— Review —

Female receptivity in butterflies and moths

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Summary

Female receptivity in butterflies and moths is influenced by a multitude of factors that vary between virgin and mated females, and is often affected by the quality and persistence of courting males. Mated females of polyandrous species frequently display a period of non-receptivity following mating, often resulting from factors transferred by the male at mating. Some of these compounds have a transient effect (e.g. anti-aphrodisiacs and mating plugs), whereas others induce long-term suppression of receptivity (i.e. sperm and seminal factors). Sperm appear to generally induce long-term suppression of female receptivity in both butterflies and moths. In some species, production of non-fertile sperm may function to fill the female's sperm storage organ and switch off receptivity, although whether this is a general phenomenon across the Lepidoptera has not yet been examined. Examination of seminal fluids suppressing female receptivity in moths suggests that more than one factor is implicated, but frequently the transfer or stimulation of Juvenile Hormone production is involved. Surprisingly, potential seminal factors influencing female receptivity in butterflies remain largely unexplored. In this review, I summarize the various factors that are known to affect female receptivity in the Lepidoptera to date, and briefly compare the function and similarity of the Pheromone Suppressing Peptide (*Hez*PSP) in moths to that of the Sex Peptide in *Drosophila melanogaster* (*Drm*SP). The exciting possibility that seminal peptides in the Lepidoptera and Diptera (e.g. *Drosophila melanogaster*) may have shared functionality is discussed.

Key words: receptivity, pheromones, sperm, ejaculate, sexual selection, PBAN, Acp, peptide.

Introduction

The decision by females to mate or not is influenced by many factors that vary in virgin and mated females. Apart from the endogenous reproductive maturation state of virgin females ensuring they are ready to reproduce, females' mating decisions can be directly affected by males' behaviour. Males have to be of the right species, but as they frequently vary in 'quality', should ideally be of high value. Male 'quality' can, for example, reflect variation in resources provided to females at mating. Males of several lepidopteran species provide females with nutrients in the ejaculate that increase female fecundity (e.g. Boggs and Gilbert, 1979; Wiklund et al., 1998), and there is variation between males in provisioning ability (e.g. Wedell, 1996). Males may also vary in genetic quality, such as viability, which can be passed to offspring (Jennions and Petrie, 2000).

Male courtship

Males frequently engage in courtship displays prior to mating. In both butterflies and moths, for example, males court females prior to mating by releasing pheromones that function as aphrodisiacs to stimulate copulation. These odours are released from specialised scent organs such as hair-pencils, coremata and modified scales (Birch et al., 1990). Male released pheromones can provide information both regarding species identity and overall quality. In the green-veined white butterfly *Pieris napi*, males release a sex-specific volatile (citral) during courtship, which entices the female to perform mate acceptance behaviours (Andersson et al., 2003). Similarly, male *Heliothis virescens* moths, display abdominal hair-pencils and release odours during courtship, promoting female mate acceptance. These compounds are species-specific (Hillier and Vickers, 2004). There is also variation in aphrodisiacs within species. In the arctiid moth *Utetheisa ornatrix*, males transfer chemical compounds to the female at mating that she bequeaths to her offspring, rendering them distasteful and thus protected against predators (Dussourd et al., 1988). Interestingly, males with more protective compounds to offer release scents that are more appealing to females than males with inferior offerings. Hence by assessing the quality of males' pheromones, females may gain information regarding the amount received, which increases

the likelihood of copulation (Dussourd et al., 1991). In many moth species, female pheromones seem to play an important role in species recognition (e.g. Roelofs and Carde, 1974), indicating that chemical communication between the sexes is an important component of courtship. In general, female moths release large odour molecules (i.e. unsaturated alcohols; Birch and Haynes, 1982) to attract males over long distances, whereas female butterfly pheromones seem to function at a shorter range (Boppré, 1984). Male butterflies and moths release small unstable volatile odour molecules during courtship to facilitate mating. Males of some moth species also use acoustic signals during courtship and to attract receptive females (Gwynne and Edwards, 1986). Males and females of some species even engage in elaborate acoustic calling bouts for extended period of time prior to mating (Sanderford and Conner, 1990), indicating a role in sexual communication. Males may also engage in elaborate aerial displays showing off their colours to impress females, a behaviour already noted by Darwin (1871).

Anti-aphrodisiacs

The majority of butterflies and moths mate multiply (Simmons, 2001). As a consequence of polyandry, male Lepidoptera frequently experience competition with rival males' sperm for fertilization of the female's ova (Parker, 1998). Selection has favoured a suite of male adaptations aimed at altering female receptivity to reduce the risk of sperm competition. These adaptations include behavioural, morphological and chemical traits.

One way in which males can reduce female receptivity is by rendering them unattractive to rival males following mating. In some species, males release pheromones that repel potential rivals (e.g. Hirai et al., 1978; Lecompte et al., 1998). In the butterfly *Danaus gilippus*, males release a pheromone-bearing dust from their hair pencils containing a flight inhibitor (a ketone) and a 'glue' that stick the inhibitor onto the female's antennae (Pliske and Eisner, 1969; Schneider, 1984). This reduces the likelihood that females mate again. Male butterflies and moths also transfer chemical compounds, or anti-aphrodisiacs, to females at mating that reduce their attractiveness. Male *Heliconius erato* butterflies transfer a pheromone to the female, which she disseminates from special storage organs called 'stink clubs' making her highly distasteful to other males (Gilbert, 1976). These odours are race-specific in this species, indicating they may be under selection. In the green veined white *Pieris napi* butterfly, males synthesize and transfer a volatile substance, methyl-salicylate, which is emitted by mated females and acts as a strong deterrent to courting males (Andersson et al., 2000). This reduces costly harassment by additional males, which is beneficial to females. However, as males in this species transfer nutrients to females this gradually turns into a conflict over remating, as females will eventually want to mate again (Wiklund et al., 1993). In the related *P. rapae*, males also synthesize and transfer anti-aphrodisiacs (methyl-salicylate and indole) to the female at mating (Andersson et al., 2003). Male tobacco budworm also appears to transfer compounds that suppress female attractiveness (Hendricks and Shaver, 1975). These anti-aphrodisiacs only tend to have a transient effect, as most females eventually remate.

Mating plugs

Males of many species transfer substances at copulation that harden to form a mating plug. Mating plugs, or sphraga, are also formed in the Lepidoptera by substances from the males' accessory glands, and can be remarkably large and elaborate. They seem to function as a means to reduce likelihood of female remating; the number of matings by females decreases with increasing elaboration or size of the mating plug across species (Simmons, 2001). The sphragis may also function as a visual deterrent to rival males, as the larger the plug the less likely males are to attempt to mate with the female in some species (Orr and Rutowski, 1991). However, it is not completely effective as females are capable of remating despite the presence of a plug (Dickinson and Rutowski, 1989; Orr and Rutowski, 1991; Matsumoto and Suzuki, 1992). In the chalcedon checkerspot butterfly, for example, females with the plug experimentally removed were just as likely to reject courting males as females with an intact plug (Dickinson and Rutowksi, 1989), indicating that other factors affect female receptivity in this species. Sphraga appear to be costly for males to produce. Some species are only able to produce a few plugs, and the size is often reduced with number of previous copulations (e.g. Matsumoto and Suzuki, 1992; Orr, 1995, 2002). Elaboration of the sphragis seems to have occurred at the expense of spermatophore (the sperm packet) size; there is an inverse relationship between spermatophore size and sphragis elaboration (Matsumoto and Suzuki, 1995; Orr, 1995). There is also evidence of adaptations by males to circumvent mating plugs. In *Heteronympha penelope* butterflies, males have specialized genitalia capable of removing rival males' sphraga (Orr, 2002).

Mechanical stimulation

In many species, the physical presence of the spermatophore is sufficient to switch off female receptivity. This was elegantly demonstrated in *Pieris rapae* butterflies, where Sugawara (1979) was able to initiate non-receptivity in females by artificially inflating their bursa (where the spermatophore is received at mating). He proposed that mechanical pressure on stretch receptors in the bursa were responsible for reduced receptivity. There is some support for this idea. Males often produce larger spermatophores on their first mating, which are associated with longer periods of female unreceptivity compared to the refractory period of females mated to males producing smaller spermatophores (e.g. Kaitala and Wiklund, 1995; Oberhauser, 1997; Cook and Wedell, 1996; Wedell and Cook, 1999). Females of many lepidopteran species have sclerotized plates or spines (signa) inside the bursa, which

show considerable variation between species. These 'teeth' puncture the spermatophore when females contract the muscles surrounding the bursa (Rogers and Wells, 1984). It is suggested signa may have evolved as means whereby females can influence the rate of spermatophore digestion and hence their receptivity (Cordero, 2005). Not all species respond to mechanical stimulation of the bursa. In *Manduca sexta*, artificially inflating the bursa has no effect on female behaviour. Spermatophores from castrated males (transferring no testicular products) have a transient effect, but only the presence of a normal spermatophore in the bursa has a lasting effect on reducing female remating behaviour (Sasaki and Riddiford, 1984).

Sperm

Not surprisingly, reduced female receptivity is associated with successful storage of sperm, which appears to have a long-term effect on suppressing female receptivity in insects. In many lepidopteran species the presence of sperm in the spermatheca (female sperm storage organ) is required to switch off female receptivity and stimulate oviposition (e.g. gypsy moths, Giebultowicz et al., 1991; silk moths, Karube and Kobayashi, 1999). Both the spermatheca and the bursa copulatrix are innervated, indicating that sperm may play a role in switching off female receptivity and stimulating oviposition (Sugawara, 1979; Kingan et al., 1995). In the butterfly *Pieris rapae*, for example, the presence of sperm in the spermatheca appears to cause neural triggering of female unreceptivity (Obara et al., 1975). Similarly, in *Plodia interpunctella*, females receiving fewer sperm from a male on his second or third mating are more likely to remate (Cook and Gage, 1995), and in the gypsy moth *Lymantria dispar*, remating is more likely to occur if there are few sperm in the spermatheca (Proshold, 1995).

Male butterflies and moths produce two types of sperm: normal, fertilizing 'eupyrene' sperm, and a large number of non-fertile, anucleate 'apyrene' sperm (Meves, 1902; Friedländer, 1997). Apyrene sperm are typically >90% of total sperm number (Cook and Wedell, 1996; Solensky, 2003), indicating that they represent a substantial investment by males. Fertilizing sperm are transferred in the spermatophore to the female in bundles containing 256 sperm per bundle (Virkki, 1969; Phillips, 1970; Richard et al., 1975; Witalis and Godula, 1993). Apyrene sperm are highly active at ejaculation, whereas eupyrene sperm usually remain in bundles. Apyrene sperm also appear to reach the female's spermatheca before the fertile sperm in both butterflies and moths (Silberglied et al., 1984; Tschudi-Rein and Benz, 1990; Watanabe et al., 2000; Marcotte et al., 2003). Non-fertile sperm seem to be critical to male reproductive success, because males do not decrease investment in apyrene relative to eupyrene sperm when reared on a restricted diet (Gage and Cook, 1994; Cook and Wedell, 1996).

Various hypotheses have been proposed to explain apyrene sperm function (reviewed in Silberglied et al., 1984). Many of these suggest that apyrene sperm have a supporting role, for example in aiding eupyrene sperm transport or activating the eupyrene sperm (e.g. Osanai et al., 1986, 1987; Sahara and Takemura, 2003). Alternatively, they may represent a nutrient source either for the fertile sperm in the spermatheca, or for the female and the developing zygotes. However, Silberglied et al. (1984) have argued that these hypotheses do not account for the fact that apyrene reach and persist within the spermatheca and do not appear to be digested. If apyrene sperm were only involved in eupyrene sperm transport or activation, it seems unlikely that they would then be stored. If apyrene sperm have a solely supporting role, a given number of non-fertile sperm should be needed for the activation or transport of a single fertile sperm, and therefore the proportion of the two sperm types should be constant within a species (Cook and Gage, 1995). This does not appear to be the case. For example, in at least two species there is a significant increase in the proportion of fertile sperm over the first two matings: in *Plodia interpunctella*, the proportion of eupyrene sperm increases from 7.5% to 10% (Gage and Cook, 1994), and in *Pieris rapae* the increase is from 11% to 15% (Cook and Wedell, 1996).

In their pioneering paper, Silberglied et al. (1984) suggested that apyrene sperm play a role in sperm competition, either by displacing or inactivating rival males' sperm, or, by remaining in the females' spermatheca they may delay female remating. Both these hypotheses predict that apyrene sperm numbers should increase with increased risk of sperm competition. If apyrene sperm displace or inactivate rival males' sperm, they may increase in response to the presence of rival male sperm. In *P. interpunctella*, males provide non-virgin females with more eupyrene, but not apyrene sperm (Cook and Gage, 1995), whereas in the green-veined white butterfly *Pieris rapae*, males provide both higher number of eupyrene and apyrene sperm to mated females (Wedell and Cook, 1999). On the other hand, if apyrene sperm influences female sexual receptivity, we expect their numbers to be related to female remating behaviour. It is of course possible that non-fertile sperm may play both these roles.

A study on the polyandrous green-veined white butterfly *P. napi*, suggests that the number of non-fertile sperm in the spermatheca is responsible for inducing reduced female receptivity (Cook and Wedell, 1999). Females that do not remate store significantly more non-fertile sperm in their spermatheca than remating females (Fig. 1). Moreover, the number of non-fertile, but not fertile, sperm stored is positively related to the duration of non-receptivity. This suggests that apyrene sperm are involved in influencing females' receptivity in the *P. napi* by filling their sperm storage organ. There is genetic variation in the tendency of females to store non-fertile sperm, which correlates with the duration of their refractory period (Wedell, 2001). Similarly, the reacquisition of female receptivity in the armyworm is associated with a pronounced decline in the number of apyrene, but not eupyrene, sperm in storage (He et al., 1995), and the presence of motile apyrene sperm in the spermatheca temporarily switches off female receptivity in *Heliothis zea* (Snow et al., 1972). Females may
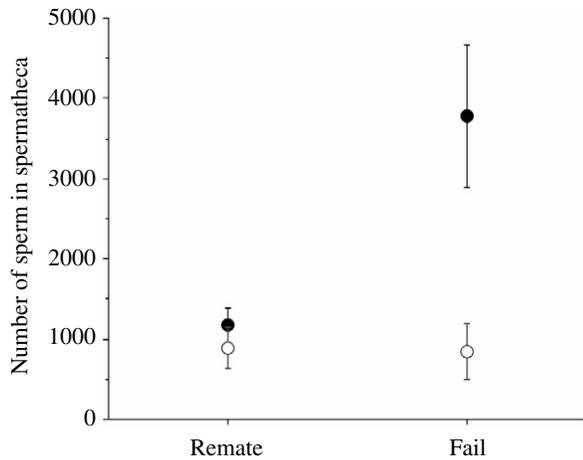
Fig. 1. The relationship between the number of fertile (open circles) and non-fertile (filled circles) sperm present in the female's sperm storage organ and the tendency to remate in the butterfly *Pieris napi*. Females that did not remate within 10 days following their initial copulation stored significantly more non-fertile sperm compared to remating females, whereas there is no difference in the number of fertile sperm stored. Values are means ± S.E.M. (redrawn after Cook and Wedell, 1999).

be able to detect the presence of sperm in their spermatheca (e.g. by the presence of mechano-receptors; Lum and Arbogast, 1980), in order to ensure high fertility. Males may have taken advantage of this system: rather than transferring many fertile sperm, males transfer large number of apyrene sperm (that are highly motile and may be cheaper to produce) that fill the females' sperm storage organ thereby switching off receptivity. It is possible that production of apyrene sperm is more efficient than a similar investment in fertile sperm to switch off female receptivity, although this is yet to be confirmed.

In some species, larger females are more likely to mate multiply and hence have elevated receptivity levels (Torres-Vila et al., 1997; Wedell and Cook, 1999). Bigger females have larger sperm storage organs (Gage, 1998), indicating they may require receipt of more sperm to induce the same duration of un-receptivity as smaller females. Intriguingly, male *Plodia interpunctella* meal moths provide more sperm to bigger females (Gage, 1998). Increased receptivity of larger females may also be due to having bigger reproductive reserves that can be converted into more eggs and hence the need for additional sperm. That is, larger females need more sperm and mate more frequently.

Seminal fluids

Juvenile hormone (JH) is a key endocrine regulator of metamorphosis, reproduction and aging. Egg development, for example, is predominantly regulated by JH through its control of vitellogenin synthesis (Nijhout, 1994). Female receptivity is intimately associated with egg maturation; the onset of

reproduction in virgin females is controlled by JH. Synthesis and release of JH is stimulated by the corpora allata (CA) and various neuropeptides produced by CNS (Nijhout, 1994; Simonet et al., 2004). These peptides have either a stimulatory (allotropins, AT), or inhibitory (allatostatic, AST) activity (Altstein, 2004; Simonet et al., 2004). Since JH is crucial to female receptivity and neuropeptides control JH production, they are central to regulating female reproduction. Ecdysteriods [e.g. the 'moulting hormone' 20-hydroxyecdysone (20E)] are also important regulators of egg maturation and are produced by the ovaries in adult females (e.g. Buszczak et al., 1999). Similarly, the testes of many Lepidoptera synthesize ecdysteriods, which stimulate development of the male reproductive tract (Loeb et al., 2001), and is also controlled by a neuropeptide (Loeb et al., 1996). Both JH and ecdysteriods come in a multitude of variants.

It is clear that substances transferred in the spermatophore play a role in switching off female receptivity and stimulating oviposition and egg maturation rate (Gillott, 2003). These substances often involve JH. In the moth *Heliothis virescens*, female egg maturation is stimulated by JH derived from males' accessory glands (Park and Ramaswamy, 1998). Similarly, in *Cecropia* silk moths, males accumulate large amounts of JH that is stored in their accessory glands, and later transferred to the female at mating (Shirk et al., 1980, 1983). In addition, it appears that male derived factors can stimulate the females' own production of JH (Park et al., 1998). Males also transfer other substances apart from JH at mating that affect female receptivity and reproductive physiology. In *Helicoverpa zea* and *H. armigera*, factors from the male accessory gland stimulate egg maturation and oviposition (Bali et al., 1996; Jin and Gong, 2001).

In most moth species, receptive females attract males by releasing pheromones during a characteristic 'calling' phase. One important neuropeptide controlling the production of sex pheromone in female moths is the female sex Pheromone Biosynthesis Activating Neuropeptide (PBAN), which is mediated either by humoral, hormonal or neural cues (e.g. Raina, 1993; Rafaeli, 2002). Mating causes cessation of female sex pheromone production in many moths: *Helicoverpa zea* (Raina, 1989), *Heliothis virescens* (Ramaswamy et al., 1996), *Lymantria dispar* (Giebultowicz et al., 1991), *Argyrotaenia velutiana* (Jurenka et al., 1993), *Epiphyas postvittana* (Foster, 1993), *Bombyx mori* (Ando et al., 1996), *Plodia interpunctella* (Rafaeli and Gileadi, 1999), *Choristoneura fumiferana* and *C. rosaceana* (Delisle and Simard, 2002), by inhibiting PBAN production.

There is large variation between species in factors that affect female pheromone production. In *Helicoverpa zea*, pheromone production is switched off by a peptide originating from the male accessory glands (Kingan et al., 1995), whilst in the closely related species *Heliothis virescens*, a testicular factor, most likely the ecdysteroid 20E, is responsible (Ramaswamy and Cohen, 1992; Ramaswamy et al., 1996). In *Choristoneura rosaceana*, despite mating resulting in increased levels of JH in females and suppressed pheromone production, this JH does

not originate from the male, as they lack the ability to synthesize and store JH in their accessory glands (Cusson et al., 1999). In other moths, suppression of calling is triggered by a neural signal, originating from the male, in the ventral nerve cord (e.g. *Bombyx mori*, Ando et al., 1996; *Lymantria dispar*, Giebultowicz et al., 1991).

In *Helicoverpa zea,* more than one factor controls the switch-off of pheromone and the cessation of calling. Males transferring a spermatophore without accessory gland products do not stop female pheromone production but do stop the calling behaviour (Kingan et al., 1993). In the silk moth, *Bombyx mori*, a combination of both mechanical stimulation of the tip of the abdomen, which takes place during copulation, together with successful receipt of sperm, trigger a neural inactivation process that suppresses production of the female sex pheromone bombykol (Karube and Kobayashi, 1999). It seems that mechanical receptors at the tip of the abdomen inhibit the release of PBAN (Ichikawa, 1998), whereas there is no evidence that any further humoral factors transferred at mating are involved. Similarly, in *Lymantria dispar* the insertion of male genitalia during copulation causes a transient suppression of female pheromone production, whereas sperm reaching the sperm storage organ are required for a more permanent switch-off (Giebultowicz et al., 1991). Both the spermatheca and the bursa are innervated (Raina et al., 1994), indicating the importance of an intact CNS for neural inactivation of pheromone production. Cessation of calling in female moths appears in general to be triggered by a combination of substances transferred in the ejaculate (i.e. peptides, juvenile hormone) and neural elements (e.g. an intact ventral nerve cord; Kingan et al., 1995; Ramaswamy et al., 1996; Marco et al., 1996; Delisle et al., 2000). In butterflies, potential seminal factors influencing female receptivity remain largely unexplored.

Male-derived factors affecting female receptivity are common in insects. In *Drosophila melanogaster*, for example, males transfer a cocktail of >80 proteins and peptides (Acps) in the seminal fluid, which reduce female receptivity and stimulate egg maturation and oviposition (Wolfner, 2002; Kubli, 2003; Chapman and Davies, 2004). The most intensively studied and characterised Acp is the sex peptide (Acp70A), which reduces female receptivity and increases egg-laying by stimulating the release of JH (Soller et al., 1997; Chapman and Davies, 2004; Wigby and Chapman, 2005). The effect appears to be caused by the C-terminal part of the peptide. Intriguingly, *D. melanogaster* sex peptide (*Drm*SP) has been found to increase JH production when injected in female *Helicoverpa armigera* moths (Fan et al., 2000). Furthermore, the C-terminal part of *Drm*SP reduces PBAN and pheromone production in *H. armigera* females, whereas the N-terminal activates CA production of JH (Fan et al., 2000).

The identification and characterisation of the male derived Pheromone Suppression Protein in moths (*Hez*PSP) has so far only been demonstrated in *Helicoverpa zea* (Kingan et al., 1993, 1995; Eliyahu et al., 2003). *Hez*PSP shows no sequence homology to the *Drosophila* sex peptide, apart from a

disulphide bridge separated by an equal number, but different amino acids (Eliyahu et al., 2003). Synthetic *D. melanogaster* SP (*Drm*SP) stimulates JH production in the related *H. armigera* moths *in vitro* in a similar way as in *D. melanogaster* (Fan et al., 2000), indicating cross-reactivity of *Drm*SP in both suppression of pheromone production and activation of JH production in *H. armigera*. Using *Drm*SP-specific antiserum, immunoreactivity in male *H. armigera* reproductive tissues was demonstrated. The antiserum was highly N-terminal specific, indicating that this is the active region, whereas none of the C-terminal peptides showed any immunoreaction (Nagalakshmi et al., 2004). These results strongly indicate that endogenous *H. armigera* proteins present in the male reproductive tract are responsible for stimulating oviposition and suppressing female receptivity, resembling *Drm*SP.

In *D. melanogaster* the N terminus of *Drm*SP binds to sperm (Kubli, 2003). Sperm function both as a carrier and a reservoir of sex peptide by slowly releasing it while stored in the female (Liu and Kubli, 2003; Peng et al., 2005). Is it possible that non-fertile sperm in the Lepidoptera may also be carriers of sex peptides in an analogous fashion to *D. melanogaster*? The so-called 'sperm effect' causing long-term suppression of receptivity in *D. melanogaster* female requires successful transfer and storage of sperm (Manning, 1972). Similarly, in butterflies and moths sperm in storage (both fertile and non-fertile) is required for inducing a long-term non-receptivity (e.g. Giebultowicz et al., 1991; Karube and Kobayashi, 1999; Cook and Wedell, 1999).

Conclusions and outlook

It is clear that many factors affect female receptivity in butterflies and moths. A female's decision about whether to mate or not is affected by the quality and persistence of courting males. Mated females of polyandrous species display a period of non-receptivity following mating, often the result of various male factors transferred at mating. Some of these compounds have a transient effect (e.g. anti-aphrodisiacs and mating plugs), whereas others induce long-term suppression of receptivity (i.e. sperm and seminal factors). In some species, production of non-fertile sperm may function to fill the female's sperm storage organ and switch off receptivity, although it is not known whether this is a general phenomenon. The effect of seminal fluids suppressing female receptivity in moths shows that more than one factor (which varies between closely related species) is implicated, but frequently the transfer or stimulation of JH production is involved. Males may have exploited females' endocrine system by hijacking the very molecules (e.g. JH, ecdysteroids, neuropeptides) involved in regulating key reproductive processes in order to manipulate females' reproductive physiology (c.f. sensory exploitation; Ryan et al., 1990). This may result in the use of different, although ancient, physiological pathways that may be largely conserved across insect groups (e.g. the Lepidoptera and Diptera). However, within the respective pathways intense selection on males to manipulate female reproductive

physiology more efficiently may promote rapid evolution of specific molecules. For example, although the regulation of female pheromone production in moths involves different physiological mechanisms, they display substantial variation similar to that of Acps in *D. melanogaster* and seminal peptides in animals generally (e.g. Swanson and Vacquier, 2002; Wolfner, 2002). Male-derived pheromone suppressing factors may be under positive selection, promoting rapid divergence among species and populations, as suggested for seminal proteins manipulating female reproductive physiology in insects and vertebrates (Wyckoff et al., 2000; Swanson and Vacquier, 2002; Dorus et al., 2004).

Another reason for variability across species in factors suppressing female receptivity may be differences in their life histories. For example, JH is an important regulator of migratory behaviours (Rankin, 1991), which may preclude its use by males as a means to stimulate female receptivity in migratory species (e.g. Delisle et al., 2000), and hence favour employment of alternative molecules. There is also growing evidence of substantial intra-specific genetic variation in female remating behaviour. For example, in female *Lobesia botrana* moths, multiple mating is a recessive, autosomally inherited trait (Torres-Vila et al., 2002). There is also additive genetic variation in *P. napi* female remating rate (Wedell, 2001). In part, genetic variation in mating rate may be a pleiotropic effect of variation in metabolic rate, because genetically monogamous *P. napi* individuals develop more slowly and lay eggs at a lower rate than genetically polyandrous females (Wedell et al., 2002). The possibility that variation in female mating rate is partly due to pleiotropy clearly needs further examination in this and other taxa. In addition, genetic correlations of reproductive traits between the sexes, either due to pleiotropy or sexually antagonistic alleles, are emerging as an important force affecting the evolvability of reproductive traits such as female receptivity (Rice, 2000; Kirkpatrick and Hall, 2004).

Intriguingly, there appears to be functional similarity between moth sex peptides and the sex peptide of *D. melanogaster*. The exciting possibility that seminal peptides have shared functionality in these two disparate insect groups clearly needs further examination, and is an intriguing research area waiting to happen. The question is: can we reconcile the potentially conserved physiological pathways present across insect groups with the observed rapid divergence of specific reproductive molecules within species? This field is likely to see a rapid expansion in the near future, given the development of new genomic and proteomic tools enabling detailed examination of gene function, even in non-model taxa. Methods where specific genes can be targeted, such as RNA interference knockdowns (e.g. Chapman et al., 2003; Fabrick et al., 2004; Wigby and Chapman, 2005) and creation of null mutants using homologous recombination (e.g. Liu et al., 2003), promise to be powerful techniques for exploring the functional characterization of gene products involved in regulating female receptivity in the Lepidoptera. The predicted research explosion will hopefully shed some light on the extent to which seminal peptides are ancient or rapidly evolving reproductive molecules in insects.

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