Assuring paternity in a promiscuous world: are there lessons for ticks among the insects?

W. R. KAUFMAN*

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9

SUMMARY

In this article I begin with a few current ideas on some physiological factors that influence mating choice in insects. Emphasis is placed on those proteins produced by the male reproductive accessory glands which increase female fecundity and reduce her receptivity to subsequent males. Strategies used by late-arriving males to favour their paternity are also mentioned. With a number of insect models as background, I then review what is currently known about several male factors in ticks (a capacitation factor, a male factor, an engorgement factor and a vitellogenesis stimulating factor) and suggest where we might focus our experimental activities in the future.

Key words: Tick and insect reproduction, fecundity enhancing substances, receptivity inhibiting substances, sperm competition, male accessory gland proteins, sperm precedence.

LIST OF ABBREVIATIONS

(In the text, a final 's' is added to an abbreviation to indicate plural)
Acp: accessory gland protein(s) of *Drosophila*; CW: critical weight (of female ixodid ticks); 20E: 20-hydroxyecdysone; EF: engorgement factor (of ixodid ticks); FES: fecundity-enhancing substance(s); JH: juvenile hormone; MAG: male accessory gland; MC: main cells of the MAG of *Drosophila*; MF: male factor (of ixodid ticks); mNSC: medial neurosecretory cell(s) (of *Rhodnius*); OSP: oviposition stimulating protein (of *Melanoplus sanguinipes*); PG: prostaglandin(s); P1, P2, Pn: the index of sperm precedence (which sperm from the 1st, 2nd or nth male to copulate with a female outcompetes the others in fertilizing the eggs); RIS: receptivity-inhibiting substance; *rec*protein: recombinant protein; SG: salivary gland (of ixodid ticks); T/VD: testis/vas deferens (of ixodid ticks); VSF: vitellogenesis-stimulating factor (of *Ornithodoros moubata*).

INTRODUCTION

There is a broad tendency among female insects for fecundity to increase with mating frequency (Ridley, 1988). A female's eggs carry forward to the next generation her genes and those of her mate. Hence, one might guess that promiscuity should be of selective advantage for both sexes. On closer inspection it is not that simple. The survival of a female's offspring is as much a function of her mate's fitness as it is of her own, so she has some interest in determining the paternity of her offspring (Birkhead, 1998; Eberhard, 1998). On the other hand, how can the male's paternity be assured if his spermatozoa have to compete with those of other males in the same arena? In this chapter I shall review some of the mechanisms used by male insects which assure their paternity in this 'promiscuous world'. I shall attempt to relate these to what is known from the less extensive literature on ticks, and ask whether there are lessons from insects which might point our way to future enquiries in ticks.

SPERM PRECEDENCE AND PATERNITY

Because of the proverbial battle of the sexes, as well as that among competing males, one encounters numerous mating strategies among terrestrial arthropods, and a fascinating literature on sperm competition to match. The degree to which sperm competition occurs in any instance is determined by a number of factors (reviewed extensively by Simmons & Siva-Jothy, 1998, and by Parker, 1998): (1) How readily will a previously mated female accept further males (sperm preemption)? (2) How much mixing of sperm occurs in the spermathecae? (3) When copulating with a female, how readily can the male either flush out or otherwise remove previously deposited sperm, so as to improve the likelihood that his will be used for fertilization (sperm displacement)? (4) Which of the following mechanisms are available to influence sperm precedence: sperm stratification (last sperm in has positional advantage), sperm loading (which male has provided the largest number of spermatozoa in the ejaculate), and sperm selection (non-random use of spermatozoa by the female)? (5) Are there substances in the male's ejaculate that can incapacitate the sperm of previous males? Finally, (6) What effort is made by the first male to avoid sperm

* Corresponding author. Tel: +1-780-492-1279. Fax: +1-780-9234. E-mail: reuben.kaufman@ualberta.ca

Parasitology (2004), **129**, S145–S160.
DOI: 10.1017/S0031182004004846 Printed in the United Kingdom

competition altogether, such as by repeated copulation prior to oviposition, or by mate-guarding? Robust experiments have rarely been conducted in specific cases to distinguish among these theoretical mechanisms, even though they influence whether the first male's sperm or the nth male's sperm is more likely to fertilize the female (P1 and Pn, respectively).

Ecological factors and the morphology of the female genital tract have a predominant influence on determining mating strategies (Thomas & Zeh, 1984). For example, some spiders possess conventional 'cul-de-sac' spermathecae – simple diverticula of the common oviduct into which sperm enters for storage and leaves for fertilization via a common opening. Other spiders possess 'conduit' spermathecae – sperm enters for storage via one channel and leaves for fertilization via another (Elgar, 1998). Considering this diversity of spermathecal morphology in spiders, Austad (1984) predicted that first male sperm priority and the occurrence of mating plugs should be the dominant pattern among species with conduit spermathecae, while last male sperm priority or mixing of sperm should be most common in species with cul-de-sac spermathecae. Austad (1984) lists the spider families correponding to the two spermathecal morphologies, and there is no overlap between these lists. But unfortunately, sperm priority has not been determined in more than a few species of spider, and for a number of reasons outlined by Austad, the data are not sufficiently reliable to adequately test the hypothesis. However, the bowl and doily spider, *Frontinella pyramitela*, with a conduit-type spermatheca, does show P1 precedence through a mechanism of remote mate-guarding. Subsequent males, arriving 24+ h after a female has first mated, readily engage in pre-insemination behaviour, but usually do not even attempt to transfer sperm (Austad, 1982). The nature of the signal conveyed by a mated female to discourage subsequent matings was not reported in this case. Austad (1982) speculated that sperm displacement would be difficult in spiders because of spermathecal morphology. In such a situation, insemination would be futile, and this might explain why males subsequent to the first one do not even attempt it.

There are a number of mechanisms whereby a male promotes his paternity. Among them are physiological effects of the reproductive accessory gland secretions.

REPRODUCTIVE ACCESSORY GLANDS

The female accessory glands

Some commonly stated functions of female accessory sex glands in insects are to synthesize material for the egg capsules, to produce the lipid waterproofing layer, to lubricate the egg while it moves through the ovipositor, and to digest the spermatophore by means of enzymes secreted into the reproductive tract (Gillott, 1988). In ticks, the female possesses three such glands: (1) a tubular accessory gland (argasid and ixodid ticks) produces a protein-rich secretion, the function of which is unknown (Diehl, Aeschlimann & Obenchain, 1982; Sonenshine, 1991), (2) a lobular accessory gland (ixodid ticks only). Lees & Beament (1948) demonstrated that it provides the initial waterproofing layer for the egg. Sonenshine (1991) suggests it is also the most likely source of the genital sex pheromone, and (3) Gené's organ (found only in ticks) deposits wax on each egg as it is delivered from the vagina. The wax coat waterproofs the eggs, causes them to stick together, and probably protects them from soil fungi and microbes (Lees & Beament, 1948; Schöl *et al*. 2001).

The male accessory glands

Semen consists of spermatozoa bathed in a seminal fluid, the latter comprising secretions from the testis and secretions from the male accessory glands (MAGs). Commonly stated functions of MAG secretions in insects (reviewed by Gillott, 1988, 1996 and 2003) are: (1) to nourish/protect the spermatozoa within the male tract, (2) to secrete the spermatophore, (3) to alter the behaviour of the female in such ways as to (a) hasten oviposition and/or increase egg production and (b) attenuate mating behaviour in the female, (4) to secrete components of the mating plug, a coagulum which helps ensure P1 sperm precedence, (5) to secrete antibacterial and antifungal agents which protect the gametes within the female reproductive tract (Lung, Kuo & Wolfner, 2001*b*), (6) to provide a nutritional supplement to the female (Friedel & Gillott, 1977), although Vahed (1998) comments that it is usually difficult to determine the extent to which spermatophore contents contribute to the overall nutritional budget of the female, and finally, (7) to extend the duration of sperm viability within the female genital tract (Tram & Wolfner, 1999; Chapman *et al*. 2000), a function also suggested for ticks (Feldman-Muhsam, 1991).

Friedel & Gillott (1977) coined the terms 'fecundity-enhancing substances' (FES) and 'receptivity-inhibiting substances' (RIS) in reference to some MAG secretions. An FES is one that (directly or indirectly) increases egg production, oviposition or egg fertility. An RIS is one that renders the female less likely to copulate with further males, and thus constitutes the mechanism for remote mate-guarding. FESs and RISs are well-documented for numerous insects (Gillott, 1988, 2003), and in this article I shall discuss a few examples.

FECUNDITY-ENHANCING SUBSTANCES

The blood-sucking bug, Rhodnius prolixus

In *Rhodnius*, transfer of a spermatophore influences fecundity in at least two ways: (1) by promoting migration of sperm to the spermathecae and (2) by stimulating ovulation and/or oviposition. Each of these effects is regulated by a distinct mechanism. (1) A spermatophore in the female's bursa copulatrix stimulates rhythmic contractions in the common oviduct, and within 5–10 min spermatozoa are found in the spermathecae. The MAG comprises three transparent lobes (which produce the spermatophore) and one opaque lobe. Davey (1958) demonstrated that a secretion from the opaque lobe stimulates peristalsis in the common oviduct, thus effecting migration of sperm to the spermathecae. This MAG secretion is not itself a myotropin, but exerts its peristaltic effect on the oviduct via the nervous system (Davey, 1958). (2) The presence of sperm within the spermathecae leads to a doubling of the rate of oviposition compared to that of fed virgins (Davey, 1965). Thus, if fed females are mated to males lacking either seminal vesicles (no sperm) or the opaque accessory gland lobes (sperm migration inhibited), the rate of oviposition equals that of normally fed virgins (Kuster & Davey, 1986). Likewise, the rate of oviposition in fed mated females surgically deprived of their spermathecae is similar to that of normally fed virgins. Finally, fed virgins receiving implants of spermathecae from mated females lay significantly more eggs than fed virgins receiving implants of virgin spermathecae. Taken together, these experiments indicate that the spermathecae secrete a factor that stimulates ovulation and/or oviposition, but only when they contain sperm.

Further experiments (Davey, 1967) demonstrated that the spermathecal factor has its accelerating effect on oviposition by stimulating medial neurosecretory cells (mNSCs) in the brain to release a myotropin. This myotropin augments contractions in the ovarian sheath and lateral oviducts, and hence ovulation and oviposition follow (Kriger & Davey, 1982, 1983). Moreover, the release of the myotropin from the mNSC depends on the presence of ecdysteroids from the ovary (Ruegg *et al*. 1981). Thus, the mNSCs require at least two inputs to release myotropin: a signal from mating (the spermathecal factor), and ovarian ecdysteroid. The myotropin of *Rhodnius* appears to be a peptide (~8·5 kDa) in the FMRF-amide family (Sevala *et al*. 1992).

Mosquitoes

Insemination produces profound effects in the female mosquito, including host-seeking, oviposition, receptivity and circadian flight activity (Klowden & Chambers, 1991). The MAGs of *Aedes aegypti* and other mosquitoes produce a secretion, originally named 'matrone' because, when injected into virgins, it elicits numerous characteristics of mated females (Fuchs, Craig & Hiss, 1968). According to earlier literature, matrone was composed of α- and β-fractions. The α-fraction alone stimulated oviposition, but both together were required to inhibit receptivity (Hiss & Fuchs, 1972; see further below). More recently, Klowden (1999) suggested that the term 'matrone' is now obsolete, since MAG extracts having matrone-activity contain at least several distinct substances which may vary from one species to another. For example, heterologous MAG substances activate different subsets of behaviour in different species, and active material from different sources come in a wide range of molecular size.

A series of elegant experiments by Adlakha & Pillai (1975) demonstrated that MAG secretions are also necessary for normal fertilization to occur, perhaps by activating the sperm or by altering the egg surface so as to facilitate penetration of the sperm.

One important behavioural change attributed to MAG secretions in mosquitoes is a switch from swarming (=mating) to host-seeking flight behaviour (Gillott, 1988). The switch to host-seeking behaviour can be regarded ultimately as a fecundity-enhancing effect, because attaining a blood meal is required for producing a batch of eggs.

It has recently been shown in *Anopheles gambiae* that oviposition does not occur if the spermathecae of blood-fed females are removed either at emergence or after mating (Klowden, 2001). It is not known whether the spermathecal signal is neural or is a humoral factor, reminiscent of the system just described for *Rhodnius* (M. J. Klowden, personal communication). Whatever the nature of the spermathecal effect, it is not mimicked by implantation of a MAG (Klowden, 2001).

Drosophila

Most experimental studies on the MAGs of insects depend on delicate surgical techniques, and the implantation of tissues or injection of homogenates into the haemocoel (most often) or into the female genital tract (less often). Even though we have learned much from these studies, they obviously do not mimic the normal mating situation. In contrast, the relative ease of generating mutant strains of *Drosophila* that lack specific components of the MAG, or that do not produce sperm, enable an experimenter to study the effects of these genetic lesions under more natural conditions. Thus, it is not surprising that we know far more about the functions of MAG secretions in *Drosophila* than we do for any other insect. To date, no fewer than 83 gene products are predicted to be accessory gland proteins (Acps), all of them produced in the 'main cells' (MC; 96% of total) of the MAG (Wolfner, 2002).

Extracts of the MAG, when injected into the haemocoel of virgin *Drosophila* females, markedly increase the number of eggs laid (Leahy & Lowe, 1967; Chen & Bühler, 1970). Kalb, DiBenedetto & Wolfner (1993) produced strains of flies in which males lacked spermatozoa or in which the MCs of the MAG were destroyed by directed cell ablation. They observed the effects on oviposition after selective cross-matings, and compared these to normal mated controls (average of 108 eggs in 5 days) and virgin controls (average of 12·2 eggs in 5 days). When mated with males producing neither spermatozoa nor MC-secretions, females laid an average of 9·3 eggs in 5 days. When mated with males producing no spermatozoa (but having functional MCs), females laid an average of 40 eggs in 5 days. Thus, MC-secretions cause only a partial, short-term fecundity-enhancing effect.

Acp62F shows sequence similarities to a neurotoxin (PhTx2-6) produced by a Brazilian spider, *Phoneutria nigriventer* (Wolfner *et al*. 1997). PhTx2-6 prolongs action potentials and increases membrane excitability, suggesting that Acp62F may behave like the myotropin of *Rhodnius* and stimulate oviposition or regulation of sperm release from the seminal receptacle. Acp62F also has significant sequence homology to a class of serine protease inhibitors from the nematode, *Ascaris* (Lung *et al*. 2002).

RECEPTIVITY-INHIBITING SUBSTANCES

Mosquitoes

Female *Aedes aegypti* and other mosquitoes mate once only, and virgin females injected with MAG homogenates remain forever virgin (Craig, 1967). Extracts of *Aedes* MAG have at least some activity across 12 species of mosquitoes from 3 genera (Craig, 1967). In fact, even the sex peptide of *Drosophila* (Chen *et al*. 1988) has biological activity in *Aedes* similar to that of α-matrone. Thus, injections into *Aedes* of extracts containing *Drosophila* sex peptide stimulates oviposition. In order to inhibit receptivity in *Aedes*, however, *Drosophila* sex peptide has to be injected together with β-matrone (Hiss & Fuchs, 1972). More recently, Klowden (2001) demonstrated that, in contrast to aedine and culicine mosquitoes, MAG substances from several anopheline mosquitoes do not control sexual receptivity as had been suggested earlier (e.g. Craig, 1967).

Drosophila

Whereas 95% of virgin females from a normal population will mate, only 3% of mated females will accept a male 1 day later (Kalb *et al*. 1993). When mated with males producing neither spermatozoa nor MC-secretions, 86% of the females mated again 1 day later. When mated with males producing MC-secretions but no spermatozoa, 43% mated again 1 day later. These partially inhibitory effects were only short-lived, however, because 90% of females mated with spermless males remated 2–7 days later. Thus, stored spermatozoa are necessary for a long-term receptivity-inhibiting effect in *Drosophila*.

The grasshopper, Gomphocerus rufus *L.*

The situation in *Gomphocerus* is unusual in that mating inhibits receptivity but does not increase the rate of egg production or oviposition (reviewed by Hartmann & Loher, 1999). A sexually immature female grasshopper will reject a male's attempt to copulate with her. This behaviour is called 'primary defence' (Hartmann & Loher, 1996). 'Secondary defence' is the rejection behaviour elicited by sexually mature females after they have mated (strong directed kicks at the male which make copulation impossible). Secondary defence endures until oviposition terminates, about 3–4 days following mating. Denervation of the spermathecae or severance of the ventral nerve cord immediately after mating abolishes secondary defense (Hartmann & Loher, 1996). Experiments by Hartmann & Loher (1996, 1999) have established that secondary defence is elicited by chemical and mechanical stimuli. The chemical stimulus comes from a specific MAG tubule (containing a white secretion), and the mechanical stimulus arises from stretch in the lateral oviducts as eggs move through them. Secondary defence in the grasshopper does not occur if the spermathecae are surgically removed prior to mating. This is because receptors for the white secretion (an identified group of sensory bristles) are found within the spermathecal bulb. In several insects discussed so far, on the other hand, the MAG secretion is effective even if injected into the haemocoel.

OTHER FUNCTIONS OF MALE ACCESSORY GLANDS

Mating plugs

In numerous insects, a mating plug constitutes a physical barrier deposited in the female genital tract by the male. The evolutionary advantage of reduced competition from other males is clear. The spermatophore itself can serve as an effective mating plug, provided it remains in place until the first male's sperm has fertilized the eggs. But as Gillott (2003) points out, the spermatophore is frequently ejected or digested *in situ* within a few days. In ticks, the (ecto)spermatophore is not even internalized within the female, and the endospermatophore, when evaginating into the female, forms a capsule around the prospermia which follow closely behind (Feldman-Muhsam, 1986). These sperm capsules can remain intact for extended periods, at least in *O. savignyi*, in which starved females still contained viable sperm within their capsules 1 year after mating

(Feldman-Muhsam, 1986). However, it is doubtful that such sperm capsules are effective as mating plugs, because at least some ticks show P2 sperm preference (see below; Yuval & Spielman, 1990).

In many insects, the mating plug is a coagulum of the seminal fluid rather than the spermatophore. In *Drosophila*, a major component of the mating plug is a 38 kDa protein (PEB-me) from the ejaculatory bulb (Lung, Kuo & Wolfner, 2001*a*). The plug also contains at least one other Acp (Acp76A; see below).

Antimicrobial proteins

In numerous organisms, antibacterial and antifungal peptides constitute a major defence mechanism against pathogens. Male *Drosophila* transfer at least three antimicrobial peptides to the female during copulation, one of which, produced by the male ejaculatory duct, is the 28 kDa andropin. These peptides are believed to protect egg and sperm while in the female genital tract, for they are likely to be expelled during oviposition, several hours after mating (Lung *et al*. 2001*b*). Although ticks do produce antimicrobial peptides (e.g. van der Goes van Naters-Yasui *et al*. 2000; Johns, Sonenshine & Hynes, 1998, 2001), it is not known whether they are a component of the seminal fluid.

Protease inhibition and sperm storage

The seminal fluid of many organisms contains protease inhibitors, which affect male fertility (see Lung *et al*. 2002, for references). Wolfner *et al*. (1997) identified genes encoding a dozen Acps in *Drosophila*, eight of which begin with putative secretion signals, and therefore are likely to be included in the semen. Acp76A, a 388 amino acid pro-protein shows a sequence homology to the serpin class of protease inhibitors. Wolfner *et al*. (1997) hypothesized the following: Acp26Aa and Acp36DE may be activated in the female by means of regulated proteolytic action. It is conceivable that this proteolytic activation does not occur in the male genital tract because Acp76A inhibits proteolysis there. Acp76A itself may be inactivated following copulation, and hence female-derived proteases would be able to activate the other Acps. Acp76A may also play a role in forming the mating plug, because it can be recovered from plugs extruded by the female (Wolfner, 1997).

Finally, Acps are also necessary for sperm to be stored in the spermathecae. Tram & Wolfner (1999) demonstrated that male *Drosophila*, deficient in MAG secretions through directed cell ablation, produced a normal amount of sperm and transferred sperm successfully. Females mated to these males, however, stored only 3–10% the amount of sperm after 6 h compared to those mated to control males. The specific peptide mediating this effect is Acp36DE (Chapman *et al*. 2000). Sperm storage in *Rhodnius* is mediated by the spermathecal factor rather than a MAG secretion (Kuster & Davey, 1986). Ticks can also store sperm for extended periods: for a year or more in the case of *O. savignyi* (Feldman-Muhsam, 1986) and for at least several months in the case of *Amblyomma americanum* (Gladney & Drummond, 1971), although in neither case is the mechanism of long-term storage known.

With the foregoing as background, let us now focus more directly on the feeding and mating strategies of ticks, and consider how information from various insect models might point the way to future studies.

There are two major families of ticks: Argasidae and Ixodidae. A third family (Nuttallielidae) consists of a single species (*Nuttalliella namaqua*), about which very little is known, because only a handful of specimens have ever been found (in Namibia and Tanzania; Keirans *et al*. 1976; Sonenshine, 1991). The Argasidae comprise five genera, in five corresponding sub-families. The Ixodidae are divided into two phyletic lines: Prostriata and Metastriata, the former comprising a single genus (*Ixodes*), and the latter comprising 12 genera in 4 sub-families (Sonenshine, 1991; and see chapter by Barker & Murrell in this Supplement). The major physiological difference between the prostriate and metastriate ticks, at least for our purposes, is that the former can mate off the host before taking a blood meal, whereas the latter mate only while on the host, and only after they have fed for at least a few days.

Feeding in ticks

In all haematophagous arthropods, the taking of a blood meal constitutes the signal for profound developmental and physiological changes. The blood-sucking insects, *Rhodnius* and *Aedes*, feed on a host for only a matter of minutes. The argasid tick, *O. moubata*, feeds for about 30–60 min (Kaufman, Kaufman & Phillips, 1981, 1982). The latter species can lay a number of egg batches, each one requiring a blood-meal. Ixodid ticks, on the other hand, remain attached to a host for 5–15 days, the specific duration depending on species, the developmental stage and other factors. Mating causes distinct behavioural and physiological changes in ixodid females (reviewed by Kaufman & Lomas, 1996; Lomas & Kaufman, 1999), and I shall return to these further below.

The extended feeding period of female ixodid ticks is divided into three phases. (1) The '*preparatory feeding phase*' occupies the first day or two, when the female anchors itself in place by means of a cement-like substance, and the feeding lesion is formed. (2) The '*slow feeding phase*' occurs over the subsequent 4–8 days, when the female mates, and increases its weight approximately 10-fold; mating normally occurs during this period. (3) The '*rapid feeding phase*' occupies only about 24 hours, during which the

female increases its weight a further 10-fold and then detaches from the host.

The salivary glands (SGs) of *Amblyomma hebraeum* degenerate within 4 days following engorgement (Harris & Kaufman, 1981), a process controlled by 20E (Harris & Kaufman, 1985; Kaufman, 1991; Mao & Kaufman, 1999). Oocyte maturation begins during the feeding period, and yolk synthesis (also controlled by 20E) and yolk uptake are underway by the 4th day post-engorgement. Oviposition begins around day 10, and continues for about 3 weeks, after which the female dies (Friesen & Kaufman, 2002).

The transition between the slow and rapid phases of feeding occurs at about 10-fold the unfed weight (named the 'critical weight' (CW) by Harris & Kaufman (1984)). Females forcibly removed from the host below the CW do not lay eggs, do reattach to a host if given the opportunity, and their SGs do not degenerate. Females forcibly removed from the host above the CW do lay eggs (the size of egg mass being proportional to the extent of engorgement), do not reattach to a host if given the opportunity, and their SGs degenerate (Kaufman & Lomas, 1996). Moreover, there are slightly different CWs for reattachment to the host, SG degeneration and egg development (Weiss & Kaufman, 2001).

Changes in sensory physiology as a result of feeding are probably responsible for the cessation of host-seeking behaviour in ticks above the CW. Anderson, Scrimgeour & Kaufman (1998) demonstrated that the ability of CO_2 to induce questing and walking in *A. hebraeum* changes dramatically with age, and feeding state. In general, ticks responded to CO_2 during periods in which they would normally be attracted to a host (e.g. when partially fed but below the CW), but were refractory to CO_2 at other times (e.g. when partially fed but above the CW, or when fully engorged).

MATING IN TICKS

The finding of a suitable mate involves a number of stereotyped behaviours that are triggered by pheromones (see chapter by Soneshine in this Supplement). In ixodid ticks there are pheromones which promote assembly and aggregation-attachment, as well as a series of sex pheromones which cause attraction, mounting and probing the female genitals. Copulatory behaviour varies significantly with the group of ticks. The following very brief description is distilled from Feldman-Muhsam (1986) and Sonenshine (1991).

Sperm development

In the Metastriata, spermatogenesis (the production of haploid spermatids) begins in the nymph with the taking of a blood-meal, and attains only the primary spermatocyte stage at the time of ecdysis to the adult. When the adult feeds, spermatogenesis is completed and spermiogenesis follows. In ticks, the two phases of spermiogenesis are: (1) growth and elongation of spermatids to form prospermia (the form in which sperm are packaged in the spermatophore and conveyed to the female), and (2) capacitation (precedes migration to, and fertilization of, the ova); capacitation occurs only after the prospermia are transferred to the female. Capacitation is triggered by a MAG substance which is discussed below under 'Mating factors in ticks'. After capacitation, the prospermium is called a 'spermiophore' (synonymous with 'spermatozoon'). A detailed description of spermiogenesis and spermatozoan structure is presented by El Said *et al.* (1981).

In contrast to the Metastriata, sperm development in the Argasidae and Prostriata advances to the prospermia stage by the time of adult ecdysis, thus accounting for these ticks being able to copulate prior to feeding.

Sperm transfer

The following pertains generally to argasid and ixodid ticks. Once the male and female are in the copulatory position, the male pushes its mouthparts through the female gonopore into the vagina, and may maintain this position for 1–2 h. At least one function for this probing behaviour is to detect the genital sex pheromone (Sonenshine, 1991; see also chapter by Sonenshine in this Supplement). But perhaps this is not the only function because (1) the duration of the behaviour is so long and (2) probing occurs in both argasid and ixodid ticks, and only the latter are known to produce a genital sex pheromone. Whether or not the male can use its mouthparts to remove sperm deposited by a previous male has not been reported, although the frequent occurrence of several spermiophore masses (from multiple males) in the female genital tract (see below under 'Inhibition of receptivity in ticks') suggests that sperm removal does not occur. The spermatophore is then formed externally in less than a minute.

The ectospermatophore is a shell-like structure formed from a coagulum of mucopolysaccharides and proteins produced by the MAG (Feldman-Muhsam, 1986). Soon after the ectospermatophore is produced, the prospermia are ejaculated into it, followed immediately by a droplet containing yeast-like sperm symbionts (*Adlerocystis* spp.; Feldman-Muhsam, 1991). The last droplet secreted, the endospermatophore, seals off the ectospermatophore (Feldman-Muhsam, 1986).

Male ticks have no specialized copulatory organ, so their mouthparts push the tip of the spermatophore into the female's gonopore. While manipulating the spermatophore, the male also secretes saliva, which is believed to serve as a lubricant. Once the tip of the spermatophore has been inserted into the

vagina, the endospermatophore evaginates into the female genital tract. There it forms capsules into which the prospermia and sperm symbionts are ultimately received. The generation of CO_2 within the ectospermatophore constitutes the force causing the evagination of the spermatophore contents into the female, although the mechanism of CO_2 production is not known (Feldman-Muhsam *et al*. 1973).

MATING FACTORS OF MALE TICKS

A 'sperm capacitation factor' from argasid and ixodid ticks

As mentioned earlier, spermiogenesis comprises two phases: formation of the prospermia, and capacitation (also called 'spermateleosis' by some authors), which takes place in the female. After copulation, an operculum at the end of each prospermium ruptures, and the prospermium evaginates to about double its original length (Sahli, Germond & Diehl, 1985) within ~60–90 min (Shepherd, Oliver & Hall, 1982). The latter authors studied capacitation of prospermia in hanging drop cultures. They exposed prospermia of *Dermacentor variabilis* and *O. moubata* for 24 h to extracts of MAGs from fed males and found a detectable capacitation response with as little as 0·01–0·03 MAG per hanging drop (2–4 μl). The capacitation factor in both species was heat-stable but destroyed by trypsin. Gel filtration revealed a single peak of activity at about 12·5 kDa for both species. In spite of the above similarities, the MAG extract of one species (tested at one concentration) was inactive on the prospermia of the other.

Because capacitation involves rupture of the operculum, Shepherd *et al*. (1982) hypothesized that the factor might be a proteolytic enzyme. However, treatment of prospermia of *O. moubata* with trypsin and pronase for several hours did not cause significantly more capacitation than observed in the controls. Moreover, these treated prospermia underwent normal capacitation when exposed to MAG extracts. They then exposed fresh prospermia to a MAG extract of *O. moubata* for 0–50 min and examined the cultures at 24 h. Percent elongation was then compared to that of similar cultures exposed to a MAG extract continuously for 24 h. As little as 2 min exposure to the MAG extract caused a significant degree of prospermial elongation (20%); elongation was 40% following 15 or 50 min exposure and was 97% after continuous exposure. Taken together, the results suggest that the capacitation factor acts more as a signalling molecule than as an enzyme. Only a brief exposure is necessary to trigger an effect which is not manifested until later. Capacitation of tick sperm is somewhat reminiscent of the acrosome reaction in sperm of vertebrates and marine invertebrates, which is triggered by Ca^{2+}, and Shepherd *et al*. (1982) speculated that a similar mechanism may apply to tick sperm.

A 'vitellogenesis-stimulating factor' (VSF) from argasid ticks

When virgin *O. moubata* feed, they undergo egg development for about a month, and then begin resorbing the yolk, a process called 'abortive vitellogenesis' (Connat *et al*. 1986). Within about 100 days after feeding, the ovary again resembles that of an unfed virgin (Sahli *et al*. 1985; Connat *et al*. 1986). If ticks are mated in the absence of a bloodmeal, oviposition does not occur. However, if a fed virgin is mated, even 4–5 months following the meal, eggs are produced and laid; however, the egg mass is only about half that laid by females which mate at the time of feeding (Sahli *et al*. 1985; Connat *et al*. 1986).

Small metal beads inserted into the genital tracts of 'abortive virgins' stimulated a resumption of oogenesis, and many of these females oviposited an egg-batch (only 30% smaller than normal) after a slightly prolonged pre-oviposition period (20–25 days; Connat *et al*. 1986). Thus, in *O. moubata*, mechanical stimulation of the female's lower reproductive tract appears to be sufficient for a significant degree of egg development and oviposition.

The following summarizes the evidence suggesting that a chemical substance from the male (called VSF) also contributes to egg development. Homogenates of spermiophores taken from the seminal vesicles of *O. moubata* induce oviposition when injected into the haemocoels of abortive virgins (Ducommun, 1984); but homogenates of testis or MAGs do not have this effect (Connat *et al*. 1986). Mature spermiophores, washed for 20 h in a physiological medium prior to injection, retain VSF-activity, indicating that the factor is not a component of the testicular fluid. The wash medium, however, also contained some VSF-activity.

Sahli *et al*. (1985) described the details of capacitation in *O. moubata* and proposed a potential secretory mechanism for VSF. Between 1–48 h after copulation, endospermatophores were dissected out of females, and the sperm masses were washed and incubated for 12 h in a physiological medium containing antibiotics. The sperm masses were centrifuged and the supernatants tested for VSF-activity in a bioassay. No activity was found in the supernatant when the spermiophores were collected 1 h post-copulation; up to this time, evagination of the prospermia was incomplete. VSF was present in the supernatant 12–48 h post-copulation (capacitation complete), with the highest activity at 12 h. Also, VSF-activity was destroyed by proteinase-K. Sahli *et al*. (1985) concluded that VSF is a protein synthesized by and contained within the prospermia while in the male, but liberated into the female genital tract during capacitation. The supernatants

from the above experiments were subjected to electrophoresis; two proteins (~100 kDa and ~200 kDa) were particularly abundant in the 12 h supernatant. Sahli *et al*. (1985) proposed that these proteins constituted VSF.

A 'male factor' (MF) from ixodid ticks

The discovery of a protein (MF) from the male gonad of *Amblyomma hebraeum*, which exerts specific changes in female behaviour and physiology, arose from the following observations. Only a small minority of virgin females spontaneously feed beyond the CW, and none of these attain full engorgement even if left on a host for several weeks (Snow, 1969; Harris & Kaufman, 1984; Lomas & Kaufman, 1992*a*). SGs of mated or virgin females removed from the host below the CW do not degenerate within 14–21 days (Kaufman, 1983; Harris & Kaufman, 1984). SGs of mated females removed from the host above the CW degenerate within 4 days, whereas those of virgin females above the CW require 8 days to degenerate (Lomas & Kaufman, 1992*a*). Injecting an homogenate of male gonad (including or excluding the MAGs) into the haemocoel of virgin females above the CW, induces SG degeneration within 4 (reduced from 8) days (Lomas & Kaufman, 1992*a*).

MF acts by stimulating the synthesis of 20E once females exceed the CW. Thus, injecting a male gonad homogenate into virgin females above the CW increases the haemolymph titre of 20E to levels characteristic of weight-matched, normal mated females (Lomas & Kaufman, 1992*b*). MF is a protein (heat labile and inactivated by proteinase-K; Lomas & Kaufman, 1992*a*), in the range 20 to 100 kDa (Kaufman & Lomas, 1996). MF is a testicular protein, because the MAG has no MF-activity, and when partially purified homogenates of testis/vas deferens (T/VD) are layered on a discontinuous sucrose density gradient, MF-activity is not associated with the spermatozoa, which sediment to the bottom (Lomas & Kaufman, 1992*a*). MF-activity is found in abundance in the gonads of fed males, but is almost undetectable in the gonads of unfed males (Harris & Kaufman, 1984; Lomas & Kaufman, 1992*a*). MF-activity is detectable in the haemolymph of mated females above and below the CW, but not in the haemolymph of virgin females of any size (Harris & Kaufman, 1984). It is not known whether the MF-activity found in the haemolymph is due to MF itself or, analogous to the spermathecal factor of *Rhodnius* (Davey, 1967), to a substance secreted by the seminal receptacle after it receives a spermatophore.

An 'engorgement factor' (EF) from ixodid ticks

It has long been known that virgin females of ixodid ticks feed to only a fraction of the normal engorged weight range of mated females (e.g. Gregson, 1944; Sonenshine, 1967; Snow, 1969; Pappas & Oliver, 1971). Pappas and Oliver (1972) showed that female *D. variabilis* did not feed to repletion when exposed to males which had been irradiated or in which the genital aperture had been blocked. These experiments suggested that an EF, produced by the male, promotes feeding to repletion.

The observations of Pappas & Oliver (1972) have recently been extended to *A. hebraeum* (Weiss *et al*. 2002). We made a cDNA library from the T/VD portion of the male gonad and used a differential cross-screening approach to identify feeding-induced genes in this tissue. Thirty-five feeding-induced transcripts were identified, only two of which (AhT/VD16 and AhT/VD146) exhibited significant homologies to sequences in the Genbank. The predicted amino acid sequence of AhT/VD16 is 53% similar to human muscle-type acylphosphatase 2, and the predicted amino acid sequence of AhT/VD146 is 44% similar to a 9 kDa basic protein (of unknown function) from *Drosophila*.

We have recently produced recombinant proteins (*rec*proteins) from those feeding-induced transcripts which had full open reading frames, and have identified *rec*AhEF using a specific bioassay (Weiss & Kaufman, 2004); *rec*AhEF is neither AhT/VD16 nor AhT/VD146. *rec*AhEF comprises two peptides: *rec*AhEFα (16·1 kD) and *rec*AhEFβ (11·6 kD). Neither peptide has EF-activity on its own. Virgin females injected with *rec*AhEF engorged normally, achieved ovary weights significantly beyond those of control virgins (though less than those of normal mated females), and their SGs degenerated within 4 days. Virgin females injected with any other of the *rec*proteins did not feed beyond the CW, did not increase their ovary weights beyond those expected for small, partially-fed virgins, and their SGs did not degenerate within 4 days.

On the following grounds, we hypothesize that MF and EF are the same protein and that they differ from the two other known male factors in ticks. (1) Both are found in homogenates of T/VD, whereas VSF is believed to be secreted by the prospermia and capacitation factor by the MAG. (2) Both are virtually undetectable in the gonads of unfed males, but are detectable at high levels in the gonads of fed males. (3) The MW of MF (20–100 kDa) is different from that of the ixodid and argasid capacitation factors (12·5 kDa) and VSF (100–200 kDa). The MW of natural EF has not yet been determined, but the combined MW of *rec*AhEFα and *rec*AhEFβ is 27·7 kDa (Weiss & Kaufman, 2004). (4) As shown immediately above, *rec*AhEF (but none of the other *rec*proteins) also triggered SG degeneration and stimulated ovary growth – two known effects of MF. We propose the name 'voraxin' for the natural EFs/MFs of ixodid ticks (from the Latin, *vorax*, 'gluttonous', 'voracious').

Site of action of the male factors

The sperm capacitation factor acts directly on the prospermia. The fact that VSF is effective when injected into the haemocoel suggests that the target tissue is outside of the female genital tract. Whether VSF acts directly on the fat body (which synthesizes vitellogenin) or on the neuroendocrine system is not known. However, the synganglion contains at least several groups of NSCs, some of which appear to become active during egg development (Shanbaky, El-Said & Helmy, 1990). Also, homogenates of synganglion from mated fed females (but not from virgin fed or unfed) stimulate oogenesis, but not oviposition (Connat *et al*. 1986).

As MF in *A. hebraeum* stimulates ecdysteroid synthesis (Lomas & Kaufman, 1992*b*), and as ecdysteroid synthesis by the epidermis of *A. hebraeum* is controlled by a neuropeptide (Lomas, Turner & Rees, 1997), MF also may act via NSCs in the synganglion. Alternatively, MF might act directly on the epidermis to stimulate ecdysteroid synthesis. Finally, it is conceivable that the EF-effect of voraxin is mediated by NSCs and the MF-effect via the epidermis.

DO PROSTAGLANDINS PLAY A ROLE IN TICK REPRODUCTION?

Prostaglandins (PGs) were originally detected in the semen of mammals, and are present there in astonishingly high concentration (20–25 μg/ml; Horrobin, 1978). Prostatic fluid stimulates sperm motility and sensitizes the uterine myometrium to seminal PGs in such a way as to cause the sperm to be sucked into the uterus (Mortimer, 1983). The pharmacological actions of PGs are far too diverse to consider here. Suffice it to say that they have direct actions on numerous smooth muscles, within and without the reproductive system, and modulate the effects of numerous other signalling molecules (Horrobin, 1978; Finn, 1983; Poyser, 1987). Thus PGs promote fecundity in mammals, including humans.

PGs also stimulate oviposition in the house cricket, *Acheta domesticus* (Destephano, Brady & Farr, 1982). Inoculation of gravid, virgin females with PGE_2 or $PGF_{2\alpha}$ increased oviposition by the next day from 2 eggs/female (control) to 114 eggs/female (Destephano & Brady, 1977). Although the pharmacological effect of PGs implies that cricket ovaries can synthesize them, PG-synthetase activity is absent from ovaries of virgin and mated females (Destephano & Brady, 1977). In contrast, mated female reproductive tracts show significant PG-synthetase activity, as do testes, seminal vesicles/vas deferentia and spermatophores. The PG-content of various tissues is even more revealing. The testes and spermatophores contain significant levels of PGs, as do the reproductive tracts of mated (but not virgin) females. PG titres in the mated female reproductive tract are much higher than can be accounted for by the contents of several spermatophores. These results indicate that mated females use the PG synthetase delivered by the spermatophore to synthesize PGs from arachidonic acid produced by the female. Support for this hypothesis come from observations that egg-laying is inhibited by over 90% when acetaminophen (a PG synthetase inhibitor) is injected into normal mated females (Destephano & Brady, 1977).

The site of PG action (whether directly on the ovaries or via the neuroendocrine system) is not known. Destephano, Brady & Lovins (1974) suggested that PGs do not stimulate contractions in the ovarian sheath. However, the fact that as little as 2 ng PGE_2 injected directly into the common oviduct induces oviposition, whereas microgram quantitites are required if injected into the haemocoel, suggests that PGs probably exert their effects within the female reproductive tract. If so, the site of action is not the spermatheca, because PGE_2 injected into the haemocoel still stimulates oviposition in spermathectomized females (Loher *et al*. 1981). More than one PG-receptor likely exists in the oviduct of *Acheta*, because PGE_2 increases cyclic AMP production marginally, whereas $PGF_{2\alpha}$ inhibits octopamine-induced cyclic AMP production in the same tissue (A. Bowman, personal communication). The transfer of PG synthetase from male to female also occurs in the Australian field cricket, *Teleogryllus commodus* (Loher *et al*. 1981).

Although a potential role for PGs in reproduction has been best elucidated in crickets, PGs have also been investigated in other insects (e.g. Yamaja Setty & Ramaiah, 1979, 1980; Lange, 1984; Wakayama, Dillwith & Blomquist, 1986; Brenner & Bernasconi, 1989; Medeiros *et al*. 2002).

Because PGs have pharmacological actions, all of which would promote the ability of ticks to feed (anti-haemostatic, vasodilatory, immunosuppressive and anti-inflammatory), it is not surprising that most studies have concentrated on the role of PGs at the host–tick interface (Bowman, Dillwith & Sauer, 1996; Aljamali *et al*. 2002). PGs are synthesized in ticks from arachidonic acid imbibed in the blood meal, and are found in tick saliva of several species at concentrations 10–100 fold higher than in mammalian inflammatory exudates (Bowman *et al*. 1996).

I am aware of only one study which reports that PGs occur in the reproductive organs of ticks. Shemesh *et al*. (1979) measured the endogenous levels of PGF and PGE_2 in a number of tissues (testis, ovary and SGs from male and female *Hyalomma anatolicum excavatum*). Shemesh *et al*. (1979) also examined the ability of these tissues to synthesize PGs over 72 h using an organ culture technique. Under these conditions, all tissues synthesized some PGF and PGE_2. The endogenous PG levels and

amounts synthesized were similar in testis and ovary. However, whether PGs are incorporated within components of the spermatophore is not known (Sonenshine, 1991).

Oliver, Pound & Andrews (1984) demonstrated in male *O. parkeri* that SG extracts, injected into the haemocoel of fed virgins, stimulated some of them to oviposit. When injected into the vagina, however, SG extracts stimulated some degree of oocyte maturation but not oviposition. As mentioned earlier, it has long been assumed that males salivate during copulation in order to lubricate the spermatophore (Feldman-Muhsam, 1986), but the results of Oliver *et al.* (1984) raise the possibility that, perhaps because of its PG-content, male saliva may also play a role in oocyte development.

Pappas & Oliver (1972) blocked the gonopores of male *D. variabilis* with wax and put them with feeding virgins. Although the males could not produce spermatophores, they still probed the female gonopore with their mouthparts and presumably could still secrete saliva. The females did not engorge fully, but they fed to a weight (mean of 160 mg; well above the CW for *Dermacentor*) significantly beyond that of control virgins not exposed to males (mean of 37 mg). They also eventually laid small, infertile egg batches. The extent to which this enhanced degree of feeding and egg development was due to the mechanical stimuli of male–female contact, or to the secretion of saliva into the female's reproductive tract, was not tested. Recall from above, however, that mechanical stimulation of the female genital tract of abortive virgin *O. moubata* is sufficient to trigger egg development and oviposition (Connat *et al.* 1986). In *H. a. excavatum*, endogenous amounts of PGs in the SGs, and the ability of these ticks to synthesize PGs were significantly higher in fed females than in fed males (Shemesh *et al.* 1979). These observations are not inconsistent with a role for male-derived PGs in reproduction for the following reason: The latter authors neglected to normalize their PG-values for amount of tissue. The SGs of fed females (*A. hebraeum*) are about 10 times heavier than those of males (Kaufman, 1976), so if a similar difference holds for *Hyalomma*, the PG-concentration in male SGs would be significantly higher than that in female glands.

Inhibition of receptivity in ticks

The weight of current evidence indicates that inhibition of receptivity does not occur in mated female ticks. As early as 1948, Lees & Beament reported finding up to 12 sperm capsules in the genital tract of *O. moubata* females. When a number of virgin *O. moubata* were kept for 24 h with 5 males each, the females engaged in multiple copulations, in most cases with several partners, and *O. tholozani* females are said to be able to copulate even while ovipositing (Feldman-Muhsam, 1986). Most argasid ticks require a meal in order to produce each batch of eggs. They will readily copulate before or after each bloodmeal, although only the initial copulation is necessary to produce several batches of fertile eggs (Aeschlimann & Grandjean, 1973; Connat *et al.* 1986).

Ixodid females produce only a single large batch of eggs and then die, so they probably require only a single copulation. Although the seminal receptacle may contain multiple sperm capsules (Feldman-Muhsam, 1986), it is not clear whether these come from the same or different males. The following study, however, demonstrates that ixodid females can also mate with multiple partners.

Sperm precedence in ticks

Yuval & Spielman (1990), taking advantage of the fact that prostriate ticks are able to mate prior to feeding (preprandial) as well as during feeding (perprandial), conducted a study on sperm precedence in *Ixodes dammini* (more commonly known as *I. scapularis*). A group of males were sterilized by exposure to cobalt-60 irradiation. When 22 such males were fed in the company of females, none of the laid eggs hatched. One group of virgin females was then kept (off the host) with irradiated males and another group with normal males for 2 weeks. Following this, the females were placed on a host, but this time in the company of the opposite group of males. Thus, females mated at least twice, each time with a different male. The results indicated that mated females remain receptive to subsequent males, and that sperm from a second mating takes precedence over that of the first. Thus, when the first male was irradiated and the second normal, more than 75% of the eggs of 78% of the ovipositing females hatched, whereas less than 25% of the eggs of the remaining females hatched. However, when the first male was normal and the second irradiated, less than 25% of the eggs of 86% of the ovipositing females hatched, and all of the eggs of the remaining females hatched.

Yuval & Spielman (1990) also showed that irradiated male *I. scapularis* can copulate normally. Twenty-five virgin females were placed on each ear of a rabbit with 20 normal plus 20 irradiated males. Of the egg batches laid by the engorged females, 49% showed no hatching, in 43% the hatch was greater than 75% and in the remaining 8% the hatch was 25–50%, results to be expected if all males were equally competitive. Irradiated males of *Argas persicus* likewise compete successfully for females (Sternberg, Peleg & Galun, 1973).

The results of Yuval & Spielman (1990) suggest that a male *I. scapularis* engaging in preprandial mating will be successful in passing his genes to the next generation only if the female, on finding a host, does not encounter another male. Otherwise, his

paternity will pass to only a minority of her eggs. (By the same token, the female benefits from preprandial mating whether or not she encounters another male on the host.) Also, the viability of sperm in the female reproductive tract falls progressively over 8 weeks (Gladney & Drummond, 1971). The foregoing arguments perhaps explain Yuval & Spielman's (1990) observation that the incidence of insemination in female *I. scapularis* collected from vegetation in the wild, though significant (30–72% had copulated preprandially), was somewhat lower than that in females collected from deer (90–100% had copulated perprandially). These results also explain the male's tendency to remain in close contact with the female throughout the feeding period (an example of proximate mate-guarding): his physical proximity would preclude the advances of other males (Kiszewski, Matuschka & Spielman, 2001). Perhaps the practice of proximate mate-guarding by male ticks accounts for the lack of remote mate-guarding (i.e. an RIS). A similar situation occurs in the male desert locust which, during the gregarious phase, breeds under crowded conditions. The male remains on the female throughout the period of oviposition, for if the pair is separated before then, the female will accept another male immediately (Seidelmann & Ferenz, 2002).

Voraxin (EF/MF) is clearly an FES, because egg production is much reduced in females which exceed the CW but which do not engorge (Kaufman & Lomas, 1996). Voraxin and the VSF in argasid ticks stimulate vitellogenesis (Weiss & Kaufman, 2004; Connat *et al*. 1986), and following stimulation, some days are required for oviposition to occur. Most of the insect FESs described earlier stimulate ovulation and/or oviposition, and so their effects are often manifested within only a matter of hours. A notable exception was the effect of MAG extracts on mosquitoes, which also acts at the level of vitellogenesis (Borovsky, 1985; Klowden & Chambers, 1991). Does this suggest that the blood-sucking lifestyle may be a factor in determining the level (vitellogenesis *vs*. ovulation or oviposition) at which mating factors act? A few prominent exceptions suggest otherwise. We have already seen that in *Rhodnius*, the male's influence is not manifested at the level of vitellogenesis but at ovulation and/or oviposition (Davey, 1967; Kriger & Davey, 1982, 1983). Also, there are at least a few instances among non-haematophagous insects from several orders, including *Drosophila*, in which MAG secretions promote egg development, apparently by stimulating JH-synthesis by the corpora allata (reviewed by Gillott, 2003).

FUTURE DIRECTIONS

Although P2 precedence has been demonstrated in an argasid (Sternberg *et al*. 1973) and an ixodid tick (Yuval & Spielman, 1990), the mechanism (sperm stratification, sperm removal by probing mouthparts, or sperm incapacitation) has not been explored. In at least one tick (*A. americanum*), sperm from fed males remained viable within the unfed female for up to 8 weeks, although viability declined progressively over that time (Gladney & Drummond, 1971). The mechanisms for sperm storage in ticks are unknown, and the viability of such sperm in partially-fed females is not known. But consider that a mated female can be dislodged from a host in the partially-fed state by grooming or by a variety of other means. If it is below the CW after removal, it will be able to seek another host to complete feeding (Kaufman & Lomas, 1996; Lomas & Kaufman, 1999). For how long can the sperm already received remain viable in relation to the time likely needed to find another host? As mentioned earlier, Feldman-Muhsam (1986) reported seeing mobile sperm in *O. savignyi* dissected 1 year following copulation, so sperm probably remains viable for extended periods.

Recall that the spermathecal factor of *Rhodnius* (Kuster & Davey, 1986) and Acp36DE of *Drosophila* (Chapman *et al*. 2000) promote sperm viability within the spermatheca. The yeast-like sperm symbiont, *Adlerocystis* spp., potentially has a similar role in ticks (Feldman-Muhsam, 1991). *Adlerocystis* develops in the posterior lobes of the MAG and is packaged in the spermatophore, separate from the sperm. The symbiont encounters the sperm only after copulation, and in argasid species (though not in ixodid species) the symbiont attaches to the spermiophores over the next day or so (Feldman-Muhsam, 1991). A unique observation of two sperm capsules within a single female *O. tholozani*, one with dead sperm (and lacking *Adlerocystis*), and the other with live sperm (containing *Adlerocystis*) suggested that the symbiont might promote sperm longevity in the female (Feldman-Muhsam, 1991). Most of these observations were made in the 1960s and 1970s, and should be extended.

It is not known whether the seminal fluid of ixodid males contains a myotropin, analogous to that of *Rhodnius*, which triggers ovulation and/or oviposition. That seems unlikely, however, because in ticks, copulation normally occurs long before vitellogenesis and oviposition. For example, Mao & Kaufman (1999) demonstrated that 69% of female *A. hebraeum* had mated by the 5th day of feeding. In this population of ticks, the normal time to engorgement was about day 12, the initiation of vitellogenesis was about day 16 and the beginning of oviposition was about day 22. However, tick semen may contain a myotropic peptide for some other function (e.g. for the transport of sperm within the genital tract, as occurs in *Rhodnius*; Davey, 1958).

The role for NSCs in the control of egg development has been demonstrated in several ticks, and the stimulation of specific NSCs by feeding has been well

documented (reviewed by Kaufman, 1997; Chang & Kaufman, 2004). It is less clear, however, whether mating also plays a role in NSC-activation, because in most previous studies, the synganglia extracts tested for stimulating egg development and/or oviposition were taken from fed, mated females.

The role for PGs in reproduction, irrespective of its source (the male's saliva, its spermatophore, or endogenous to the female), has not yet been adequately explored in ticks. Kaufman & Lomas (1996) considered the possibility that MF might be a PG or PG-synthetase. But PGs infused into the haemocoel of virgin females above the CW, did not reduce salivary fluid secretion, as would have been expected for a substance having MF-activity. Quite the contrary, $PGF_{2\alpha}$ and PGE_2 both significantly increased salivary fluid secretion, which is not surprising considering that PGE_2 also stimulates protein secretion by SGs (Sauer, Essenberg & Bowman, 2000). Even though MF is unlikely to be a PG-synthetase, PGs may play other roles in reproduction.

Does the seminal fluid of ticks contain antimicrobial substances? Tick haemolymph contains defensin (van der Goes van Naters-Yasui *et al*. 2000; Johns *et al*. 1998, 2001) but the extent to which it appears in various secretions is not known. A substance in tick egg wax inhibits bacterial growth (unpublished observations, and P. A. Diehl, personal communication). This substance might be secreted by Gené's organ (the major source of the egg wax), although it remains to be seen whether it is a defensin (peptide) or a sterol amide-like substance similar to boophiline (Potterat *et al*. 1997).

The morphology and histology of the MAGs have been described for several ticks (e.g. Khalil, 1970). The MAG is probably the largest gland in the male body, and consists of 14 lobes in *O*. (*Pavlovskyella*) *erraticus*, showing characteristic histological differences (El Shoura, 1987) but, as outlined in this review, very little is known about the variety of substances produced by tick MAGs.

Proximate mate-guarding has been well-established in ticks, and this seems understandable considering the crowded conditions under which they often feed. Moreover, Wang *et al*. (1998) demonstrated that male *Rhipicephalus appendiculatus* secrete an immunoglobulin-binding protein (IgBP) into the feeding site shared with the female. This IgBP leads to a 24% enhancement of female engorged weight, which in turn would lead to a larger egg batch carrying his genes. But the existence of mating plugs (unlikely), or of putative pheromones inhibiting rival males, have not been adequately investigated.

The VSF of *Ornithodoros* and the voraxin of *A. hebraeum* both stimulate fecundity. However, the differences between them (voraxin acts by stimulating engorgement, VSF does not; voraxin is in the testicular fluid, VSF is produced in the prospermia; their molecular sizes are different) may not be as substantial as appear at first sight. Both may be transported from the seminal receptacle to the haemolymph, and a reasonable hypothesis for both is that they act at the synganglion. Molecular homology between the two factors should be investigated.

In conclusion, this comparative view of male factors that affect female reproductive behaviour and physiology indicates that less is known about the male tick's influences on the female than is known for several insects. Many of the most recent citations on male tick reproductive physiology referred to here are 10 or more years old, a sad reflection of how little attention has been devoted to this topic in recent years. However, we know enough already to formulate some interesting questions that can be easily answered with current biochemical and molecular biological techniques. Let us hope that more experimenters will answer the call.

ACKNOWLEDGEMENTS

I am most grateful to Dr Cedric Gillott, Department of Biology, University of Saskatchewan, Canada, who thoroughly reviewed an early draft of the manuscript and recommended numerous, substantial revisions. I also thank Dr Brian Weiss, Department of Epidemiology and Public Health, Yale School of Medicine, Dr A. S. Bowman, Department of Zoology, University of Aberdeen, Scotland and Dr P. A. Diehl, Institut de Zoologie, Université de Neuchâtel, Switzerland, for their valuable insights on various sections of the manuscript. Research in my laboratory is generously supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada.

REFERENCES

ADLAKHA, V. & PILLAI, M. K. K. (1975). Involvement of male accessory gland substances in the fertility of mosquitoes. *Journal of Insect Physiology* **21**, 1453–1455.

AESCHLIMANN, A. A. & GRANDJEAN, O. (1973). Observations on fecundity in *Ornithodoros moubata* Murray (Ixodoidea: Argasidae). Relationships between mating and oviposition. *Acarologia* **15**, 206–217.

ALJAMALI, M., BOWMAN, A. S., DILLWITH, J. W., TUCKER, J. S., YATES, G. W., ESSENBERG, R. C. & SAUER, J. R. (2002). Identity and synthesis of prostaglandins in the lone star tick, *Amblyomma americanum* (L.), as assayed by radio-immunoassay and gas chromatography/mass spectrometry. *Insect Biochemistry and Molecular Biology* **32**, 331–341.

ANDERSON, R. B., SCRIMGEOUR, G. J. & KAUFMAN, W. R. (1998). Responses of the tick, *Amblyomma hebraeum* (Acari: Ixodidae), to carbon dioxide. *Experimental and Applied Acarology* **22**, 667–681.

AUSTAD, S. N. (1982). First male sperm priority in the bowl and doily spider, *Frontinella pyramitela* (Walckenaer). *Evolution* **36**, 777–785.

AUSTAD, S. N. (1984). Evolution of sperm priority patterns in spiders. In *Sperm Competition and the Evolution of Animal Mating Systems* (ed. Smith, R. L.), pp. 223–249. Orlando and San Diego, Academic Press.

BIRKHEAD, T. R. (1998). Cryptic female choice: Criteria for establishing female sperm choice. *Evolution* **52**, 1212–1218.

BOROVSKY, D. (1985). The role of male accessory gland fluid in stimulating vitellogenesis in *Aedes taeniorhynchus*. *Archives of Insect Biochemistry and Physiology* **2**, 405–413.

BOWMAN, A. S., DILLWITH, J. W. & SAUER, J. R. (1996). Tick salivary prostaglandins: Presence, origin and significance. *Parasitology Today* **12**, 388–396.

BRENNER, R. R. & BERNASCONI, A. (1989). Prostaglandin biosynthesis in the gonads of the hematophagus insect *Triatoma infestans*. *Comparative Biochemistry and Physiology* **93B**, 1–4.

CHANG, E. S. & KAUFMAN, W. R. (2005). Endocrinology of Crustacea and Chelicerata. In *Volume 3* (*Endocrinology*) *Comprehensive Insect Science* (ed. Gilbert, L. I., Iatrou, K. & Gill, S. S.), Oxford, Elsevier, pp. 805–842.

CHAPMAN, T., NEUBAUM, D. M., WOLFNER, M. F. & PARTRIDGE, L. (2000). The role of male accessory gland protein Acp36DE in sperm competition in *Drosophila melanogaster*. *Proceedings of the Royal Society of London B* **267**, 1097–1105.

CHEN, P. S. & BÜHLER, R. (1970). Paragonial substance (sex peptide) and other free ninhydrin-positive components in male and female adults of *Drosophila melanogaster*. *Journal of Insect Physiology* **16**, 615–627.

CHEN, P. S., STUMM-ZOLLINGER, E., AIGAKI, T., BALMER, J., BIENZ, M. & BOHLEN, P. (1988). A male accessory gland peptide that regulates reproductive behaviour of female *D. melanogaster*. *Cell* **54**, 291–298.

CONNAT, J.-L., DUCOMMUN, J., DIEHL, P.-A. & AESCHLIMANN, A. A. (1986). Some aspects of the control of the gonotrophic cycle in the tick, *Ornithodoros moubata* (Ixodoidea, Argasidae). In *Morphology, Physiology and Behavioural Biology of Ticks* (ed. Sauer, J. R. & Hair, J. A.), pp. 194–216. Chichester, UK, Ellis Horwood Publishers.

CRAIG, G. B. Jr. (1967). Mosquitoes: Female monogamy induced by male accessory gland substance. *Science* **156**, 1499–1501.

DAVEY, K. G. (1958). The migration of spermatozoa in the female of *Rhodnius prolixus* Ståhl. *Journal of Experimental Biology* **35**, 694–701.

DAVEY, K. G. (1965). Copulation and egg production in *Rhodnius prolixus*: The role of the spermathecae. *Journal of Experimental Biology* **42**, 373–378.

DAVEY, K. G. (1967). Some consequences of copulation in *Rhodnius prolixus*. *Journal of Insect Physiology* **13**, 1629–1636.

DESTEPHANO, D. B. & BRADY, U. E. (1977). Prostaglandin and prostaglandin synthetase in the cricket *Acheta domesticus*. *Journal of Insect Physiology* **23**, 905–911.

DESTEPHANO, D. B., BRADY, U. E. & FARR, C. A. (1982). Factors influencing oviposition behaviour in the cricket *Acheta domesticus*. *Annals of the Entomological Society of America* **75**, 111–114.

DESTEPHANO, D. B., BRADY, U. E. & LOVINS, R. E. (1974). Synthesis of prostaglandins by reproductive tissue of the house cricket, *Acheta domesticus*. *Prostaglandins* **6**, 71–79.

DIEHL, P. A., AESCHLIMANN, A. A. & OBENCHAIN, F. D. (1982). Tick Reproduction: Oogenesis and Oviposition. In *Physiology of Ticks* (ed. Obenchain, F. D. & Galun, R.), pp. 277–350. Oxford, Pergamon Press.

DUCOMMUN, J. (1984). Contribution á la connaissance de la reproduction chez la tique *Ornithodoros moubata*, Murray, 1877; sensu Walton, 1962 (Ixodoidea: Argasidae). Thesis, University of Neuchâtel, Switzerland, 138 pp.

EBERHARD, W. G. (1998). Female roles in sperm competition. In *Sperm Competition and Sexual Selection* (ed. Birkhead, T. R. & Møller, A. P.), pp. 91–116. San Diego, Academic Press.

ELGAR, M. A. (1998). Sperm competition and sexual selection in spiders and other arachnids. In *Sperm Competition and Sexual Selection* (ed. Birkhead, T. R. & Møller, A. P.), pp. 307–339. San Diego, Academic Press.

EL SAID, A., SWIDERSKI, Z., AESCHLIMANN, A. & DIEHL, P. A. (1981). Fine structure of spermiogenesis in the tick *Amblyomma hebraeum* (Acari: Ixodidae): Late stages of differentiation and structure of the mature spermatozoon. *Journal of Medical Entomology* **18**, 464–476.

EL SHOURA, S. (1987). Fine structure of the vasa deferentia, seminal vesicle, ejaculatory duct and accessory gland of male *Ornithodoros* (*Pavloskkyella*) *erraticus* (Acari: Ixodoidea: Argasidae). *Journal of Medical Entomology* **24**, 235–242.

FELDMAN-MUHSAM, B. (1986). Observations on the mating behaviour of ticks. In *Morphology, Physiology and Behavioural Biology of Ticks* (ed. Sauer, J. R. & Hair, J. A.), pp. 217–232. Chichester, UK, Ellis Horwood Publishers.

FELDMAN-MUHSAM, B. (1991). The role of *Adleorcystis* sp. in the reproduction of argasid ticks. In *The Acari: Reproduction, Development and Life-history Strategies* (ed. Schuster, R. & Murphy, P. W.), pp. 179–190. London, Chapman & Hall.

FELDMAN-MUHSAM, B., BORUT, S., SALITERNIK-GIVANT, S. & EDEN, C. (1973). On the evacuation of sperm from the spermatophore of the tick *Ornithodoros savignyi*. *Journal of Insect Physiology* **19**, 951–962.

FINN, C. A. (ed.) (1983). *Oxford Reviews of Reproductive Biology*, Vol. 5. Oxford, Clarendon Press.

FRIEDEL, T. & GILLOTT, C. (1977). Contribution of male-produced proteins to vitellogenesis in *Melanoplus sanguinipes*. *Journal of Insect Physiology* **23**, 145–151.

FRIESEN, K. & KAUFMAN, W. R. (2002). Quantification of vitellogenesis and its control by 20-hydroxyecdysone in the ixodid tick, *Amblyomma hebraeum*. *Journal of Insect Physiology* **48**, 773–782.

FUCHS, M. S., CRAIG, G. B. Jr. & HISS, E. A. (1968). The biochemical basis of female monogamy in mosquitoes. I. Extraction of the active principle from *Aedes aegypti*. *Life Sciences* **7**, 835–839.

GILLOTT, C. (1988). Arthropoda–Insecta. In *Reproductive Biology of Invertebrates*, Volume III, *Accessory Sex Glands* (ed. Adiyodi, K. G. & Adiyodi, R. G.), pp. 319–471. Chichester, New York, Brisbane, Toronto, Singapore, John Wiley & Sons.

GILLOTT, C. (1996). Male insect accessory glands: Functions and control of secretory activity. *Invertebrate Reproduction and Development* **30**, 199–205.

GILLOTT, C. (2003). Male accessory gland secretions: Modulators of female reproductive physiology and behavior. *Annual Reveiw of Entomology* **48**, 163–184.

GLADNEY, W. J. & DRUMMOND, R. O. (1971). Spermatophore transfer of lone star ticks off the host. *Annals of the Entomological Society of America* **64**, 379–381.

GREGSON, J. D. (1944). The influence of fertility on the feeding rate of the female of the Wood Tick, *Dermacentor andersoni* Stiles. *Entomological Society of Ontario* **74**, 46–47.

HARRIS, R. A. & KAUFMAN, W. R. (1981). Hormonal control of salivary gland degeneration in the ixodod tick *Amblyomma hebraeum*. *Journal of Insect Physiology* **27**, 241–248.

HARRIS, R. A. & KAUFMAN, W. R. (1984). Neural involvement in the control of salivary gland degeneration in the ixodid tick *Amblyomma hebraeum*. *Journal of Experimental Biology* **109**, 281–290.

HARRIS, R. A. & KAUFMAN, W. R. (1985). Ecdysteroids: Possible candidates for the hormone which triggers salivary gland degeneration in the ixodid tick *Amblyomma hebraeum*. *Experientia* **41**, 740–742.

HARTMANN, R. & LOHER, W. (1996). Control mechanisms of the behavior 'secondary defense' in the grasshopper *Gomphocerus rufus* L. (Gomphocerinae: Orthoptera). *Journal of Comparative Physiology A* **178**, 329–336.

HARTMANN, R. & LOHER, W. (1999). Post-mating effects in the grasshopper, *Gomphocerus rufus* L. mediated by the spermatheca. *Journal of Comparative Physiology A* **184**, 325–332.

HISS, E. A. & FUCHS, M. S. (1972). The effect of matrone on oviposition in the mosquito, *Aedes aegypti*. *Journal of Insect Physiology* **18**, 2217–2225.

HORROBIN, D. F. (1978). *Prostaglandins: Physiology, Pharmacology and Clinical Significance*. Montreal, Eden Press.

JOHNS, R., SONENSHINE, D. E. & HYNES, W. L. (1998). Control of bacterial infections in the hard tick, *Dermacentor variabilis* (Acari: Ixodidae): Evidence for the existence of antimicrobial proteins in tick hemolymph. *Journal of Medical Entomology* **35**, 458–464.

JOHNS, R., SONENSHINE, D. E. & HYNES, W. L. (2001). Identification of a defensin from the hemolymph of the American dog tick, *Dermacentor variabilis*. *Insect Biochemistry and Molecular Biology* **31**, 857–865.

KALB, J. M., DiBENEDETTO, A. J. & WOLFNER, M. F. (1993). Probing the function of *Drosophila melanogaster* accessory glands by directed cell ablation. *Proceedings of the National Academy of Sciences, USA* **90**, 8093–8097.

KAUFMAN, W. R. (1976). The influence of various factors on fluid secretion by *in vitro* salivary glands of ixodid ticks. *Journal of Experimental Biology* **64**, 727–742.

KAUFMAN, W. R. (1983). The function of tick salivary glands. *Current Topics in Vector Research* **1**, 215–247.

KAUFMAN, W. R. (1991). Correlation between haemolymph ecdysteroid titre, salivary gland degeneration and ovarian development in the ixodid tick, *Amblyomma hebraeum* Koch. *Journal of Insect Physiology* **37**, 95–99.

KAUFMAN, W. R. (1997). Arthropoda–Chelicerata. In *Reproductive Biology of Invertebrates* (series editors: Adiyodi, K. G. & Adiyodi, R.) Vol. 8, Part A, *Progress in Reproductive Endocrinology* (ed. Adams, T. S.), pp. 211–245. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India, and Wiley, NY.

KAUFMAN, S. E., KAUFMAN, W. R. & PHILLIPS, J. E. (1981). Fluid balance in the argasid tick, *Ornithodorus moubata*, fed on modified blood meals. *Journal of Experimental Biology* **93**, 225–242.

KAUFMAN, S. E., KAUFMAN, W. R. & PHILLIPS, J. E. (1982). Mechanism and characteristics of coxal fluid execretion in the argasid tick *Ornithodorus moubata*. *Journal of Experimental Biology* **98**, 343–352.

KAUFMAN, W. R. & LOMAS, L. O. (1996). 'Male Factors' in ticks: Their role in feeding and egg development. *Invertebrate Reproduction and Development* **30**, 191–198.

KEIRANS, J. E., CLIFFORD, C. M., HOOGSTRAAL, H. & EASTON, E. R. (1976). Discovery of *Nuttalliella namaqua* Bedford (Acarina: Ixodoidea: Nutalliellidae) in Tanzania and redescription of the female based on scanning electron microscopy. *Annals of the Entomological Society of America* **69**, 926–932.

KHALIL, G. M. (1970). Biochemical and physiological studies of certain ticks (Ixodoidea). Gonad development and gametogenesis in *Hyolomma* (*H.*) *anatolicum excavatum* Koch (Ixodidae). *Journal of Parasitology* **56**, 596–610.

KIZEWSKI, A. E., MATUSCHKA, F.-R. & SPIELMAN, A. (2001). Mating strategies and spermiogenesis in ixodid ticks. *Annual Review of Entomology* **46**, 167–182.

KLOWDEN, M. J. (1999). The check is in the male: Male mosquitoes affect female physiology and behavior. *Journal of the American Mosquito Control Association* **15**, 213–220.

KLOWDEN, M. J. (2001). Sexual receptivity in *Anopheles gambiae* mosquitoes: Absence of control by male accessory gland substances. *Journal of Insect Physiology* **47**, 661–666.

KLOWDEN, M. J. & CHAMBERS, G. M. (1991). Male accessory gland substances activate egg development in nutritionally stressed *Aedes aegypti* mosquitoes. *Journal of Insect Physiology* **37**, 721–726.

KRIGER, F. L. & DAVEY, K. G. (1982). Ovarian motility in mated *Rhodnius prolixus* requires an intact cerebral neurosecretory system. *General and Comparative Endocrinology* **48**, 130–134.

KRIGER, F. L. & DAVEY, K. G. (1983). Ovulation in *Rhodnius prolixus* Stål is induced by an extract of neurosecretory cells. *Canadian Journal of Zoology* **61**, 684–686.

KUSTER, J. E. & DAVEY, K. G. (1986). Mode of action of cerebral neurosecretory cells on the function of the spermatheca in *Rhodnius prolixus*. *International Journal of Invertebrate Reproduction and Development* **10**, 59–69.

LANGE, A. B. (1984). The transfer of prostaglandin-synthesizing activity during mating in *Locusta migratoria*. *Insect Biochemistry* **14**, 551–556.

LEAHY, M. G. & LOWE, M. L. (1967). Purification of the male factor increasing egg deposition in *D. melanogaster*. *Life Sciences* **6**, 151–156.

LEES, A. D. & BEAMENT, J. W. L. (1948). An egg-waxing organ in ticks. *Quarterly Journal of Microscopical Science* **89**, 291–232.

LOHER, W., GANJIAN, I., KUBO, I., STANLEY-SAMUELSON, D. & TOBE, S. S. (1981). Prostaglandins: Their role in egg-laying of the cricket *Teleogryllus commodus*. *Proceedings of the National Academy of Sciences, USA* **78**, 7835–7838.

LOMAS, L. O. & KAUFMAN, W. R. (1992*a*). The influence of a factor from the male genital tract on salivary gland degeneration in the female ixodid tick *Amblyomma hebraeum*. *Journal of Insect Physiology* **38**, 595–601.

LOMAS, L. O. & KAUFMAN, W. R. (1992*b*). An indirect mechanism by which a protein from the male gonad

hastens salivary gland degeneration in the female ixodid tick *Amblyomma hebraeum*. *Archives of Insect Biochemistry and Physiology* **21**, 169–178.

LOMAS, L. O. & KAUFMAN, W. R. (1999). What is the meaning of 'critical weight' to female ixodid ticks?: A 'grand unification theory'! In *Acarology IX*, Volume 2, Symposia (ed. Needham, G. R., Mitchell, R., Horn, D. J. & Welbourn, W. C.), pp. 481–485. Columbus, Ohio, The Ohio Biological Survey.

LOMAS, L. O., TURNER, P. C. & REES, H. H. (1997). A novel neuropeptide–endocrine interaction controlling ecdysteroid production in ixodid ticks. *Proceedings of the Royal Society of London B* **264**, 589–596.

LUNG, O., KUO, M. F. & WOLFNER, M. F. (2001*a*). Identification and characterization of the major *Drosophila melanogaster* mating plug protein. *Insect Biochemistry and Molecular Biology* **31**, 543–551.

LUNG, O., KUO, M. F. & WOLFNER, M. F. (2001*b*). *Drosophila* males transfer antibacterial proteins from their accessory gland and ejaculatory duct to their mates. *Journal of Insect Physiology* **47**, 617–622.

LUNG, O., TRAM, U., FINNERTY, C. M., EIPPER-MAINS, M. A., KALB, J. M. & WOLFNER, M. F. (2002). The *Drosophila melanogaster* seminal fluid protein Acp62F is a protease inhibitor that is toxic upon ectopic expression. *Genetics* **160**, 211–224.

MAO, H. & KAUFMAN, W. R. (1999). Profile of the ecdysteroid hormone and its receptor in the salivary gland of the adult female tick, *Amblyomma hebraeum*. *Insect Biochemistry and Molecular Biology* **29**, 33–42.

MEDEIROS, M. N., OLIVEIRA, D. N. P., PAIVA-SILVA, G. O., SILVA-NETO, M. A. C., ROMEIRO, A., BOZZA, M., MASUDA, H. & MACHADO, E. A. (2002). The role of eicosanoids on *Rhodnius* heme-binding protein (RHBP) endocytosis by *Rhodnius prolixus* ovaries. *Insect Biochemistry and Molecular Biology* **32**, 537–545.

MORTIMER, D. (1983). Sperm transfer in the human female reproductive tract. In *Oxford Reviews of Reproductive Biology*, Vol. 5 (ed. Finn, C. A.), pp. 30–61. Oxford, Clarendon Press.

OLIVER, J. H. Jr., POUND, J. M. & ANDREWS, R. H. (1984). Induction of egg maturation and oviposition in the tick, *Ornithodoros parkeri* (Acari: Argasidae). *Journal of Parasitology* **70**, 337–342.

PAPPAS, P. J. & OLIVER, J. H. Jr. (1971). Mating necessary for complete feeding of female *Dermacentor variabilis* (Acari: Ixodidae). *Journal of the Georgia Entomological Society* **6**, 122–124.

PAPPAS, P. J. & OLIVER, J. H. Jr. (1972). Reproduction in ticks (Acari: Ixodoidea). 2. Analysis of the stimulus for rapid and complete feeding of female *Dermacentor variabilis* (Say). *Journal of Medical Entomology* **9**, 47–50.

PARKER, G. A. (1998). Sperm competition and the evolution of ejaculates: Towards a theory base. In *Sperm Competition and Sexual Selection* (ed. Birkhead, T. R. & Møller, A. P.), pp. 3–54. San Diego and London, Academic Press.

POTTERAT, O., HOSTETTMANN, K., HÖLTZEL, A., JUNG, G., DIEHL, P. A. & PETRINI, O. (1997). Boophiline, an antimicrobial sterol amide from the cattle tick *Boophilus microplus*. *Helvetica Chimica Acta* **80**, 2066–2072.

POYSER, N. L. (1987) Prostaglandins and ovarian function. In *Eicosanoids and Reproduction* (ed. Hillier, K.), pp. 1–29. Lancaster, England, MTP Press Ltd.

RIDLEY, M. (1988). Mating frequency and fecundity in insects. *Biological Reviews* **63**, 509–549.

RUEGG, R. P., KRIGER, F. L., DAVEY, K. G. & STEEL, C. G. H. (1981). Ovarian ecdysone elicits release of a myotropic ovulation hormone in *Rhodnius* (Insecta: Hemiptera). *International Journal of Invertebrate Reproduction* **3**, 357–361.

SAHLI, R., GERMOND, J. E. & DIEHL, P. A. (1985). *Ornithodoros moubata*: Spermateleosis and secretory activity of the sperm. *Experimental Parasitology* **60**, 383–395.

SAUER, J. R., ESSENBERG, R. C. & BOWMAN, A. S. (2000). Salivary glands in ixodid ticks: Control and mechanism of secretion. *Journal of Insect Physiology* **46**, 1069–1078.

SCHÖL, H., SIEBERZ, J., GÖBEL, E. & GOTHE, R. (2001). Morphology and structural organization of Gené's organ in *Dermacentor reticulatus* (Acari: Ixodidae). *Experimental and Applied Acarology* **25**, 327–352.

SEIDELMANN, K. & FERENZ, H.-J. (2002). Courtship inhibition pheromone in desert locusts, *Schistocerca gregaria*. *Journal of Insect Physiology* **48**, 991–996.

SEVALA, V. L., SEVALA, V. M., DAVEY, K. G. & LOUGHTON, B. G. (1992). A FMRFamide-like peptide is associated with the myotropic ovulation hormone in *Rhodnius prolixus*. *Archives of Insect Biochemistry and Physiology* **20**, 193–203.

SHANBAKY, N. M., EL-SAID, A. & HELMY, N. (1990). Changes in neurosecretory cell activity in female *Argas* (*Argas*) *hermanni* (Acari: Argasidae). *Journal of Medical Entomology* **27**, 975–981.

SHEMESH, M., HADANI, A., SHKLAR, A., SHORE, L. S. & MELEGUIR, F. (1979). Prostaglandins in the salivary glands and reproductive organs of *Hyalomma anatolicum excavatum* Koch (Acari: Ixodidae). *Bulletin of Entomological Research* **69**, 381–385.

SHEPHERD, J., OLIVER, J. H. Jr. & HALL, J. D. (1982). A polypeptide from male accessory glands which triggers maturation of tick spermatozoa. *International Journal of Invertebrate Reproduction* **5**, 129–137.

SIMMONS, L. W. & SIVA-JOTHY, M. T. (1998). Sperm competition in insects: Mechanisms and the potential for selection. In *Sperm Competition and Sexual Selection* (ed. Birkhead, T. R. & Møller, A. P.), pp. 341–434. San Diego, Academic Press.

SNOW, K. R. (1969). Egg maturation in *Hyalomma anatolicum anatolicum* Koch, 1844 (Ixodoidea, Ixodidae). In *Proceedings of the 2nd International Congress of Acarology, 1967* (ed. Owen Evans, G.), pp. 349–355. Budapest, Akademiai Kiodo.

SONENSHINE, D. E. (1967). Feeding time and oviposition of *Dermacentor variabilis* (Acarina: Ixodidae) as affected by delayed mating. *Annals of the Entomological Society of America* **60**, 489–490.

SONENSHINE, D. E. (1991). *Biology of Ticks*. Vol. 1, Oxford, Oxford University Press.

STERNBERG, S., PELEG, B. A. & GALUN, R. (1973). Effect of irradiation on mating competitiveness of the male tick, *Argas persicus* (Oken). *Journal of Medical Entomology* **10**, 137–142.

THOMAS, R. W. & ZEH, D. W. (1984). Sperm transfer and utilization strategies in arachnids: Ecological and

morphological constraints. In *Sperm Competition and the Evolution of Animal Mating Systems* (ed. Smith, R. L.), pp. 179–221. Orlando, Academic Press.

TRAM, U. & WOLFNER, M. F. (1999). Male seminal fluid proteins are essential for sperm storage in *Drosophila melanogaster*. *Genetics* **153**, 837–844.

VAHED, K. (1998). The function of nuptial feeding in insects: A review of empirical studies. *Biological Reviews* **73**, 43–78.

VAN DER GOES VAN NATERS-YASUI, A., TAYLOR, D., SHONO, T. & YAMAKAWA, M. (2000). Purification and partial amino acid sequence of antibacterial peptides from the hemolymph of the soft tick, Ornithodoros moubata, (Acari: Argasidae). In *Proceedings of the 3rd International Conference 'Ticks and Tick-Borne Pathogens: Into the 21st Century'* (ed. Kazimirová, M., Labuda, M. & Nuttall, P. A.), pp. 189–194. Bratislava, Slovakia, Institute of Zoology, Slovak Academy of Sciences.

WAKAYAMA, E. J., DILLWITH, J. W. & BLOMQUIST, G. J. (1986). Occurrence and metabolism of prostaglandins in the housefly, *Musca domestica* (L.). *Insect Biochemistry* **16**, 895–902.

WANG, H., PAESEN, G. C., NUTTALL, P. A. & BARBOUR, A. G. (1998). Male ticks help their mates to feed. *Nature* **391**, 753–754.

WEISS, B. L. & KAUFMAN, W. R. (2001). The relationship between 'critical weight' and 20-hydroxyecdysone in the female ixodid tick, *Amblyomma* hebraeum. *Journal of Insect Physiology* **47**, 1261–1267.

WEISS, B. L. & KAUFMAN, W. R. (2004). Two feeding-induced proteins from the male gonad trigger engorgement of the female tick, *Amblyomma hebraeum*. *Proceedings of the National Academy of Science, USA* **101**, 5874–5879.

WEISS, B. L., STEPCZYNSKI, J. M., WONG, P. & KAUFMAN, W. R. (2002). Identification and characterization of genes differentially expressed in the testis/vas deferens of the fed male tick, *Amblyomma hebraeum*. *Insect Biochemistry and Molecular Biology* **32**, 785–793.

WOLFNER, M. F. (1997). Tokens of love: functions and regulation of *Drosophila* male accessory gland products. *Insect Biochemistry and Molecular Biology* **27**, 179–192.

WOLFNER, M. F. (2002). The gifts that keep on giving: Physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* **88**, 85–93.

WOLFNER, M. F., HARADA, H. A., BERTRAM, M. J., STELICK, T. J., KRAUS, K. W., KALB, J. M., LUNG, Y. O., NEUBAUM, D. M., PARK, M. & TRAM, U. (1997). New genes for male accessory gland proteins in *Drosophila melanogaster*. *Insect Biochemistry and Molecular Biology* **10**, 825–834.

YAMAJA SETTY, B. N. & RAMAIAH, T. R. (1979). Isolation and identification of prostaglandins from the reproductive organs of male silkmoth, *Bombyx mori* L. *Insect Biochemistry* **9**, 613–617.

YAMAJA SETTY, B. N. & RAMAIAH, T. R. (1980). Effect of prostaglandins and inhibitors of prostaglandin biosynthesis on oviposition in the silkmoth, *Bombyx mori*. *Indian Journal of Experimental Biology* **18**, 539–541.

YUVAL, B. & SPIELMAN, A. (1990). Sperm precedence in the deer tick *Ixodes dammini*. *Physiological Entomology* **15**, 123–128.