1984) showed that *D. mojavensis* are likely to undergo frequent nutritional stress. Markow et al. (1990) showed that *D. mojavensis* females kept on low-quality diets experienced enhanced fecundity from the receipt of male ejaculate, and suggested that nutritive ejaculates are more likely to evolve when adults are subject to nutrient limitation. Consistent with the enhanced fecundity hypothesis (Tallamy, 1994), females in some cactophilic, and presumably nutrient-limited, *Drosophila* species show a high degree of male ejaculate incorporation (Pitnick et al., 1997). In addition, Markow et al. (2001) found that in *Drosophila nigrospiracula*, a cactophilic species subject to larval phosphorus limitation, mated females incorporate phosphorus derived from male ejaculates into oocytes. Therefore, variation in resource availability among *Drosophila* species may be one factor in the evolution of nutritive male ejaculates that contribute to female somatic maintenance and reproduction.

The presence of nutritive male ejaculates also shows strong phylogenetic correlations with other features of *Drosophila* mating systems (Markow, 2002). Across 21 *Drosophila* species, Markow (2002) found strong congruence between female mating frequency and exaggerated male ejaculates (these include nutritive male ejaculates). Additionally, Markow (2002) suggested that the evolution of nutritive male ejaculates may have been preceded by higher female remating rates. However, because so many reproductive traits covary with mating systems, additional work is needed to test the sequence of these evolutionary transitions. The extensive knowledge base for *Drosophila* concerning host use, life histories, and reproductive traits makes this a compelling system for examining specific factors that promote the evolution of endogenous genital gifts.

In summary, in this section we present previous work that has taken a comparative phylogenetic approach to describe and test hypotheses about nuptial gift evolution. To rigorously test the various evolutionary scenarios that have been proposed for nuptial gifts, it will be essential to map gifts and other relevant traits onto robust phylogenies developed for particular taxa. We emphasize how valuable it is to include life-history and ecological traits in such evolutionary analyses. This approach should provide insight into the evolutionary sequence of gift transitions and will also allow formal tests of correlated evolution between male gifts and other traits that can influence their evolutionary trajectory.

D. Evolutionary Rates of *Drosophila* Seminal Proteins

Drosophila male ejaculates are certainly the most well-characterized of all endogenous nuptial gifts. Their nonsperm components comprise a complex cocktail of molecules produced by male accessory glands and secretory tissues in the male ejaculatory duct. While many different types of molecules are transferred within *Drosophila* male ejaculates, research has focused on seminal fluid proteins (SFPs). Once transferred, these SFPs engage in dynamic molecular interactions within the female reproductive tract, and this sexual interplay is likely to influence SFP evolution. Because these molecules have been so well-studied, research on SFP evolutionary rates can contribute to a broader understanding of nuptial gift evolution.

Nearly 150 different SFPs have been identified from the ejaculate of *D. melanogaster* males, and these proteins initiate many physiological and behavioral changes within mated females (reviewed by Avila et al., 2011). Significant changes in female gene expression are seen 1–3 h following the receipt of ejaculate and are maximized at 6 h postmating. Conformational changes of the female reproductive tract allow for sperm storage, and the oviduct shows increased innervation and enhanced formation of myofibrils (Adams and Wolfner, 2007; Kapelnikov et al., 2008). Specific SFPs are necessary for female sperm storage and release, while others improve sperm survival (Ravi Ram and Wolfner, 2007a; Xue and Noll, 2000). Male SFPs increase female egg production and ovulation (Heifetz et al., 2000; Ravi Ram and Wolfner, 2007a,b), initiate the formation of a mating plug (a gelatinous mass containing sperm; Bretman et al., 2010; Lung and Wolfner, 2001), and cause females to actively reject courting males. Female activity levels also increase following mating, with increased foraging (Carvalho et al., 2006) and 70% less sleep (Isaac et al., 2010), possibly leading to shorter life spans for mated females (Isaac et al., 2010; Wigby and Chapman, 2005).

Notably, rapid evolution of genes encoding male SFPs has been documented in *Drosophila* as well as in other taxa (Clark et al., 2006; Swanson and Vacquier, 2002; Vacquier, 1998). Comparisons between *D. melanogaster* and *D. simulans* demonstrated high rates of nonsynonymous nucleotide substitution in SFP genes compared to non-SFP genes (Swanson et al., 2001). Sequence comparisons between *D. melanogaster* and *D. pseudoobscura* of 52 SFP-encoding genes from male reproductive accessory glands detected only 58% conserved as true orthologs (Mueller et al., 2005). Such rapid and dynamic evolution of SFPs is likely due to postcopulatory sexual selection (Clark et al., 2006; Panhuis et al., 2006; Swanson and Vacquier, 2002). Sperm competition (Birkhead and Moller, 1998), cryptic female choice (Eberhard, 1996), and sexual conflict (Parker, 1979) may all contribute to a coevolutionary arms race between and within sexes over control of reproductive outcomes.

Comparisons between *Drosophila* species can be used to test the prediction that SFP evolution will proceed more rapidly when postcopulatory sexual selection is more intense. Mating systems and reproductive ecology differ dramatically between species in the *repleta* group and those in the *melanogaster* group. *D. repleta* males transfer a nutritive ejaculate, and females remate more frequently (Markow, 2002; Markow and Ankney, 1984; Pitnick et al., 1997). In addition, many *repleta* species show an insemination reaction, consisting of an opaque mass that develops within the female vagina after mating. This is thought to prevent females from remating, thus protecting the male's nutritional investment from cuckholdry by rival males (Markow and Ankney, 1984, 1988). Based on these differences in reproductive ecology, species in the *D. repleta* group appear subject to more intense postcopulatory sexual selection and thus are predicted to show faster rates of SFP evolution compared to *D. melanogaster*. Supporting this prediction, several studies have shown that SFP genes expressed by male accessory glands in the *repleta* group evolve more rapidly than those in the *D. melanogaster* group (Almeida and DeSalle, 2009; Wagstaff and Begun, 2005, 2007). In the *repleta* group, SFP genes also show high rates of gene duplication, which is suggested to facilitate adaptive protein evolution (Ohno, 1970; Walsh, 2003). Thus, *repleta* SFPs appear to be undergoing rapid evolution, potentially due to differences in their reproductive ecology.

Consistent with the prediction that sexual coevolution is responsible for rapid evolutionary changes in male gifts, some female reproductive proteins in the *repleta* group also show rapid adaptive evolution, and gene duplication has also been important in the evolution of these proteins (Kelleher et al., 2007). Of particular interest are several digestive proteases, which Kelleher et al. (2007) suggest might play a role in breaking down the mating-induced insemination reaction. Interestingly, male ejaculates in *D. mojavensis* contain protease inhibitors (Wagstaff and Begun, 2005), two of which have also experienced lineage-specific gene duplication events (Kelleher et al., 2009). The reproductive tract of female *D. arizonae* (a close sister species to *D. mojavensis*) shows exceptionally high proteolytic activity that is negatively regulated by mating (Kelleher and Pennington, 2009). Taken together, these results from different *repleta* species suggest active sexually antagonistic coevolution around the insemination reaction, with male protease inhibitors acting to prevent male ejaculate components getting broken down by female proteases.

Thus, rapid evolution of *Drosophila* nuptial gifts appears to be driven by a complex sexual interplay taking place at the molecular level. While some male-derived proteins are incorporated into female oocytes and somatic tissue, other SFPs may have evolved to counter defenses mounted by

females to prevent male manipulation. Further exploration of these dynamic sexual interactions should provide many insights into the constantly shifting balance between the costs and benefits of nuptial gifts.

VI. Conclusions and Future Directions

Animal nuptial gifts take multitudinous forms, and their evolutionary stories promise to be just as diverse. In this overview, we have tried to offer a fresh perspective on the evolution of animal nuptial gifts. We argue for a broader definition of nuptial gifts that can accommodate anticipated lability of nuptial gift structure and function arising from coevolutionary interactions both between and within the sexes. By systematically classifying nuptial gifts according to how they are produced (endogenous vs. exogenous) and how they are absorbed by the recipient (oral, genital, or transdermal), we hope to establish a robust framework for testing predictions about how gifts influence both male and female fitness. Rather than attempting to place potential benefits gained by gift-giving males into the falsely dichotomous categories of parental investment versus mating effort, we illustrate how nuptial gifts might enhance male fitness across multiple selection episodes that occur before, during, and after mating. Finally, we highlight some studies that have greatly advanced our understanding by using comparative phylogenetic methods to examine how nuptial gifts and associated life-history traits have changed over evolutionary time.

We hope this foundation will inspire future research efforts to enhance our understanding of nuptial gift evolution. Despite many advances, there remain several areas that clearly call out for more focused research efforts:

- We have detailed morphological descriptions of the glands which are responsible for manufacturing many endogenous gifts (e.g., Leopold, 1976; Liu and Hua, 2010). In many taxa, nuptial gifts are the combined productions of multiple glands, yet much work remains to fully characterize these glandular products. Transcriptome studies of gene expression within gift-manufacturing glands will provide insight into differences and similarities in their gene products and associated functions. For example, to what extent has convergent evolution occurred between those male reproductive glands that produce oral versus genital gifts, or between reproductive and salivary glands?

- In considering selection for nutritive nuptial gifts, the geometric framework developed for nutritional ecology (Raubenheimer, 2011; Raubenheimer et al., 2009) provides a powerful tool for testing whether male gifts evolved to support female reproduction. Does

selection shape male glandular products to provide novel nutritional mixtures that will supplement females' dietary resources, that is, do such gifts act as vectors that specifically target the requirements of vitellogenesis? We need more detailed biochemical analyses of different types of nuptial gifts to test many of the predictions laid out here.

- Most importantly, there is a compelling need for additional phylogenetic analyses of nuptial gift traits that can provide insight into the evolutionary origin and maintenance of nuptial gifts across different taxonomic groups. Continuing to examine evolutionary patterns within the Orthoptera will be especially interesting, because their nuptial gift types are so variable. Phylogenetic analysis would also be worthwhile in the Lepidoptera, where reconstructing ancestral character states could shed light on possible trajectories of spermatophore evolution. Finally, because nuptial gifts lie at the intersection of nutritional ecology, sexual selection, and life-history evolution, testing informed predictions concerning evolutionary associations between nuptial gifts and relevant ecological and life-history traits is of fundamental importance.

References

Adams, E.M., Wolfner, M.F., 2007. Seminal proteins but not sperm induce morphological changes in the *Drosophila melanogaster* female reproductive tract during sperm storage. J. Insect Physiol. 53, 319–331.

Alexander, R.D., Borgia, G., 1979. On the origin and basis of the male-female phenomenon. In: Blum, M.S., Blum, N.A. (Eds.), Sexual Selection and Reproductive Competition in the Insects. Academic Press, New York, pp. 417–440.

Alexandre, D., Linhares, R.T., Queiroz, B., Fontoura, L., Uchoa, A.F., Samuels, R.I., et al., 2011. Vicilin-derived peptides are transferred from males to females as seminal nuptial gift in the seed-feeding beetle *Callosobruchus maculatus*. J. Insect Physiol. 57, 801–808.

Almeida, F.C., DeSalle, R., 2009. Orthology, function and evolution of accessory gland proteins in the *Drosophila repleta* group. Genetics 181, 235–245.

Alonzo, S.H., Pizzari, T., 2010. Male fecundity stimulation: conflict and cooperation within and between the sexes. Am. Nat. 175, 174–185.

Andersson, M., 1994. Sexual Selection. Princeton University Press, New Jersey.

Andersson, J., Borg-Karlson, A., Wiklund, C., 2004. Sexual conflict and anti-aphrodisiac titre in a polyandrous butterfly: male ejaculate tailoring and absence of female control. Proc. Biol. Sci. 271, 1765–1770.

Andrade, M.C.B., 1996. Sexual selection for male sacrifice in the Australian redback spider. Science 271, 70–72.

Andres, J.A., Maroja, L.S., Bogdanowicz, S.M., Swanson, W.J., Harrison, R.G., 2006. Molecular evolution of seminal proteins in field crickets. Mol. Biol. Evol. 23, 1574–1584.

Andres, J.A., Maroja, L.S., Harrison, R.G., 2008. Searching for candidate speciation genes using a proteomic approach: seminal proteins in field crickets. Proc. R. Soc. Lond. B 275, 1975–1983.

Arnqvist, G., Nilsson, T., 2000. The evolution of polyandry: multiple mating and female fitness in insects. Anim. Behav. 60, 145–164.

Arnqvist, G., Rowe, L., 2005. Sexual Conflict. Princeton University Press, Princeton, NJ.

Arnqvist, G., Jones, T.M., Elgar, M.A., 2003. Reversal of sex roles in nuptial feeding. Nature 424, 387.

Austad, S.N., Thornhill, R., 1986. Female reproductive variation in a nuptial-feeding spider, *Pisaura mirabilis*. Bull. Cr. Arachnol. Soc. 7, 48–52.

Avila, F., Sirot, L.K., Laflamme, B.A., Rubinstein, C.D., Wolfner, M.F., 2011. Seminal fluid proteins: identification and function. Annu. Rev. Entomol. 56, 21–40.

Baer, B., Heazlewood, J.L., Taylor, N.L., Eubel, H., Millar, A.H., 2009. The seminal fluid proteome of the honeybee *Apis mellifera*. Proteomics 9, 2085–2097.

Bailey, W.J., Withers, P.C., Endersby, M., Gaull, K., 1993. The energetic costs of calling in the bushcricket *Requena verticalis* (Orthoptera: Tettigoniidae: Listroscelidinae). J. Exp. Biol. 178, 21–37.

Bidochka, M.J., Snedden, W.A., 1985. Effect of nuptial feeding on the mating behaviour of female ground crickets. Can. J. Zool. 63, 207–208.

Birkhead, T.R., Moller, A.P., 1998. Sperm Competition and Sexual Selection. Academic Press, London.

Bissoondath, C.J., Wiklund, C., 1996. Effect of male mating history and body size on ejaculate size and quality in two polyandrous butterflies, *Pieris napi* and *Pieris rapae* (Lepidoptera: Pieridae). Funct. Ecol. 10, 457–464.

Boggs, C.L., 1990. A general model of the role of male-donated nutrients in female insects' reproduction. Am. Nat. 136, 598–617.

Boggs, C.L., 1995. Male nuptial gifts: phenotypic consequences and evolutionary implications. In: Leather, S.R., Hardie, J. (Eds.), Insect Reproduction. CRC Press, Boca Raton, FL, pp. 215–242.

Boggs, C.L., 2009. Understanding insect life histories and senescence through a resource allocation lens. Funct. Ecol. 23, 27–37.

Boggs, C.L., Gilbert, L.E., 1979. Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. Science 206, 83–84.

Bowen, B.J., Codd, C.G., Gwynne, D.T., 1984. The katydid spermatophore (Orthoptera: Tettigoniidae); male investment and its fate in the mated female. Aust. J. Zool. 32, 23–31.

Braswell, W.E., Andres, J.A., Maroja, L.S., Harrison, R.G., Howard, D.J., Swanson, W.J., 2006. Identification and comparative analysis of accessory gland proteins in Orthoptera. Genome 49, 1069–1080.

Brazner, J., Aberdeen, V., Starmer, W.T., 1984. Host-plant shift and adult survival in the cactus breeding *Drosophila mojavensis*. Ecol. Entomol. 9, 375–381.

Bretman, A., Lawniczak, M.K., Boone, J., Chapman, T., 2010. A mating plug protein reduces early female remating in *Drosophila melanogaster*. J. Insect Physiol. 56, 107–113.

Brown, W.D., 1997. Courtship feeding in tree crickets increases insemination and female reproductive life span. Anim. Behav. 54, 1369–1382.

Bussiére, L.F., Basit, H.A., Gwynne, D.T., 2005. Preferred males are not always good provi-
    ders: female choice and male investment in tree crickets. Behav. Ecol. 16, 223–231.
Carayon, J., 1964. Un cas d'offrande nuptiale chez les Heteropteres. C. R. Hebd. Acad. Sci.
    259, 4815–4818.
Cardoso, M.Z., Gilbert, L.E., 2007. A male gift to its partner? Cyanogenic glycosides in the
    spermatophore of longwing butterflies (*Heliconius*). Naturwissenschaften 94, 39–42.
Carvalho, G.B., Kapahi, P., Anderson, D.J., Benzer, S., 2006. Allocrine modulation of feeding
    behavior by the sex peptide of *Drosophila*. Curr. Biol. 16, 692–696.
Chapman, T., 2008. The soup in my fly: evolution, form and function of seminal fluid proteins.
    PLoS Biol. 6, 1379–1382.
Chapman, T., Davies, S.J., 2004. Functions and analysis of the seminal fluid proteins of
    Drosophila melanogaster fruit flies. Peptides 25, 1477–1490.
Clark, N.L., Aagaard, J.E., Swanson, W.J., 2006. Evolution of reproductive proteins from
    animals and plants. Reproduction 131, 11–22.
Collins, A.M., Caperna, T.J., Williams, V., Garrett, W.M., Evans, J.D., 2006. Proteomic
    analyses of male contributions to honey bee sperm storage and mating. Insect Mol. Biol.
    15, 541–549.
Cordero, C., 1996. On the evolutionary origins of nuptial seminal gifts in insects. J. Insect
    Behav. 9, 969–974.
Cratsley, C.K., Lewis, S.M., 2005. Seasonal variation in mate choice of *Photinus ignitus* fireflies.
    Ethology 111, 89–100.
Cratsley, C.K., Rooney, J., Lewis, S.M., 2003. Limits to nuptial gift production by male fireflies
    Photinus ignitus. J. Insect Behav. 16, 361–370.
Cronk, L., Dunham, B., 2007. Amounts spend on engagement rings reflect aspects of male and
    female mate quality. Hum. Nat. 18, 329–333.
Cumming, J.M., 1994. Sexual selection and the evolution of dance fly mating systems (Diptera:
    Empididae). Can. Entomol. 126, 907–920.
Davey, K.G., 1960. The evolution of spermatophores in insects. Proc. R. Entomol. Soc. Lond.
    35, 107–113.
Davies, P.M., Dadour, I.R., 1989. A cost of mating by male *Requena verticalis* (Orthoptera:
    Tettigoniidae). Ecol. Entomol. 14, 467–469.
Demary, K.C., Lewis, S.M., 2007. Male reproductive allocation in fireflies (*Photinus* spp.).
    Invertebr. Biol. 126, 74–80.
Dodson, G.N., Morris, G.K., Gwynne, D.T., 1983. Mating behaviour of the primitive orthop-
    teran genus *Cyphoderris* (Haglidae). In: Gwynne, D.T., Morris, G.K. (Eds.), Orthopteran
    Mating Systems: Sexual Competition in a Diverse Group of Insects. Westview Press,
    Boulder, CO, pp. 305–318.
Eberhard, W.G., 1996. Female Control: Sexual Selection by Cryptic Female Choice. Princeton
    University Press, Princeton, NJ.
Eggert, A.K., Sakaluk, S.K., 1994. Sexual cannibalism and its relation to male mating success in
    sagebrush crickets, *Cyphoderris strepitans* (Haglidae: Orthoptera). Anim. Behav. 47,
    1171–1177.
Eisner, T., Meinwald, J., 1995. The chemistry of sexual selection. Proc. Natl. Acad. Sci. USA
    92, 50–55.
Eisner, T., Smedley, S.R., Young, D.K., Eisner, M., Roach, B., Meinwald, J., 1996. Chemical
    basis of courtship in a beetle (*Neopyrochroa flabellata*): cantharidin as "nuptial gift" Proc.
    Natl. Acad. Sci. USA 93, 6499–6503.
Elgar, M.A., Schneider, J.M., 2004. Evolutionary significance of sexual cannibalism. Adv. Stud.
    Behav. 34, 135–163.

Engqvist, L., 2007a. Nuptial gift consumption influences female remating in a scorpionfly: male or female control of mating rate? Evol. Ecol. 21, 41–61.

Engqvist, L., 2007b. Sex, food and conflicts: nutrition dependent nuptial feeding and pre-mating struggles in scorpionflies. Behav. Ecol. Sociobiol. 61, 703–710.

Engqvist, L., Dekomien, G., Lippmann, T., Epplen, J.T., Sauer, K.P., 2007. Sperm transfer and paternity in the scorpionfly *Panorpa cognata*: large variance in traits favoured by post-copulatory episodes of sexual selection. Evol. Ecol. 21, 801–816.

Estrada, C., Schulz, S., Yildizhan, S., Gilbert, L.E., 2011. Sexual selection drives the evolution of antiaphrodisiac pheromones in butterflies. Evolution 65, 2843–2854.

Fabre, J.H., 1918. The Life of the Grasshopper. Hodder and Stoughton, London.

Fedorka, K.M., Mousseau, T.A., 2003. Tibial spur feeding in ground crickets: larger males contribute larger gifts (Orthoptera: Gryllidae). Fla. Entomol. 85, 317–323.

Gillott, C., 2003. Male accessory gland secretions: modulators of female reproductive physiology and behavior. Annu. Rev. Entomol. 48, 163–184.

Gwynne, D.T., 1984. Courtship feeding increases female reproductive success in bushcrickets. Nature 307, 361–363.

Gwynne, D.T., 1986. Courtship feeding in katydids: investment in offspring or in obtaining fertilisations? Am. Nat. 128, 342–352.

Gwynne, D.T., 1988a. Courtship feeding in katydids benefits the mating male's offspring. Behav. Ecol. Sociobiol. 23, 373–377.

Gwynne, D.T., 1988b. Courtship feeding and the fitness of female katydids. Evolution 42, 545–555.

Gwynne, D.T., 1990. Testing parental investment and the control of sexual selection in katydids: the operational sex ratio. Am. Nat. 136, 474–484.

Gwynne, D.T., 1991. Sexual competition among females: what causes courtship-role reversal? Trends Ecol. Evol. 6, 118–121.

Gwynne, D.T., 1995. Phylogeny of the Ensifera (Orthoptera): a hypothesis supporting multiple origins of acoustical signalling, complex spermatophores and maternal care in crickets, katydids and weta. J. Orthop. Res. 4, 203–218.

Gwynne, D.T., 1997. The evolution of edible ''sperm sacs'' and other forms of courtship feeding in crickets, katydids and their kin (Orthoptera: Ensifera). In: Choe, J., Crespi, B. (Eds.), The Evolution of Mating Systems in Insects and Arachnids. Cambridge University Press, Cambridge, UK, pp. 110–129.

Gwynne, D.T., 2001. Katydids and Bushcrickets: Reproductive Behaviour and Evolution of the Tettigoniidae. Cornell University Press, Ithaca, NY.

Gwynne, D.T., 2008. Sexual conflict over nuptial gifts in insects. Annu. Rev. Entomol. 53, 83–101.

Gwynne, D.T., Simmons, L.W., 1990. Experimental reversal of courtship roles in an insect. Nature 346, 172–174.

Gwynne, D.T., Snedden, A.W., 1995. Paternity and female remating in *Requena verticalis* (Orthoptera: Tettigoniidae). Ecol. Entomol. 20, 191–194.

Gwynne, D.T., Bowen, B.J., Codd, C.G., 1984. The function of the katydid spermatophore and its role in fecundity and insemination. Aust. J. Zool. 32, 15–22.

Happ, G., 1969. Multiple sex pheromones of the mealworm beetle, *Tenebrio molitor* L. Nature 222, 180–181.

Hayashi, F., Suzuki, H., 2003. Fireflies with and without prespermatophores: evolutionary origins and life-history consequences. Entomol. Sci. 6, 3–10.

Heifetz, Y., Lung, O., Frongillo Jr., E.A., Wolfner, M.F., 2000. The *Drosophila* seminal fluid protein Acp26Aa stimulates release of oocytes by the ovary. Curr. Biol. 10, 99–102.

Herberstein, M.E., Schneider, J.M., Harmer, A.M.T., Gaskett, A.C., Robinson, K., Shaddick, K., et al., 2011. Sperm storage and copulation duration in a sexually cannibalistic spider. J. Ethol. 29, 9–15.

Houston, A.I., Stephens, P.A., Boyd, I.L., Harding, K.C., McNamara, J.H., 2007. Capital or income breeding? A theoretical model of female reproductive strategies. Behav. Ecol. 18, 241–250.

Hoving, H.J.T., Laptikhovsky, V., 2007. Getting under the skin: autonomous implantation of squid spermatophores. Biol. Bull. 212, 177–179.

Isaac, R.E., Li, C., Leedale, A.E., Shirras, A.D., 2010. *Drosophila* male sex peptide inhibits siesta sleep and promotes locomotor activity in the post-mated female. Proc. Biol. Sci. 277, 65–70.

Jacobellis v. Ohio, 1964. No. 378-184. Supreme Court of the United States, 22 June 1964.

Jeng, M.L., 2008. Comprehensive phylogenetics, systematics, and evolution of neoteny of Lampyridae (Insecta: Coleoptera). Unpublished PhD thesis, University of Kansas.

Jervis, M.A., Ferns, P.N., 2004. The timing of egg maturation in insects: ovigeny index and initial egg load as measures of fitness and of resource allocation. Oikos 107, 449–460.

Jervis, M.A., Boggs, C.L., Ferns, P.N., 2005. Egg maturation strategy and its associated trade-offs: a synthesis focusing on Lepidoptera. Ecol. Entomol. 30, 359–375.

Kapelnikov, A., Rivlin, P.K., Hoy, R.R., Heifetz, Y., 2008. Tissue remodeling: a mating-induced differentiation program for the *Drosophila* oviduct. BMC Dev. Biol. 8, 114.

Karlsson, B., 1995. Resource allocation and mating systems in butterflies. Evolution 49, 955–961.

Karlsson, B., Leimar, O., Wiklund, C., 1997. Unpredictable environments, nuptial gifts and the evolution of size dimorphism in insects: an experiment. Proc. Biol. Sci. 264, 475–479.

Kelleher, E.S., Pennington, J.E., 2009. Protease gene duplication and proteolytic activity in *Drosophila* female reproductive tracts. Mol. Biol. Evol. 26, 2125–2134.

Kelleher, E.S., Swanson, W.J., Markow, T.A., 2007. Gene duplication and adaptive evolution of digestive proteases in *Drosophila arizonae* female reproductive tracts. PLoS Genet. 3, 1541–1549.

Kelleher, E.S., Watts, T.D., LaFlamme, B.A., Haynes, P.A., Markow, T.A., 2009. Proteomic analysis of *Drosophila mojavensis* male accessory glands suggests novel classes of seminal fluid proteins. Insect Biochem. Mol. Biol. 39, 366–371.

Khalifa, A., 1949. Spermatophore production in Tricoptera and some other insects. Trans. R. Entomol. Soc. Lond. 100, 449–479.

Klowden, M.J., 2007. Physiological Systems in Insects. Academic Press, San Diego, CA.

Koene, J.M., Schulenburg, H., 2005. Shooting darts: co-evolution and counter-adaptation in hermaphroditic snails. BMC Evol. Biol. 5, 25.

Koene, J.M., Ter Maat, A., 2001. ''Allohormones'': a class of bioactive substances favoured by sexual selection. J. Comp. Physiol. A 187, 323–326.

Koene, J.M., Pfortner, T., Michiels, N.K., 2005. Piercing the partner's skin influences sperm uptake in the earthworm *Lumbricus terrestris*. Behav. Ecol. Sociobiol. 59, 243–249.

Lack, D., 1940. Courtship feeding in birds. Auk 57, 169–178.

Lebas, N.R., Hockham, L.R., 2005. An invasion of cheats: the evolution of worthless nuptial gifts. Curr. Biol. 15, 64–67.

Leimar, O., Karlsson, B., Wiklund, C., 1994. Unpredictable food and sexual size dimorphism in insects. Proc. R. Soc. Lond. B 258, 121–125.

Leopold, R.A., 1976. The role of male accessory glands in insect reproduction. Annu. Rev. Entomol. 21, 199–221.

Lewis, S.M., Cratsley, C.K., 2008. Flash signal evolution, mate choice, and predation in fireflies. Annu. Rev. Entomol. 53, 293–321.

Lewis, S.M., Cratsley, C.K., Rooney, J.A., 2004. Nuptial gifts and sexual selection in *Photinus* fireflies. Integr. Comp. Biol. 44, 234–237.

Lewis, S.M., South, A., Burns, R., Al-Wathiqui, N., 2011. Nuptial gifts. Curr. Biol. 21, R644–R645.

Liu, S., Hua, B., 2010. Histology and ultrastructure of the salivary glands and salivary pumps in the scorpionfly *Panorpa obtusa* (Mecoptera:Panorpidae). Acta Zool. 91, 457–465.

Lung, O., Wolfner, M.F., 2001. Identification and characterization of the major *Drosophila melanogaster* mating plug protein. Insect Biochem. Mol. Biol. 31, 543–551.

Lynam, A.J., Morris, S., Gwynne, D.T., 1992. Differential mating success of virgin female katydids *Requena verticalis* (Orthoptera: Tettigoniidae). J. Insect Behav. 5, 51–59.

Mann, T., 1984. Spermatophores: Development, Structure, Biochemical Attributes and Role in the Transfer of Spermatozoa. Springer, Berlin.

Markow, T.A., 2002. Female remating, operational sex ratio, and the arena of sexual selection in *Drosophila* species. Evolution 56, 1725–1734.

Markow, T.A., Ankney, P.F., 1984. *Drosophila* males contribute to oogenesis in a multiple mating species. Science 224, 302–303.

Markow, T.A., Ankney, P.T., 1988. Insemination reaction in *Drosophila*: found in species whose males contribute material to oocytes before fertilization. Evolution 42, 1097–1101.

Markow, T.A., O'Grady, P.M., 2005. Evolutionary genetics of reproductive behavior in *Drosophila*: connecting the dots. Annu. Rev. Genet. 39, 263–291.

Markow, T.A., O'Grady, P.M., 2008. Reproductive ecology of *Drosophila*. Funct. Ecol. 22, 747–759.

Markow, T.A., Gallagher, P.D., Krebs, R.A., 1990. Ejaculate derived nutritional contribution and female reproductive success in *Drosophila mojavensis* (Patterson and Crow). Funct. Ecol. 4, 67–73.

Markow, T.A., Raphael, B., Dobberfuhl, D., Breitmeyer, C.M., Elser, J.J., 1999. Elemental stoichiometry of *Drosophila* and their hosts. Funct. Ecol. 13, 78–84.

Markow, T.A., Coppola, A., Watts, T.D., 2001. How *Drosophila* males make eggs: it is elemental. Proc. Biol. Sci. 268, 1527–1532.

Mays, D.L., 1971. Mating behaviour of nemobiini crickets *Hygronemobius*, *Nemobius* and *Pteronemobius* (Orthoptera: Gryllidae). Fla. Entomol. 54, 113–126.

Mehdi, R., 2003. Danish law and the practice of *mahr* among Muslim Pakistanis in Denmark. Int. J. Sociol. Law 31, 115–129.

Michiels, N.K., Koene, J.M., 2006. Sexual selection favors harmful mating in hermaphrodites more than in gonochrists. Integr. Comp. Biol. 46, 473–480.

Molleman, F., 2010. Puddling: from natural history to understanding how it affects fitness. Entamol. Exp. Appl. 134, 107–113.

Molleman, F., Zwaan, B.J., Brakefield, P.M., 2004. The effect of male sodium diet and mating history on female reproduction in the puddling squinting bush brown *Bicyclus anynana* (Lepidoptera). Behav. Ecol. Sociobiol. 56, 404–411.

Morris, G.K., 1979. Mating systems, paternal investment and aggressive behaviour of acoustic orthoptera. Fla. Entomol. 62, 9–17.

Mougeot, F., Arroyo, B.E., Bretagnolle, V., 2006. Paternity assurance responses to first-year and adult male territorial intrusions in a courtship-feeding raptor. Anim. Behav. 71, 101–108.

Mueller, J.L., Ravi Ram, K., McGraw, L.A., Bloch Qazi, M.C., Siggia, E.D., Clark, A.G., et al., 2005. Cross-species comparison of *Drosophila* male accessory gland protein genes. Genetics 171, 131–143.

Ohno, S., 1970. Evolution by Gene Duplication. Springer-Verlag, Berlin/Heidelberg, Germany/New York.

Oxford English Dictionary, 1989. Online September 2011. Oxford University Press. (Accessed 16 September 2011). http://www.oed.com/view/Entry/78177?rskey=pENIVG&result=98.

Panhuis, T.M., Clark, N.L., Swanson, W.J., 2006. Rapid evolution of reproductive proteins in abalone and *Drosophila*. Philos. Trans. R. Soc. Lond. B Biol. Sci. 361, 261–268.

Parker, G.A., 1979. Sexual selection and sexual conflict. In: Blum, M.S., Blum, N.A. (Eds.), Sexual Selection and Reproductive Competition in Insects. Academic Press, London, pp. 123–166.

Piascik, E.K., Judge, K.A., Gwynne, D.T., 2010. Polyandry and tibial spur chewing in the Carolina ground cricket (*Eunemobius carolinus*). Can. J. Zool. 88, 988–994.

Pitnick, S., Spicer, G.S., Markow, T., 1997. Phylogenetic examination of female incorporation of ejaculate in *Drosophila*. Evolution 51, 833–845.

Pivnick, K.A., McNeil, J.N., 1987. Puddling in butterflies: sodium affects reproductive success in *Thymelicus lineola*. Physiol. Entomol. 12, 461–472.

Poiani, A., 2006. Complexity of seminal fluid: a review. Behav. Ecol. Sociobiol. 60, 289–310.

Preston-Mafham, K.G., 1999. Courtship and mating in *Empis* (*Xanthempis*) *trigramma* Meig., *E. tessellate* F. and *E.* (*Polyblepharis*) *opaca* F. (Diptera: Empididae) and the possible implications of 'cheating' behavior. J. Zool. 247, 239–246.

Qazi, M.C., Wolfner, M.F., 2003. An early role for the *Drosophila melanogaster* male seminal protein Acp36DE in female sperm storage. J. Exp. Biol. 206, 3521–3528.

Raubenheimer, D., 2011. Toward a quantitative nutritional ecology: the right-angled mixture triangle. Ecol. Monogr. 81, 407–427.

Raubenheimer, D., Simpson, S.J., Mayntz, D., 2009. Nutrition, ecology, and nutritional ecology: toward an integrated framework. Funct. Ecol. 23, 4–16.

Ravi Ram, K., Wolfner, M.F., 2007a. Sustained post-mating response in *Drosophila melanogaster* requires multiple seminal fluid proteins. PLoS Genet. 3, e238.

Ravi Ram, K., Wolfner, M.F., 2007b. Seminal influences: *Drosophila Acps* and the molecular interplay between males and females during reproduction. Integr. Comp. Biol. 47, 427–445.

Reinhardt, K., Naylor, R.A., Siva-Jothy, M.T., 2009. Ejaculate components delay reproductive senescence while elevating female reproductive rate. Proc. Natl. Acad. Sci. USA 106, 21743–21747.

Rice, W.R., 1998. Intergenomic conflict, interlocus antagonistic coevolution, and the evolution of reproductive isolation. In: Howard, D.J., Berlocher, S.H. (Eds.), Endless Forms: Species and Speciation. University Press, Oxford, pp. 161–270.

Rogers, D.W., Chase, R., 2001. Dart receipt promotes sperm storage in the garden snail *Helix aspersa*. Behav. Ecol. Sociobiol. 50, 122–127.

Rooney, J.A., Lewis, S.M., 1999. Differential allocation of male-derived nutrients in two lampyrid beetles with contrasting life-history characteristics. Behav. Ecol. 10, 97–104.

Rooney, J., Lewis, S.M., 2002. Fitness advantage from nuptial gifts in female fireflies. Ecol. Entomol. 27, 373–377.

Sakaluk, S.K., 1984. Male crickets feed females to ensure complete sperm transfer. Science 223, 609–610.

Sakaluk, S.K., 1986. Is courtship feeding by male insects parental investment? Ethology 73, 161–166.

Sakaluk, S.K., 2000. Sensory exploitation as an evolutionary origin to nuptial food gifts in insects. Proc. Biol. Sci. 267, 339–343.

Sauer, K.P., Lubjuhn, T., Sindern, J., Kullmann, H., Kurtz, J., Epplen, C., et al., 1998. Mating system and sexual selection in the scorpionfly *Panorpa vulgaris* (Mecoptera: Panorpidae). Naturwissenschaften 85, 219–228.

Schilthuizen, M., 2005. The darting game in snails and slugs. Trends Ecol. Evol. 20, 581–584.

Simmons, L.W., 1995. Male bushcrickets tailor spermatophores in relation to their remating intervals. Funct. Ecol. 9, 881–886.

Simmons, L.W., 2001. Sperm Competition and Its Evolutionary Consequences in the Insects. Princeton University Press, New Jersey.

Simmons, L.W., Achmann, R., 2000. Microsatellite analysis of sperm-use patterns in the bushcricket *Requena verticalis*. Evolution 54, 942–952.

Simmons, L.W., Bailey, W.J., 1990. Resource influenced sex roles of zaprochiline tettigoniids (Orthoptera:Tettigoniidae). Evolution 44, 1853–1868.

Simmons, L.W., Parker, G.A., 1989. Nuptial feeding in insects: mating effect versus paternal investment. Ethology 81, 332–343.

Simmons, L.W., Teale, R.J., Maier, M., Standish, R.J., Bailey, W.J., Withers, P.C., 1992. Some costs of reproduction for male bushcrickets, *Requena verticalis* (Orthoptera: Tettigoniidae): allocating resources to mate attraction and nuptial feeding. Behav. Ecol. Sociobiol. 31, 57–62.

Simmons, L.W., Craig, M., Llorens, T., Schinzig, M., Hosken, D., 1993. Bushcricket spermatophores vary in accord with sperm competition and parental investment theory. Proc. R. Soc. Lond. B Biol. Sci. 251, 183–186.

Simmons, L.W., Llorens, T., Schinzig, M., Hosken, D., Craig, M., 1994. Sperm competition selects for male mate choice and protandry in the bushcricket, *Requena verticalis* (Orthoptera: Tettigoniidae). Anim. Behav. 47, 117–122.

Simmons, L.W., Beveridge, M., Kennington, W.J., 2007. Polyandry in the wild: temporal changes in female mating frequency and sperm competition intensity in natural populations of the tettigoniid *Requena verticalis*. Mol. Ecol. 16, 4613–4623.

Sirot, L.K., Poulson, R.L., McKenna, M.C., Girnary, H., Wolfner, M.F., Harrington, L.C., 2008. Identity and transfer of male reproductive gland proteins of the dengue vector mosquito, *Aedes aegypti*: potential tools for control of female feeding and reproduction. Insect Biochem. Mol. Biol. 38, 176–189.

Sirot, L.K., Wolfner, M.F., Wigby, S., 2011. Protein-specific manipulation of ejaculate composition in response to female mating status in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 108, 9922–9926.

Smedley, S.R., Eisner, T., 1996. Sodium: a male moth's gift to its offspring. Proc. Natl. Acad. Sci. USA 93, 809–813.

Sonenshine, D.E., Bissinger, B.W., Egekwu, N., Donohue, K.V., Khalil, S.M., Roe, M., 2011. First transcriptome of the testis-vas deferens-male accessory gland and proteome of the spermatophore from *Dermacentor variabilis* (Acari: Ixodidae). PLoS One 6, e24711.

South, A., Lewis, S.M., 2011. The influence of male ejaculate quantity on female fitness: a meta-analysis. Biol. Rev. Camb. Philos. Soc. 86, 299–309.

South, A., Lewis, S.M., 2012a. Determinants of reproductive success across sequential episodes of sexual selection in a firefly. Proc. R. Soc. Lond. B. in press.

South, A., Lewis, S.M., 2012b. Effects of male ejaculate on female reproductive output and longevity in *Photinus* fireflies. Can. J. Zool. in press.

South, A., Sota, T., Abe, N., Yuma, M., Lewis, S.M., 2008. The production and transfer of spermatophores in three Asian species of *Luciola* fireflies. J. Insect Physiol. 54, 861–866.

South, A., Sirot, L.K., Lewis, S.M., 2011a. Identification of predicted seminal fluid proteins in *Tribolium castaneum*. Insect Mol. Biol. 20, 447–456.

South, A., Stanger-Hall, K., Jeng, M.-L., Lewis, S.M., 2011b. Correlated evolution of female neoteny and flightlessness with male spermatophore production in fireflies (Coleoptera: Lampyridae). Evolution 65, 1099–1113.

Stanger-Hall, K.F., Lloyd, J.E., Hillis, D.M., 2007. Phylogeny of North American fireflies (Coleoptera:Lampyridae): implications for the evolution of light signals. Mol. Phylogenet. Evol. 45, 33–49.

Stearns, S.C., 1992. The Evolution of Life Histories. Oxford University Press, Oxford.

Steele, R.J., 1986. Courtship feeding in *Drosophila subobscura*. I. The nutritional significance of courtship feeding. Anim. Behav. 34, 1087–1098.

Stutt, A.D., Siva-Jothy, M.T., 2001. Traumatic insemination and sexual conflict in the bed bug *Cimex lectularius*. Proc. Natl. Acad. Sci. USA 98, 5683–5687.

Swanson, W.J., Vacquier, V.D., 2002. Reproductive protein evolution. Annu. Rev. Ecol. Syst. 33, 161–179.

Swanson, W.J., Clark, A.G., Waldrip-Dail, H.M., Wolfner, M.F., Aquadro, C.F., 2001. Evolutionary EST analysis identifies rapidly evolving male reproductive proteins in *Drosophila*. Proc. Natl. Acad. Sci. USA 98, 7375–7379.

Tallamy, D.W., 1994. Nourishment and the evolution of paternal investment in subsocial arthropods. In: Hunt, J.H., Nalepa, C.A. (Eds.), Nourishment and Evolution in Insect Societies. Westview Press, Boulder, CO, pp. 21–56.

Thornhill, R., 1976. Sexual selection and paternal investment in insects. Am. Nat. 110, 153–163.

Thornhill, R., 1981. *Panorpa* (Mecoptera: Panorpidae) scorpionflies: systems for understanding resource-defense polygyny and alternative male reproductive efforts. Ann. Rev. Ecol. Syst. 12, 355–386.

Thornhill, R., Alcock, J., 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, MA.

Trivers, R., 1972. Parental investment and sexual selection. In: Campbell, B. (Ed.), Sexual Selection and the Descent of Man. Aldine-Atherton, Chicago, pp. 136–179.

Vacquier, V.D., 1998. Evolution of gamete recognition proteins. Science 281, 1995–1998.

Vahed, K., 1998. The function of nuptial feeding in insects: a review of empirical studies. Biol. Rev. 73, 43–78.

Vahed, K., 2007. All that glisters is not gold: sensory bias, sexual conflict and nuptial feeding in insects and spiders. Ethology 113, 105–127.

van Der Reijden, E., Monchamp, J., Lewis, S.M., 1997. The formation, transfer, and fate of male spermatophores in *Photinus* fireflies (Coleoptera: Lampyridae). Can. J. Zool. 75, 1202–1205.

Wagstaff, B.J., Begun, D.J., 2005. Molecular population genetics of accessory gland protein genes and testis-expressed genes in *Drosophila mojavensis* and *D. arizonae*. Genetics 171, 1083–1101.

Wagstaff, B.J., Begun, D.J., 2007. Adaptive evolution of recently duplicated accessory gland protein genes in desert *Drosophila*. Genetics 177, 1023–1030.

Walsh, B., 2003. Population-genetic models of the fates of duplicate genes. Genetica 118, 279–294.

Walters, J.R., Harrison, R.G., 2010. Combined EST and proteomic analysis identifies rapidly evolving seminal fluid proteins in *Heliconius* butterflies. Mol. Biol. Evol. 27, 2000–2013.

Wedell, N., 1992. Protandry and mate assessment in the wartbiter *Decticus verrucivorus* (Orthoptera: Tettigoniidae). Behav. Ecol. Sociobiol. 31, 301–308.

Wedell, N., 1994. Variation in nuptial gift quality in bush crickets (Orthoptera: Tettigoniidae). Behav. Ecol. 5, 418–425.

Wedell, N., 2005. Sperm competition in butterflies and moths. In: Fellowes, M.D.E., Holloway, G.J., Rolff, J. (Eds.), Insect Evolutionary Ecology. CABI Publishing, London, pp. 49–81.

Wedell, N., Tregenza, T., Simmons, L.W., 2008. Nuptial gifts fail to resolve a sexual conflict in an insect. BMC Evol. Biol. 8, 204.

Welke, K.W., Schneider, J.M., 2012. Sexual cannibalism benefits offspring survival. Anim. Behav. 83, 201–207.

Wheeler, D., 1996. The role of nourishment in oogenesis. Annu. Rev. Entomol. 41, 407–431.

Wickler, W.Z., 1985. Stepfathers in insects and their pseudo- parental investment. Z. Tierpsychol. 69, 72–78.

Wigby, S., Chapman, T., 2005. Sex peptide causes mating costs in female *Drosophila melanogaster*. Curr. Biol. 15, 316–321.

Wolfner, M.F., 2007. "S.P.E.R.M". (seminal proteins (are) essential reproductive modulators): the view from *Drosophila*. Soc. Reprod. Fertil. Suppl. 65, 183–199.

Wolfner, M.F., 2009. Battle and ballet: molecular interactions between the sexes in *Drosophila*. J. Hered. 100, 399–410.

Xue, L., Noll, M., 2000. *Drosophila* female sexual behavior induced by sterile males showing copulation complementation. Proc. Natl. Acad. Sci. USA 97, 3272–3275.

Zeh, D.W., Smith, R.L., 1985. Paternal investment by terrestrial arthropods. Am. Zool. 25, 785–805.