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Counter-perfume: using pheromones to prevent female remating

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Counter-perfume: using pheromones to prevent female remating

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Dedication

A mon grand-père, Michel Boussot,
with whom anything is possible.

Counter-perfume: using pheromones to prevent female remating

by

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The University of Texas at Austin, 2018

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Strong selection to secure paternity in polyandrous species leads to the evolution of numerous chemicals in the male's seminal content. These include antiaphrodisiac pheromones, which are transmitted from the male to the female during mating to render her unattractive to subsequent males. An increasing number of species have been shown to use these chemicals. Herein, I examine the taxonomic distribution of species using antiaphrodisiac pheromones, the selection pressures driving their evolution in both males and females, and the ecological interactions in which these pheromones are involved. The literature review shows a highly skewed distribution of antiaphrodisiac use; all species currently known to use them are insects with the exception of the garter snakes *Thamnophis sirtalis parietalis* and *T. radix*. Nonetheless, many taxa have not yet been tested for the presence of antiaphrodisiacs, in groups both closely and distantly related to species known to express them. Within the Insecta, there have been multiple cases of convergent evolution of antiaphrodisiac pheromones using different chemical compounds and methods of transmission. Antiaphrodisiacs usually benefit males, but their effect on females is variable as they can either prevent them from mating multiple times or help them reduce male harassment when they are unreceptive. Some indirect costs of antiaphrodisiacs also impact both males and females, but more research is needed to determine how general this pattern is. Additional research is also important to understand how antiaphrodisiacs interact with the reproductive biology and sexual communication in different species.

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I. INTRODUCTION: SECURING PATERNITY WITH CHEMICALS

In species where females reproduce with more than one partner, male reproductive success is influenced by competition pre-copulation for access to females and post-copulation by sperm competition and cryptic female choice. Post-copulatory competition can lead to the evolution of complex ejaculates consisting of additional compounds besides sperm (Gillott, 2003; Perry, Sirot & Wigby, 2013). Some of these ejaculate contents enhance female fertility and survival by stimulating egg production (Wedell, 2005) and providing nuptial gifts. Others increase male competitiveness by neutralizing previous sperm (Birkhead & Pizzari, 2002) or producing molecules that reduce sperm competition by hindering female remating (Takami *et al.*, 2008). This chemical mate guarding can be achieved by producing mating plugs, which physically prevent other males from transferring sperm to the female, or the use of chemicals to make the female unreceptive or unattractive. Both strategies are less costly for the male than physical mate guarding, which requires extended proximity to the female after copulation (Brent & Byers, 2011). The chemicals used to reduce female receptivity are usually pheromones and they occur in both vertebrates and invertebrates (Poiani, 2006). The pheromones specifically reducing female attractiveness are called antiaphrodisiacs.

Antiaphrodisiacs (AA) are compounds transferred to the female during mating that make her unattractive to subsequent males for periods of time (Happ, 1969). They act by signalling to subsequent males the mating status of a female and directly repelling them rather than by manipulating the female's sex pheromones (Gillott, 2003; Brent & Byers, 2011). This is in contrast to male-transferred chemicals inducing hormonal changes in

females that either stop her from producing male-attracting chemicals or induce her to produce male-repelling ones. AAs are usually transmitted in the seminal fluid, but other transmission modes exist such as cuticular contact or mating plugs (Carlson & Schlein, 1991; Schulz *et al.*, 2008; Brent & Byers, 2011). AAs are classified as pheromones because they are molecules released by one individual to communicate with a conspecific, but it should be noted that the female releasing the pheromone is not the individual who produced it. Their effect can be so strong that males will not court a virgin female to which an AA pheromone has been artificially applied (Andersson, Borg-Karlson & Wiklund, 2000).

Despite an increasing number of species observed to use AA pheromones, few studies of seminal fluids take them into account, and there are no reviews of their taxonomic distribution. Here, I review all studies of AAs across the animal kingdom found in a thorough bibliographic search. All studies concur that AAs benefit males, but their effects on females are more controversial. I explore whether these chemicals are adaptive for both sexes or if they result from sexual conflict between males and females. Finally, I discuss their implication in other ecological interactions and potential applications.

II. TAXONOMIC DISTRIBUTION OF SPECIES USING ANTIAPHRODISIACS

AAs have been described in 14 genera, 13 of which are in the Insecta (Table 1). Most studies documenting these pheromones are recent, which suggests growing interest in this topic, but the overall number of studies remains small.

Next pages: Table 1. Species known to use antiaphrodisiac pheromones. *Suspected; 1debate remains for this species.

Lepidoptera <small>©butterfliesoffrance.com</small> <small>© GFDL</small> <small>© Bob Moul</small> <small>© GFDL</small> <small>© Ken Smith heliconius.co.uk</small> <small>© Ken Childs</small>	<i>Pieris brassicae</i>	Benzyl cyanide	Spermatophore	Andersson <i>et al.</i> (2003)
	<i>Pieris napi</i>	Methyl salicylate	Spermatophore	Andersson <i>et al.</i> (2000)
	<i>Pieris rapae</i>	Methyl salicylate and indole	Spermatophore	Andersson <i>et al.</i> (2003)
	<i>Heliconius erato</i>	Beta-ocimene and dihydrofarnesenic acid	Unknown	Gilbert (1976)
	<i>Heliconius melpomene</i>	Beta-ocimene	Unknown	Schulz <i>et al.</i> (2008)
Hemiptera <small>© J. Fabrick ars.usda.gov</small> <small>© Bill Johnson 2015</small>	<i>Pseudaletia unipuncta</i>	Benzaldehyde*	Unknown	Hirai <i>et al.</i> (2013)
	<i>Lygus hesperus</i>	Myristyl acetate	Spermatophore	Brent & Byers (2011)
	<i>Lygus lineolaris</i> *	Myristyl acetate	Spermatophore	Brent & Byers (2011)

Table 1 (continued)

2011 Jim Moore	<i>Lygus elisus*</i>	Myristyl acetate	Spermatophore	Brent & Byers (2011)
Coleoptera © Kerbtier.de	<i>Aleochara curtula</i>	Unknown	Spermatophore_	Schlechter-Helas <i>et al.</i> (2011)
© Biopix: N Sloth	<i>Tenebrio molitor</i>	Unknown	Spermatophore*	Happ (1969)
Diptera © istockphoto/janeff	<i>Drosophila melanogaster</i>	7-tricosene	Cuticular contact	Scott (1986)
		Cis-vaccenyl acetate	Seminal fluid	Ejima <i>et al.</i> (2007)
		CH ₅ O ₃	Seminal fluid*	Yew <i>et al.</i> (2009)
© Ecol. Evol.	<i>Drosophila arizonae</i>	Triacylglycerides and acetyldienyl acetates	Seminal fluid	Chin <i>et al.</i> (2014)
© Ecol. Evol.	<i>Drosophila mojavensis</i>	Triacylglycerides and acetyldienyl acetates	Seminal fluid	Chin <i>et al.</i> (2014)
No picture available	<i>Drosophila navojoa</i>	Triacylglycerides and acetyldienyl acetates	Seminal fluid	Chin <i>et al.</i> (2014)
© Ray Wilson	<i>Glossina morsitans</i>	Polymethylalkenes (19,23-dimethyltrtriacont-1-ene)	Cuticular contact	Carlson & Schlein (1991)

Table 1 (continued)

Hymenoptera

© Lynette Schimming
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*Lasioglossum
zephyrum*¹

Unknown

Unknown

Kukuk (1985);
Wcislo (1987);
Ayasse *et al.* (2001)

© Andreas Haselböck
naturspaziergang.de

*Lasioglossum
malachurum*

Hexadecyl
octadecenoate

Cuticular contact

Ayasse *et al.* (2001)

© kentwildlifetrust.org.uk

Bombus terrestris

Linoleic acid

Mating plug

Baer *et al.* (2001); Baer
& Boomsama (2004)

© Alex Wild

*Solenopsis invicta**

Linoleic acid

Mating plug

Mikheyev (2003)

© entomart.be

Osmia rufa

(Z)-7-ethyl
hexadecenoate

Wing–stermite
contact

Ayasse *et al.* (2001)

© Rollin Coville

Centris adani

Unknown

Unknown

Ayasse *et al.* (2001)

Squamata

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*Thamnophis sirtalis
parietalis*

Unknown

Seminal fluid

Shine *et al.* (2000);
Friesen *et al.* (2013)

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Thamnophis radix

Unknown

Mating plug

Ross & Crews (1978)

Table 1 (continued)

(1) Coleoptera

The first record of an AA pheromone was in the mealworm beetle *Tenebrio molitor* (Happ, 1969). This is a highly polygynandrous species in which the AA pheromone acts shortly after mating by reducing long-distance attractiveness of females (Carazo, Fernández-Perea & Font, 2012). The only other coleopteran known to express an AA is the rove beetle *Aleochara curtula*, which transfers the pheromone through a spermatophore (capsule encompassing the spermatozoa and containing nutrients transferred from males to females during copulation; Schlechter-Helas, Schmitt & Peschke, 2011). Both species have very high last-male sperm precedence (Simmons, 2001; Drnevich, 2003), *i.e.* most of the eggs are fertilized by the sperm of the last male to mate with a female before oviposition. Some other species of Coleoptera have been argued to use AAs, but they do not act directly as a repelling signal; instead, they mask female pheromones (*Trypodendron lineatum*; Nijholt, 1973) or induce a refractory period in females that makes them unreceptive (*Leptocarabus procerulusi*; Takami *et al.*, 2008).

(2) Lepidoptera

AAs have been best documented in lepidopterans. Gilbert (1976) first recorded them in *Heliconius erato*, with recent studies investigating further *Heliconius* species (Schulz *et al.*, 2008; Estrada *et al.*, 2011). The chemical compound was identified as beta-ocimene in *H. melpomene* (Schulz *et al.*, 2008). In the most comprehensive study of AA evolution to date (Estrada *et al.*, 2011) AAs compounds were identified and compared across 11 *Heliconius* species. This study showed an absence of phylogenetic signal,

suggesting a rapid diversification among species. *Heliconius* butterflies encompass two clades: one is monoandrous with pupal mating and the other polyandrous. Estrada *et al.* (2011) found a faster rate of divergence in AA chemicals in the polyandrous group with more sperm competition as opposed to the pupal-mating group. Another comparison of AAs among species of a single genus was performed by Andersson, Borg-Karlson & Wiklund (2000 and 2003) in pierid butterflies. *Pieris napi* uses methyl salicylate as an AA (Andersson *et al.*, 2000), which is also used by *P. rapae* in association with indole, whereas *P. brassicae* uses benzyl cyanide (Andersson *et al.*, 2003). In the latter species, the fragrance transferred is a male-specific pheromone, suggesting that mated females become unattractive by expressing a male signal. The three pierid butterflies seem to transfer their AA through the spermatophore (Andersson *et al.*, 2000, 2003). Both genera of butterflies described here exhibit a last-male sperm precedence (Gilbert, 1976; Simmons, 2001; Andersson *et al.*, 2003).

The most recent lepidopteran recorded to express AA pheromones is the armyworm moth, *Pseudaletia unipuncta* (Noctuidae; Hirai, Shorey & Gaston, 2013). Unlike other lepidopteran species using AAs, this moth has relatively low last-male sperm precedence (the proportion of offspring sired by the last male in a 2 males experiment $p_2 = 0.47$; Simmons, 2001). The specific AA compound has not been identified, but mated females have a scent which is associated with the expression of benzaldehyde, making this molecule the suspected AA. In other moth species, females become unattractive or unreceptive after mating because male-transferred molecules switch off the production of

sex pheromones in the female, therefore acting only indirectly as an AA (Raina, 1989; Giebultowicz *et al.*, 1991; Gillott, 2003).

(3) Diptera

AAs have also been studied in species of *Drosophila* and in the tsetse fly *Glossina morsitans* (Table 1; Thomas, 2011). Both *D. melanogaster* and *G. morsitans* have cuticular pheromones transferred by contact during mating that function as AAs (Scott, 1986; Carlson & Schlein, 1991). In addition to these, *D. melanogaster* transfers two other AAs in the seminal fluid: the compound cis-vaccenyl acetate and the long-chained acetate CH₅O₃ (Ejima *et al.*, 2007; Yew *et al.*, 2009). Cis-vaccenyl acetate is typically found in the ejaculatory bulb and cuticle of males only, making it a male-specific signal. Thus, recently mated females become unattractive by expressing a male fragrance, similar to the situation in the lepidopteran *P. brassicae*. However, the cis-vaccenyl acetate is only expressed for up to two days after copulation. so the only AA that female fruit flies express for a longer time is the CH₅O₃, which is present for at least ten days post-copulation on the surface the anogenital region (Yew *et al.*, 2009). *D. melanogaster* is the only species recorded to use a combination of different AAs that are chemically distinct and are transmitted through different modes. This may be due to the unusually high last-male sperm precedence in this species ($p_2 = 0.93$ compared to $p_2 = 0.11\text{--}0.45$ in *G. morsitans*; Simmons, 2001) imposing a strong selection pressure on males to be the last to mate with a female before fertilization. The three other *Drosophila* species found to use AAs are part of the *D. repleta* group – drosophilids adapted to desert environments that feed on fermented cacti. Their AA

compounds are a mixture of triacylglycerides (lipids typically found in fat bodies that function in as energy storage) acting synergistically with long-chain acetyldienyl acetates (Chin *et al.*, 2014). These are unusual chemicals to be used as pheromones, particularly when compared to the volatile compounds used as AAs in most other species, but this may be related to the unique diet of these desert-living flies. Alternatively, such chemical compounds may be present as AAs in other species too, but have not been identified in pheromone studies because they are not detectable with the typical methods used to identify volatile compounds (gas chromatography and mass spectrometry; Yew *et al.*, 2009). Chin *et al.* (2014) found that the triacylglycerides used as pheromones are conserved across the *D. repleta* group as well as the related *D. virilis* and *D. robusta* taxa. It is thus likely that AA pheromones are used in all 11 species making up these three taxa.

(4) Hymenoptera

AAs have been recorded in several hymenopteran families. The male bee *Osmia rufa* (Megachilidae) uses cuticular transfer to transmit 7-ethylhexadecenoate to the female by rubbing the female's wings with its sternites after copulation (Ayasse, Paxton & Teng, 2001). Other bees in which AA pheromones are known include *Centris adani* (Ayasse *et al.*, 2001), the sweat bee *Lasioglossum malachurum*, and possibly *Lasioglossum zephyrum*, which was initially reported to use an AA (Kukuk, 1985) although behavioural experiments failed to confirm this (Wcislo, 1987). Finally, the bumble bee *Bombus terrestris* uses linoleic acid as an AA in a mating plug (Baer & Boomsma, 2004). The mating plug itself is not enough to preclude subsequent mating, as other males can displace it, whereas the

presence of linoleic acid is effective. Linoleic acid has also been suspected to act as an AA in the fire ant *Solenopsis invicta*. The same fatty acids are produced in this ant as in the bumble bee *B. terrestris*, including linoleic acid which is transferred to the female during mating (Mikheyev, 2003). Its use as an AA remains to be proven but seems highly plausible.

(5) Hemiptera

Only one hemipteran species, *Lygus hesperus*, has been shown to use an AA (Brent & Byers, 2011). As in pierid butterflies, this compound – myristyl acetate – is transmitted through the spermatophore (Brent & Byers, 2011). Two species of the same genus, *L. lineolaris* and *L. elisus*, are also suspected to use myristyl acetate as an AA but this remains to be confirmed. Myristyl acetate is present in males and mated females but not in virgin females, which is similar to the pattern observed in *L. hesperus*.

(6) Orthoptera

A pheromone similar to an AA has been identified in the desert locust *Schistocerca gregaria* and the term AA has been used to describe it (Seidelmann & Ferenz, 2002). Because there is total sperm precedence in this species ($p_2 = 1$; Simmons, 2001), gregarious males remain in close proximity to the female to prevent remating until egg laying. But in crowded situations other males are able to separate the pair and do so if the repelling pheromone phenylacetone nitrile is not emitted. However, this phenylacetone nitrile is secreted directly by the male, therefore not falling strictly into the definition of an AA pheromone

which must be transferred to the female. A similar finding has been reported in the sweat bee *Lasioglossum malachurum* (Ayasse, 1994) where an ester emitted from the cuticle of males during copulation repels other males. The inhibitory effect on other males is specifically efficient with individuals from the same population.

(7) Squamata

The only records of AA pheromones outside of the Insecta are in the garter snakes *Thamnophis radix* (Ross & Crews, 1978) and *T. sirtalis parietalis* (Friesen *et al.*, 2013). These pheromones are transferred through a mating plug in *T. radix*, whereas the transfer is more likely in the seminal fluid and independent of the mating plug in *T. sirtalis parietalis* (Shine, Olsson, & Mason, 2000). The AA compound has yet to be identified, although Mason & Parker (2010) suggested that it might be squalene. Squalene is a potential sex identifier since it is found in the skin of male garter snakes but not females, and Shine *et al.* (2005) showed that experimentally applying squalene on females repels males. However, artificially adding a male-specific molecule to females would make them unattractive even if this compound is not used as an AA in nature, and thus the role of squalene as an AA awaits further investigation.

(8) Convergent evolution?

The above review shows that AAs are present in many different taxa within the insects, with garter snakes the sole example outside this group. Although AAs are found in multiple insect orders, only a few species within each order use these pheromones,

suggesting multiple cases of independent evolution of AAs. This is also supported by the diversity of transmission modes: seminal fluid, cuticular contact, mating plug, and spermatophore. Indeed, transmission mode does not correlate strictly with taxon, although closely related species often use the same transmission mode.

In some instances, the AA compound is the same as a male-specific pheromone, thus making mated females signal themselves with a male identifier to repel potential mates. This strategy seems less costly and easier to evolve than the use of a new chemical, which may be why it arose in three different taxa. In particular, using a male-specific odour as an AA can be highly beneficial in species where the AA is not an honest signal of female receptivity. In that case, selection pressures would favour other males to evolve resistance to AA signal, which could be avoided if the signal is also used as honest information in male–male communication.

The convergent evolution of AA pheromones among different taxa suggests substantial benefits to their acquisition. If such benefits are present, why are AAs restricted to these few taxa, and are the benefits the same for males and females?

III. WHY ARE ANTIAPHRODISIACS MORE COMMON IN INSECTS?

There are several possible explanations for the phylogenetic bias in AA distribution. First, insects might be particularly likely to use AAs because of their usually promiscuous and polyandrous mating systems. Because females mate with many males, there is strong intra-sexual competition and potential for the evolution of traits to secure paternity. In line with this hypothesis, the only vertebrates known to use AAs are garter snakes, whose life history promotes a highly male-biased operational sex ratio: males emerges from brumation (the low-metabolism state in which garter snakes spend the winter months) at higher density than females in early spring and remain in the vicinity of emerging females to mate multiple times, whereas females emerge at lower density throughout the season and disperse after the first mating (Ross & Crews, 1978). In insects, the importance of sperm competition and selection to prevent female remating is enhanced because of last-male sperm precedence. Nevertheless, this sperm precedence pattern is not universal among insect species, including those using AAs. It may strengthen the potential for AAs but other factors are no doubt in play. Moreover, not all insect species are polyandrous and some non-insects are, raising the question of why AAs did not evolve in these other groups too. The importance of mating system and sperm precedence in the evolution of AAs could be tested by examining the distribution of these pheromones in closely related species where one is monoandrous and the other polyandrous, as well as in polyandrous species with and without sperm precedence. If these factors are driving forces in the evolution of AAs, we should find more AA pheromones in the promiscuous and last-male sperm precedence species than in others.

Second, insects use chemical communication more than many other taxa (Symonds & Elgar, 2008), making them more likely to evolve specific pheromones, such as the AAs. Selection pressures might be similar in different groups, but the chance of having AAs appear may be higher in insects due to the existence of other pheromones and pheromonal pathways in their metabolism. This hypothesis could also be tested using the comparative method, looking at the distribution of AAs between groups which vary in their use of pheromones. We would expect the presence of AAs to be correlated with extensive use of chemical communication. Furthermore, the chemical structure of AAs and their metabolic pathways could be compared to other pheromones to see if they have evolved from a common ancestral molecule.

Third, AAs might be present in other groups where they have not been investigated. There are only four species in which an absence of AAs has been documented, all part of the Insecta: the house cricket *Acheta domesticus* (Fleischman & Sakaluk, 2004), the ant *Leptothorax gredleri* (in which the cuticular pheromones change after mating due to a change in the female's physiology; Oppelt & Heinze, 2009), the honey bee *Apis mellifera*, where a mating sign transferred from males to females during copulation increases their chance of remating (Koeniger, 1990), and the African migratory locust *Locusta migratoria* (Seidelmann & Ferenz, 2002). To my knowledge, no study of AAs has been published in other groups and further research should investigate whether AAs in groups other than insects are absent or simply undocumented.

If AAs are present in other taxa, they would be expected to evolve in species showing internal fertilization, multiple mating with last-male precedence and strong use of

pheromonal communication. For instance, many mammals use olfactory senses and pheromones as a major communication route, especially in the context of reproduction (Vandenbergh, 1983). In addition, they often have multiple matings leading to sperm competition. This is the case in rodents, where some species like prairie voles and hamsters have also been shown to have high last-male precedence (Dewsbury, 1984), thus exhibiting all conditions likely to favour the evolution of AAs. It should be noted that other rodent species like house mice exhibit opposite patterns of sperm precedence and are therefore unlikely to use AA pheromones. Other taxa with characteristics relevant to the development of AAs include some species of fish and birds. Poeciliidae are live-bearing fishes, most of them with internal fertilization. They use pheromones as part of their reproductive behaviour, and the existence of multiple mating with last-male precedence (Constantz, 1984) makes them a highly suitable target for investigating the presence of AAs. Birds also have strong last-male precedence (Briskie, 1996), and although chemical communication has usually been considered less significant in this group, recent studies suggest that olfactory communication is present and that pheromones may well be employed (Caro & Balthazart, 2010).

Knowing the full distribution of AAs would help understand their evolutionary and ecological impacts. Besides, these impacts may be different for males and females.

IV. DO ANTIAPHRODISIACS BENEFIT BOTH SEXES?

(1) Males

In promiscuous species, as well as in socially monogamous species where females exhibit extra-pair matings, males are subject to strong intra-sexual competition in order to access females and secure paternity. This leads to numerous morphological, physiological, chemical and behavioural adaptations. Examples of such adaptations include the evolution of weapons to fight other males for access to females (Emlen, 2008), increased testosterone levels (Martínez-Padilla *et al.*, 2014), sperm competition between the seminal fluid of different males (Edward, Stockley & Hosken, 2015) and physical mate guarding (Edward *et al.*, 2015). Sperm competition can either affect the evolution of the sperm itself, for instance by increasing its size or longevity (Parker, 1998), or it can affect the other molecules present in the ejaculate (Cameron, Day & Rowe, 2007). These seminal fluid contents transmitted along with the sperm increase reproductive success in three different ways: they have an ‘offence’ effect when they replace sperm already present in the female; a ‘defence’ one when they protect their sperm from being displaced by future males; or an ‘avoidance’ effect when they prevent sperm competition from happening altogether (Dhole & Servedio, 2014). Antiaphrodisiacs fall into the avoidance category: they make the female appear unattractive and thus prevent future sperm competition from happening. This is a strong benefit for males, particularly in species with a high degree of last sperm precedence.

If the selection pressure to secure paternity through access to females is strong enough to lead to extreme morphological adaptations like the eyestalks of the stalk-eyed fly, or the horns of Hercules beetles (Emlen, 2008), we can expect a similar pressure on

the evolution of the ejaculate compounds transmitted to females during mating. Any such compound that provides reduced competition from the sperm of other males would thus be highly beneficial to the individual producing it.

Additionally, AAs can benefit males in two other ways. First, when females have a refractory period after mating AAs can function as honest signals of females' lack of receptivity and prevent subsequent males from wasting time and energy courting unreceptive females (Daly, 1978; Ross & Crews, 1978; Andersson *et al.*, 2003; Estrada *et al.*, 2011). This is especially important in species where copulation cannot be forced, such as some species of Lepidoptera (*e.g.* Pieridae and *Heliconius* species; Gilbert, 1976; Andersson *et al.*, 2003). Second, if males court females despite the expression of AAs and copulation ensues, they can use these pheromones as information that there is likely to be sperm competition due to the female's previous mating. Having that information can influence their mating behaviour to maximize fitness, as has been observed in *P. napi*. The males of this butterfly species use the expression of the AA methyl salicylate to assess the female's mating status, and transmit an ejaculate on average 26% larger to mated females than to virgin ones to improve their chances of fertilizing the eggs in the presence of competition with the sperm of a previous male (Larsdotter-Mellström *et al.*, 2015).

The use of AA pheromones thus has multiple advantages for males, the major one being an increased chance of fertilizing eggs by reducing sperm competition. The effects on females, however, are more complex.

(2) Females

Even though the optimal number of partners is lower for females than for males (Bateman's principle), they can still benefit from multiple matings (Eberhard, 1998). These benefits might be direct by providing fertility insurance and nutrients to the female (Hosken & Blanckenhorn, 1983), as is the case in some lepidopteran species. For example, female pierid butterflies gain nuptial gifts during copulation events (Andersson *et al.*, 2003), and in *Heliconius erato* additional spermatophores increase female fecundity (Gilbert, 1976). Benefits of multiple matings might also be genetic by compensating for low-quality partners (Hosken & Blanckenhorn, 1983), avoiding genetic incompatibilities (Hosken & Blanckenhorn, 1983), acquiring 'good genes' (Yasui, 1998), and enhancing the genetic diversity of offspring – which favours different gene by environment interactions (Yasui, 1998). AAs incur a cost to females by reducing their chances of remating and thus of accessing these various benefits of multiple mating.

Nevertheless, most females have a refractory period after mating. In this case, it is advantageous for them to have an honest signal of their mating status that will reduce harassment from males, therefore saving females time and energy (Gilbert, 1976; Andersson *et al.*, 2003; Estrada *et al.*, 2011). In garter snakes, for example, removing the mating plug containing AAs makes the female attractive again, but she is still unreceptive. In this species, as in many others, avoiding male harassment is especially important because mating increases exposure to predators. Other organisms might experience similar trade-offs, such as between mating and laying eggs (*e.g.* pierid butterflies; Andersson *et al.*, 2003). In addition to reducing male harassment, AAs can benefit females in species

where mating damages females' organs, as is for example the case in *D. melanogaster* where the male wounds the female's vaginal wall during copulation (Mattei *et al.*, 2015). The expression of AA pheromones in this species might allow females to avoid unnecessary matings and the ensuing internal damage. This could explain why *D. melanogaster* has several different molecules used as AAs; a sex pheromone is more likely to evolve if it has significant benefits for both sexes. Alternatively, this apparent higher diversity of AA pheromones in the fruit fly may simply result from the higher number of studies on this model organism.

In *Heliconius erato*, females seem to have evolved specific structures called 'stink-clubs' to disseminate AAs (Eltringham, 1925; Gilbert, 1976), suggesting selection pressures favouring AAs in this sex. The impact of AAs on female fitness then depends on the control females have on the release of the pheromones. An honest signal is beneficial for females, but if AAs are expressed for longer than the female is unreceptive this becomes a cost (Andersson *et al.*, 2003; Estrada *et al.*, 2011). In most species, it seems that females do not have such control on the release of AAs, but further research is needed to explore this. There is no evidence of evolution of resistance to AAs by females, which would be expected if these pheromones were highly detrimental to their fitness, but this has not been investigated systematically.

In several species, there is evidence for both evolutionary advantages and disadvantages to AAs in females. In *H. erato* for instance, females suffer the cost of a reduced number of spermatophores, and the resources they contain if they mate only once, but have nonetheless evolved structures to disseminate male-transferred AA. Similarly, it

is unclear whether females of the rove beetle *Aleochara curtula* benefit or suffer a cost from AAs: mated females do not alter their production of aphrodisiac pheromones, which suggests that they are still willing to mate after copulation, but females without AA refuse to do so and spend 60% of their time repulsing males (Peschke, 1987; Schlechter-Helas *et al.*, 2011). Whether AAs are favourable or costly to females would influence how these pheromones interact with other components of the ejaculate transferred during mating, notably in situations where AAs are transferred through a mating plug or spermatophore. Mating plugs are detrimental to females' fitness since they prevent the females from remating without any apparent female control on the presence of the plug. Thus, the expression of AA pheromones transmitted through this route is probably not controlled by females and is presumably deleterious to them. Alternatively, AAs may act as a signal when transferred with a plug, preventing subsequent males from wasting time courting a female that cannot mate. Even if the female would ultimately benefit from a second mating, the plug does not allow this and her best strategy would be to not waste time courting or rejecting other males. Spermatophores, on the other hand, often benefit females by providing them with resources (Boggs, 1995). We could therefore expect AAs to be beneficial for females in species where they are transmitted through this route, as females may have more control on their expression. But it could also be a successful evolutionary strategy for males to transmit costly pheromones with beneficial resources since that would make the evolution of resistance in females less likely.

V. ECOLOGICAL IMPACTS OF ANTIAPHRODISIACS

In some cases, both sexes may suffer a cost from AAs. Natural enemies like parasitoids often intercept chemical communication to locate their hosts, and there are examples of AAs being used in this way. The parasitic wasps *Trichogramma brassicae* and *T. evanescens* use AAs to find their hosts *Pieris brassicae* and *P. rapae*. This happens in two different ways: first, both *Trichogramma* species hitch-hike on *P. brassicae* by using the AA to locate mated females and then ride on them to the oviposition site to infect the eggs (Fatouros *et al.*, 2005; Huigens *et al.*, 2009); second, this interaction can be plant-mediated, which is not an unexpected adaptation since it is advantageous to the plant to facilitate parasitoid attraction for protection against herbivorous insects. Mated *P. brassicae* and *P. rapae* females prompt the production of synomones by Brussels sprouts to attract the *Trichogramma* parasitoids *T. brassicae* and *T. evanescens* (Fatouros *et al.*, 2008, 2009; Pashalidou *et al.*, 2010). The latter species responds only to the plant cues induced by *P. rapae*. The two *Trichogramma* species use different AAs but both molecules are also produced by the host plant, suggesting potential interactions between the plant and insect chemicals (Andersson *et al.*, 2003).

The use of AAs by plants and natural enemies has not been explored in other systems and additional studies are needed to determine whether this is a general pattern or not. The plant-mediated impact of natural enemies could be used for biocontrol in Pieridae, as well as to provide new insights for management of other pest species if similar patterns are present. But the possible use of AA pheromones in biological control is not restricted to species interacting with parasitoids; AAs could also be incorporated in biological control

programs for invasive species, insect pests (Brent & Byers, 2011) and disease vectors (e.g. Tsetse fly) to reduce females' attractiveness and thus reproduction.

VI. ANTIAPHRODISIACS AND SPECIATION

In addition to their ecological implications, AAs also have evolutionary consequences. Because they are a type of chemical communication, they can be involved in species recognition. Among the few studies comparing AA compounds between closely related species, two out of three genera have different molecules used by the different species (Table 1; Andersson *et al.*, 2003; Brent & Byers, 2011; Estrada *et al.*, 2011). The mode of transfer, however, is shared by all species of each genus. This suggests that the transfer of AAs evolved ancestrally in these genera with the signal then becoming species-specific. This hypothesis of ancestral signal evolution followed by more recent divergence is also supported by the fact that AAs in closely related species are different but share some of their compounds, which is not the case for AA pheromones of distantly related species (Table 1).

Rapid signal diversification is observed in the lepidopteran genera *Pieris* and *Heliconius* (Andersson *et al.*, 2003; Estrada *et al.*, 2011). In addition, AAs can be race-specific as in *Heliconius erato* (Gilbert, 1976), which could create isolation between races possibly leading to speciation. It is unknown whether the signal diversification in most species occurred before or after speciation and how it interacted with the speciation process. This could be explored by investigating the coalescence time for genes encoding AA pheromones and comparing this to species divergence times. Increasing the number of studies comparing AA pheromones in closely related species would also help to understand how AAs contribute to speciation and show whether rapid signal diversification is a widespread phenomenon. Inter-sexual coevolution due to post-copulation sexual selection

has been shown to be a potential driver of reproductive isolation and eventually speciation in other systems, but the extent of this phenomenon is not clearly defined (Birkhead & Pizzari, 2002). AAs could be used to study this question.

VII. CONCLUSIONS

(1) Antiaphrodisiacs are pheromones transmitted from males to females during copulation to make the female unattractive to subsequent males. They can be transferred as part of the seminal fluid, in a spermatophore, through cuticular contact or in a mating plug.

(2) The repeated evolution of these pheromones in different insect families across five orders suggests strong selection pressures favouring their appearance and maintenance. The strong phylogenetic signal towards insects in AA distribution might be due to the importance of chemical communication in this group, making them more prone to evolve such pheromones. But the discovery of AA pheromones in two species of *Thamnophis* snakes suggests a potentially more widespread distribution.

(3) The benefit of AAs on males' reproductive success is clear: AAs help males to avoid sperm competition and secure paternity. The effect on females, however, is more ambiguous. On one side, AAs are likely to be detrimental to females' fitness since they reduce their number of mating partners and thus the benefits of multiple mating. But on the other side, they may be beneficial by reducing male harassment when females are not receptive, especially if females have control over their release.

(4) Investigating AA distribution and evolution is crucial for a full understanding of reproductive biology and could explain patterns of attractiveness in species where the presence of AAs has not been tested yet. AAs also play an important role in sperm competition and would thus benefit from being included in studies of this topic, as well as in research on sexual conflict.

(5) AAs have been shown to be involved in speciation in several taxa and can be used to study signal evolution. They are also used in some ecological interactions and could potentially be used for biological control.

(6) There is still much to learn on the topic of AA pheromones, in particular in the following areas: (a) to understand the impact of AAs on females' fitness, we need further investigation of the physiology of AA pheromones, how they are produced and perceived by males, as well as stored and expressed by the females. (b) There is a lack of studies looking at how AA pheromones interact with other pheromones involved in sexual communication, such as those produced directly by females to attract or deter males, as well as with other communication systems such as visual or acoustic cues. (c) Further research is needed to explore the existence of AAs in other species, both within the Insecta and in other taxonomic groups. In particular, AAs are likely to be present in species that have high sperm competition and use pheromonal communication extensively. (d) Increasing the number of comparative studies of AAs within closely related species would help to clarify the evolution of AA pheromonal communication.

REFERENCES

- ANDERSSON, J., BORG-KARLSON, A.-K. & WIKLUND, C. (2000) Sexual cooperation and conflict in butterflies: a male-transferred anti-aphrodisiac reduces harassment of recently mated females. *Proceeding of the Royal Society of London B*. **267**, 1271–1275.
- ANDERSSON, J., BORG-KARLSON, A.-K. & WIKLUND, C. (2003) Antiaphrodisiacs in pierid butterflies: a theme with variation! *Journal of Chemical Ecology* **29**, 1489–1499.
- AYASSE, M. (1994) The meaning of male odor in the mating biology of sweat bees (Hymenoptera: Halictidae). *Les Insectes Sociaux—Proceedings of the 12th International Congress of IUSSI*, 88.
- AYASSE, M., PAXTON, R.J. & TENG, J. (2001) Mating behavior and chemical communication in the order Hymenoptera. *Annual Review of Entomology* **46**, 31–78.
- BAER, B. & BOOMSAMA, J.J. (2004) Male reproductive investment and queen mating-frequency in fungus-growing ants. *Behavioral Ecology* **15**, 426–432.
- BIRKHEAD, T.R. & PIZZARI, T. (2002) Postcopulatory sexual selection. *Nature Reviews Genetics* **3**, 262–273.
- BOGGS, C.L. (1995) Male nuptial gifts: phenotypic consequences and evolutionary implications. In *Insect reproduction* (eds S.R. LEATHER & J. HARDIE), pp. 215–242. CRC Press.
- BRENT, C.S. & BYERS, J. A. (2011) Female attractiveness modulated by a male-derived antiaphrodisiac pheromone in a plant bug. *Animal Behaviour* **82**, 937–943. Elsevier Ltd.
- BRISKIE, J. V (1996) Spatiotemporal of sperm storage and last-male patterns in birds sperm precedence. *Functional Ecology* **10**, 375–383.
- CAMERON, E., DAY, T. & ROWE, L. (2007) Sperm competition and the evolution of ejaculate composition. *American Naturalist* **169**, E158–E172.
- CARAZO, P., FERNÁNDEZ-PEREA, R. & FONT, E. (2012) Quantity estimation based on numerical cues in the mealworm beetle (*Tenebrio molitor*). *Frontiers in Psychology* **3**, article 502.

- CARLSON, D.A. & SCHLEIN, Y. (1991) Unusual polymethyl alkenes in tsetse flies acting as abstinon in *Glossina morsitans*. *Journal of Chemical Ecology* **17**, 267–284.
- CARO, S.P. & BALTHAZART, J. (2010) Pheromones in birds: myth or reality? *Journal of Comparative Physiology A* **196**, 751–766.
- CHIN, J.S.R., ELLIS, S.R., PHAM, H.T., BLANKSBY, S.J., MORI, K., KOH, Q.L., ETGES, W.J. & YEW, J.Y. (2014) Sex-specific triacylglycerides are widely conserved in *Drosophila* and mediate mating behavior. *eLife* **2014**, 1–18.
- CONSTANTZ, G.D. (1984) Sperm Competition in Poeciliid Fishes. In *Sperm Competition and the Evolution of Animal Mating Systems* (ed R.L. SMITH), pp. 465–485. Academic Press.
- DALY, M. (1978) The Cost of Mating. *The American Naturalist* **112**, 771–774.
- DEWSBURY, D.A. (1984) Sperm Competition in Muroid Rodents. In *Sperm Competition and the Evolution of Animal Mating Systems* (ed R.L. SMITH), pp. 547–571. Academic Press.
- DHOLE, S. & SERVEDIO, M.R. (2014) Sperm competition and the evolution of seminal fluid composition. *Evolution* **68**, 3008–3019.
- DRNEVICH, J.M. (2003) Number of mating males and mating interval affect last-male sperm precedence in *Tenebrio molitor* L. *Animal Behaviour* **66**, 349–357.
- EBERHARD, W.G. (1998) Females Roles in Sperm Competition. In *Sperm competition and sexual selection* (eds T.R. BIRKHEAD & A.P. MØLLER), pp. 91–116. Academic Press.
- EDWARD, D. A, STOCKLEY, P. & HOSKEN, D.J. (2015) Sexual Conflict and Sperm Competition. *Cold Spring Harb Perspect Biol.*
- EJIMA, A., SMITH, B.P.C., LUCAS, C., VAN DER GOES VAN NATERS, W., MILLER, C.J., CARLSON, J.R., LEVINE, J.D. & GRIFFITH, L.C. (2007) Generalization of courtship learning in *Drosophila* is mediated by cis-vaccenyl acetate. *Current biology* **17**, 599–605.
- ELTRINGHAM, H. (1925) On the Abdominal Glands in *Heliconius* (Lepidoptera). *Transactions of the Royal Entomological Society of London*.
- EMLEN, D.J. (2008) The Evolution of Animal Weapons. *Annual review of Ecology, Evolution, and Systematics* **39**, 387–413.

- ESTRADA, C., SCHULZ, S., YILDIZHAN, S. & GILBERT, L.E. (2011) Sexual selection drives the evolution of antiaphrodisiac pheromones in butterflies. *Evolution* **65**, 2843–2854.
- FATOUROS, N.E., BROEKGAARDEN, C., BUKOVINSZKINE'KISS, G., VAN LOON, J.J. A, MUMM, R., HUIGENS, M.E., DICKE, M. & HILKER, M. (2008) Male-derived butterfly anti-aphrodisiac mediates induced indirect plant defense. *Proceedings of the National Academy of Sciences U.S.A.* **105**, 10033–10038.
- FATOUROS, N.E., HUIGENS, M.E., VAN LOON, J.A., DICKE, M. & HILKER, M. (2005) Butterfly anti-aphrodisiac lures parasitic wasps. *Nature* **433**, 704.
- FATOUROS, N.E., PASHALIDOU, F.G., APONTE CORDERO, W. V, VAN LOON, J.J. A, MUMM, R., DICKE, M., HILKER, M. & HUIGENS, M.E. (2009) Anti-aphrodisiac compounds of male butterflies increase the risk of egg parasitoid attack by inducing plant synomone production. *Journal of Chemical Ecology* **35**, 1373–1381.
- FLEISCHMAN, R.R. & SAKALUK, S.K. (2004) Sexual conflict over remating in house crickets: no evidence of an anti-aphrodisiac in males' ejaculates. *Behaviour* **141**, 633–646.
- FRANKIE, G.W., VINSON, S.B. & COVILLE, R.E. (1980) Territorial behavior of *Centris adani* and its reproductive function in the Costa Rican dry forest (Hymenoptera : Anthophoridae). *Journal of the Kansas Entomological Society* **53**, 837–857.
- FRIESEN, C.R., SHINE, R., KROHMER, R.W. & MASON, R.T. (2013) Not just a chastity belt: the functional significance of mating plugs in garter snakes, revisited. *Biological Journal of the Linnean Society* **109**, 893–907.
- GIEBULTOWICZ, J.M., RAINA, A.K., UEBEL, E.C. & RIDGWAY, R.L. (1991) Two-step regulation of sex-pheromone decline in mated gypsy moth females. *Archives of Insect Biochemistry and Physiology* **16**, 95–105.
- GILBERT, L.E. (1976) Postmating female odor in *Heliconius* butterflies : a male-contributed antiaphrodisiac ? *Science* **193**, 419–420.
- GILLOTT, C. (2003) Male accessory gland secretions: modulators of female reproductive physiology and behavior. *Annual Review of Entomology* **48**, 163–184.
- HAPP, G.M. (1969) Multiple sex pheromones of the mealworm beetle, *Tenebrio molitor* L. *Nature* **222**, 180–181.

- HIRAI, K., SHOREY, H.H. & GASTON, L.K. (2013) Competition among courting male moths : male-to-male inhibitory pheromone. *Science* **202**, 644–645.
- HOSKEN, D.J. & BLANCKENHORN, W.U. (1983) Female multiple mating, inbreeding avoidance, and fitness: it is not only the magnitude of costs and benefits that counts. *Behavioral Ecology* **10**, 462–464.
- HUIGENS, M.E., PASHALIDOU, F.G., QIAN, M.-H., BUKOVINSZKY, T., SMID, H.M., VAN LOON, J.J. A, DICKE, M. & FATOUROS, N.E. (2009) Hitch-hiking parasitic wasp learns to exploit butterfly antiaphrodisiac. *Proceedings of the National Academy of Sciences U.S.A.* **106**, 820–825.
- KOENIGER, G. (1990) The role of the mating sign in honey bees, *Apis mellifera* L.:does it hinder or promote multiple mating? *Animal Behaviour* **39**, 444–449.
- KUKUK, P. (1985) Evidence for an antiaphrodisiac in the sweat bee *Lasioglossum zephyrum* (Dialictus). *Science* **227**, 656–657.
- LARSDOTTER-MELLSTRÖM, H., ERIKSSON, K., JANZ, N., NYLIN, S. & CARLSSON, M. A (2015) Male butterflies use an anti-aphrodisiac pheromone to tailor ejaculates. *Functional Ecology* **30**, 255–261.
- MARTÍNEZ-PADILLA, J., PÉREZ-RODRÍGUEZ, L., MOUGEOT, F., LUDWIG, S. & REDPATH, S.M. (2014) Intra-sexual competition alters the relationship between testosterone and ornament expression in a wild territorial bird. *Hormones and Behavior* **65**, 435–444.
- MASON, R.T. & PARKER, M.R. (2010) Social behavior and pheromonal communication in reptiles. *Journal of Comparative Physiology A* **196**, 729–749.
- MATTEI, A.L., RICCIO, M.L., AVILA, F.W. & WOLFNER, M.F. (2015) Integrated 3D view of postmating responses by the *Drosophila melanogaster* female reproductive tract, obtained by micro-computed tomography scanning. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 8475–8480.
- MIKHEYEV, A.S. (2003) Evidence for mating plugs in the fire ant *Solenopsis invicta*. *Insectes Sociaux* **50**, 401–402.
- NIJHOLT, W.W. (1973) The effect of male *Trypodendron lineatum* (Coleoptera : Scolytidae) on the response of field populations to secondary attraction. *Canadian Entomologist* **105**, 583–590.

- OPPELT, A. & HEINZE, J. (2009) Mating is associated with immediate changes of the hydrocarbon profile of *Leptothorax gredleri* ant queens. *Journal of Insect Physiology* **55**, 624–628.
- PARKER, G.A. (1998) Sperm Competition and the Evolution of Ejaculates: Toward a Theory Base. In *Sperm Competition and Sexual Selection* (eds T.R. BIRKHEAD & A.P. MØLLER), pp. 3–54. Academic Press.
- PASHALIDOU, F.G., HUIGENS, M.E., DICKE, M. & FATOUROS, N.E. (2010) The use of oviposition-induced plant cues by *Trichogramma* egg parasitoids. *Ecological Entomology* **35**, 748–753.
- PERRY, J.C., SIROT, L. & WIGBY, S. (2013) The seminal symphony: how to compose an ejaculate. *Trends in Ecology and Evolution* **28**, 414–422. Elsevier Ltd.
- PESCHKE, K. (1987) Male aggression, female mimicry and female choice in the rove beetle, *Aleochara curtula* (Coleoptera, Staphylinidae). *Ethology* **75**, 265–284.
- POIANI, A. (2006) Complexity of seminal fluid: a review. *Behavioral Ecology and Sociobiology* **60**, 289–310.
- RAINA, A.K. (1989) Male-induced termination of sex pheromone production and receptivity in mated females of *Heliothis zea*. *Journal of Insect Physiology* **35**, 821–826.
- ROSS, P. & CREWS, D. (1978) Stimuli influencing mating behavior in the garter snake, *Thamnophis radix*. *Behavioral Ecology and Sociobiology* **4**, 133–142.
- SCHLECHTER-HELAS, J., SCHMITT, T. & PESCHKE, K. (2011) A contact anti-aphrodisiac pheromone supplied by the spermatophore in the rove beetle *Aleochara curtula*: mode of transfer and evolutionary significance. *Naturwissenschaften* **98**, 855–862.
- SCHULZ, S., ESTRADA, C., YILDIZHAN, S., BOPPRÉ, M. & GILBERT, L.E. (2008) An antiaphrodisiac in *Heliconius melpomene* butterflies. *Journal of Chemical Ecology* **34**, 82–93.
- SCOTT, D. (1986) Sexual mimicry regulates the attractiveness of mated *Drosophila melanogaster* females. *Proceedings of the National Academy of Sciences U.S.A.* **83**, 8429–8433.
- SEIDELMANN, K. & FERENZ, H.J. (2002) Courtship inhibition pheromone in desert locusts, *Schistocerca gregaria*. *Journal of Insect Physiology* **48**, 991–996.

- SHINE, R., OLSSON, M.M. & MASON, R.T. (2000) Chastity belts in gartersnakes : the functional significance of mating plugs. *Biological Journal of the Linnean Society* **70**, 377–390.
- SHINE, R., WALL, M., LANGKILDE, T., MASON, R.T., THE, S., NATURALIST, A. & JUNE, N. (2005) Do Female Garter Snakes Evade Males to Avoid Harassment or to Enhance Mate Quality ? *The American Naturalist* **165**, 660–668.
- SIMMONS, L.W. (2001) *Sperm competition and its evolutionary consequences in insects*. Princeton University Press.
- SYMONDS, M.R.E. & ELGAR, M. A. (2008) The evolution of pheromone diversity. *Trends in Ecology and Evolution* **23**, 220–228.
- TAKAMI, Y., SASABE, M., NAGATA, N. & SOTA, T. (2008) Dual function of seminal substances for mate guarding in a ground beetle. *Behavioral Ecology* **19**, 1173–1178.
- THOMAS, M.L. (2011) Detection of female mating status using chemical signals and cues. *Biological Reviews* **86**, 1–13.
- VANDENBERGH, J. (ed) (1983) *Pheromones and Reproduction in Mammals*. New York : Academic Press.
- WCISLO, W.T. (1987) The role of learning in the mating biology of a sweat bee *Lasioglossum zephyrum* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology* **20**, 179–185.
- WEDELL, N. (2005) Female receptivity in butterflies and moths. *Journal of Experimental Biology* **208**, 3433–3440.
- YASUI, Y. (1998) The ‘genetic benefits’ of female multiple mating reconsidered. *Trends in Ecology and Evolution* **13**, 246–250.
- YEW, J.Y., DREISEWERD, K., LUFTMANN, H., MÜTHING, J., POHLENTZ, G. & KRAVITZ, E. A. (2009) A New Male Sex Pheromone and Novel Cuticular Cues for Chemical Communication in *Drosophila*. *Current Biology* **19**, 1245–1254.