



## Articles

## Female attractiveness modulated by a male-derived antiaphrodisiac pheromone in a plant bug

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Males of the plant bug *Lygus hesperus* prefer to court virgins over recently mated females. Because a male delivers a large spermatophore mass to the female during copulation that contains more than just sperm, we investigated whether males transferred an odorant molecule rendering females less attractive. We found that topical application of homogenates of the spermatophore, or of the male accessory glands (AG) from which this mass is derived, made virgin females less acceptable as potential mates. Additionally, we found that the fatty molecule myristyl acetate is present in male accessory glands and in the seminal receptacles of recently mated females, but is absent in virgin females. The same distribution of myristyl acetate was also found in *Lygus elisus* and *Lygus lineolaris*. We hypothesized that myristyl acetate has a repellent effect on *L. hesperus* males seeking an appropriate mate. Using topically applied synthetic myristyl acetate at biological concentrations, we found that myristyl acetate was as effective as the AG homogenate at reducing the attractiveness of virgin females. Collectively these results indicate that males use myristyl acetate as a seminally transferred antiaphrodisiac for passive mate guarding, and usage of the compound may be widespread among *Lygus* bug species.

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The attractiveness of a female to a conspecific male can vary widely depending on her developmental state and the external stimuli to which she has been exposed. In insects, mature, fecund, virgin females are usually the most attractive to potential mates. Many female insects lose this attractiveness either temporarily or permanently after mating (reviewed in Gillot 2003). In some instances, this is an endogenous effect due to a reduction in the release of an attractant sex pheromone (e.g. Raina 1989; Kingan et al. 1993; Ayasse et al. 1999; Eliyahu et al. 2003; Fukuyama et al. 2007; Oku & Yasuda 2010), or possibly the induced production of a repellent pheromone (Fuyama & Ueyama 1997; Schiestl & Ayasse 2000). In other species, males may release an antisex pheromone during courtship or mating that counteracts the female's sex pheromone (Zhang & Aldrich 2003). These volatile pheromones may mask the female's odour, marking her and the area around her with a scent that is strongly associated with the presence of a male, indicating that she is either guarded or likely to be unreceptive to further courtship attempts. A third mechanism to reduce female attractiveness is by the transfer of a male-derived antiaphrodisiac during copulation (Happ 1969). These pheromonal compounds function by directly signalling males about the mating status of a female rather than by

manipulating or masking the female's sex pheromone. They act as a passive form of mate guarding and may work in conjunction with a male-induced postmating loss of sexual receptivity in the females (Gillot 2003). While antiaphrodisiac-like effects have been documented in several insects (Happ 1969; Gilbert 1976; Tompkins & Hall 1981a, b; Kukuk 1985; Andersson et al. 2000), definitive behavioural and chemical evidence is quite rare. Only in *Drosophila melanogaster* (Jallon et al. 1981; Scott 1986; Yew et al. 2009), *Heliconius melpomene* (Schulz et al. 2008), and a few closely related pierid butterflies (Andersson et al. 2000, 2003) have the active compounds involved been identified and proven to inhibit male courtship. Even these few cases are not without issue. The *Drosophila* pheromone, *cis*-vaccenyl acetate, has a context-dependent function (Ejima et al. 2007; Everaerts et al. 2010), and its antiaphrodisiac function has been disputed (Vander Meer et al. 1986; Scott & Richmond 1987; Tram & Wolfner 1998). Of the three pierid species examined, males in one are simply masking female odour with their own rather than transferring a unique compound (Andersson et al. 2003).

In the western tarnished plant bug, *Lygus hesperus* Knight, there is evidence that recently mated females are less attractive to males than are virgins (Strong et al. 1970; Brent 2010a). Prior to mating, a male will approach a female and begin tapping his antennae (antennate) against the posterior tip of her abdomen. If the male is interested in the female after the initial assessment, he will begin courting her by shaking his body while moving to mount her (Strong et al. 1970). In contrast, males that antennate a recently

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mated female will often back away quickly (Brent 2010a). It has been suggested for *Lygus* spp. that the spermatophore may contain factors that change female fertility signalling (Aldrich et al. 1988), as appears to occur in the mirids *Lygocoris pabulinus* (Groot et al. 2001), *Trigonotylus caelestialium* (Fukuyama et al. 2007) and *Stenotus rubrovittatus* (Oku & Yasuda 2010). However, such a female fertility signal has yet to be identified in *Lygus* bugs and the loss of female attractiveness occurs so quickly after mating that it is more likely to be the result of a transferred chemical marker from the male. During copulation of the mirid *Phytocoris difficilis*, males convey metathoracic gland compounds to females that act as antisex pheromones (Zhang & Aldrich 2003; Zhang et al. 2007). However, these compounds are widely broadcast by the males and might be generally repellent. While other males may learn to associate this odour with a mated female and modify their courtship behaviour appropriately, the odour compound may not necessarily be produced specifically as an antiaphrodisiac. Additionally, both sexes of *L. hesperus* have metathoracic glands that contain large amounts of the same noxious volatiles that are immediately released when attacked by predators (Byers 2006), suggesting the postmating repulsion effect is associated with another source. Unpublished work in conjunction with analysis of male and female defensive volatiles in the metathoracic glands (Byers 2006) indicated an internal male-specific volatile, myristyl acetate, not contained within the metathoracic gland. The function, if any, of the volatile was unknown, but in light of the 4–5 day postcopulatory period during which mated females remain unattractive to males (Brent 2010b), the compound might be utilized as an antiaphrodisiac. The likeliest mechanism by which a male could deliver an antiaphrodisiac would be along with the other components of the spermatophore during mating. The compound could then be released slowly through a mated female's vaginal pore, where it is detected by antennating males.

To determine whether *Lygus* males deliver an antiaphrodisiac to females, we conducted behavioural assays to assess the effect of the spermatophore contents on male courtship behaviour in *L. hesperus*. Similar assays were used to test the antiaphrodisiac properties of myristyl acetate identified from male *Lygus*. Analytical–chemical studies employing gas chromatography–mass spectrometry (GC–MS) were used to localize the site of storage of this male-specific compound and to test for its presence in the spermatophore. To make an initial assessment of whether this compound is widely used among *Lygus*, additional analyses were conducted to measure expression in the females and males of two sympatric sister species, *Lygus elisus* and *Lygus lineolaris*.

## METHODS

### Insects

We obtained *Lygus hesperus* from an established colony at the USDA-ARS Arid Land Agricultural Research Center (Maricopa, AZ, U.S.A.). Colony health and genetic diversity were maintained by periodic outbreeding with locally caught conspecifics. Insects were reared at 25 °C, 20% relative humidity, under a 14:10 h light:dark cycle. Adults were produced from groups of mixed-sex nymphs reared in 1890 ml waxed chipboard cups (Huhtamaki, De Soto, KS, U.S.A.). Each container was provisioned with approximately 20 g of fresh green bean pods (*Phaseolus vulgaris* L.) and 12 g of artificial diet (Debolt 1982) packaged in Parafilm M (Pechiney Plastic Packaging, Chicago, IL, U.S.A.) (Patana 1982). Provisions were replaced every 48 h. Containers were covered with nylon mesh to ensure adequate ventilation and light exposure. Daily monitoring allowed adults to be collected within 24 h of emergence. Cohorts of same-aged adults were separated by gender and reared under

conditions matching those for nymphs. Nymphs and adults were reared at densities known to minimally affect their development (50–100/container; Brent 2010c).

Additional insects were collected directly from nearby alfalfa (*Medicago sativa* L.) fields. These were used to produce whole body extracts for interspecific composition comparisons between *L. hesperus* and the sympatric mirid species *L. elisus* and *L. lineolaris*. Age and mating status were unknown for these individuals, but they were separated by sex.

### Behavioural Effects of Tissue Homogenates

We used a no-choice behavioural assay to test male courtship response to females treated with various tissue homogenates. All individuals were aged 7 days posteclosion to ensure they were sexually mature and willing to copulate (Brent 2010a). They were isolated from the opposite gender throughout adulthood to prevent their behaviour towards prospective mates from being influenced by postmating refractoriness (Strong et al. 1970; Brent 2010b) and to guarantee that the males were naïve with regard to the odour associated with mated, unreceptive females (Brent 2010b). Females were typically treated with a solvent control or one of three male-derived tissues. Tissues were harvested from 10 individuals and homogenized in 60 µl of 95% ethanol (Sigma–Aldrich). Tissues included (1) spermatophores removed from females immediately after mating, (2) lateral and medial accessory glands (AGs) from virgin 7-day-old males and (3) male thoracic muscle of an equivalent mass. Muscle served as a negative control because it has no detectable odorant properties or volatiles (by GC–MS). Homogenates were centrifuged at 0.4 g for 1 min to clarify the supernatant. To the posterior tip of the abdomen of each female was applied 0.6 µl of supernatant (0.1 organ equivalents). Individual females were placed in clean 1.5 × 5.0 cm covered glass petri dishes. After the applications dried for 10 min, a male was introduced to each dish. Pairs were observed for 1 h, during which all instances of male courtship were recorded. Courtships were distinguished from incidental approaches by characteristic male behaviours indicating the intent to mate (Strong et al. 1970). A total of 116 trials were conducted, and during each trial all four treatments were run concurrently. All individuals were dissected after each trial to ensure they were sexually mature and had not previously mated (i.e. no spermatophore for females or depleted accessory glands for males; Brent 2010b).

### Collection and Analysis of Odorants

Body washes of bugs were conducted in 2 ml vials containing 100 µl of hexane with internal standards (1–2 ng/µl of (+)-carvone and ethyl heptanoate). Contents were swirled for 10 s, then solvent was pipetted into a glass insert (VWR, Radnor, PA, U.S.A.) and stored at –20 °C until analysis by GC–MS within a few days. Macerations of body parts (head and thorax combined, abdominal cuticle, hind- and midgut combined, testes and seminal vesicles combined, medial and lateral accessory glands separately, or spermatophore) or whole bugs (newly emerged virgin males, or female or male adults at 7 days postemergence that were either virgin or mated within the previous 2 h) in 100 µl of hexane in conical glass vials (Wheaton Scientific Products, Millville, NJ, U.S.A.) were done with a blunt, nickel-plated tapestry needle (Prym-Dritz Corp., Spartanburg, SC, U.S.A.) and the supernatant was analysed as above (Byers 2006). Compounds in the *Lygus* bug extracts were separated on a Varian 3800 GC (Varian, Inc., Palo Alto, CA, U.S.A.) equipped with a 60 m × 0.25 mm (inner diameter) fused-silica capillary column coated with 0.25 µm permethylated β-cyclodextrin (Cyclodex-B column, J&W Scientific of Agilent Technologies, Santa Clara, CA).

The helium carrier gas was programmed for constant flow of 1.2 ml/min. Samples of 1  $\mu$ l were injected by a Varian CP-8400 autosampler into the GC port at 250 °C. The injection mode was split-less for 0.75 min, then 60:1 split for 5 min, thereafter 20:1. The oven/column temperature programme for the chiral column was initially held at 40 °C for 2 min, and then increased to 100 °C at a rate of 15 °C/min. After 10 min at 100 °C, the temperature was increased at 3 °C/min to 150 °C and then to 230 °C at 20 °C/min before being held at 230 °C for 10 min. For chemical identification, the mass spectra of compounds separated by GC were determined using a Varian Saturn 2000 mass spectrometer (MS, ion trap, 70 eV EI) using the spectral databases NIST08 (National Institute of Standards, U.S.A.) and Wiley7 (Wiley Registry of Mass Spectral Data, Hoboken, NJ, U.S.A.) and comparison to spectra of commercial standards (Sigma–Aldrich, St Louis, MO, U.S.A.). The reconstructed ion chromatogram was filtered for ions 69, 83, 97 and 111 by Varian Saturn software (v.6.41) to ensure specificity. Quantification of myristyl acetate per insect was based on the MS response factor of the compound relative to internal standard on the chiral column, amount of internal standard per solvent extract volume and number of insects or parts per extract. The detection limit for myristyl acetate was 0.3 ng/insect. The identification of myristyl acetate was confirmed by additional comparisons of retention times and mass spectra of male *Lygus* extracts and commercial standards on GC nonpolar (Varian CP-Sil 8 CB; Byers 2006) and polar columns (Varian CP Wax 52CB, 30 m  $\times$  0.25 mm (inner diameter)  $\times$  0.25  $\mu$ m coating; 40 °C initially, then increased to 110 °C at 10 °C/min, held 7 min and then increased at 20 °C/min to 230 °C and held for 20 min; otherwise, same conditions as above).

#### Behavioural Effects of Myristyl Acetate

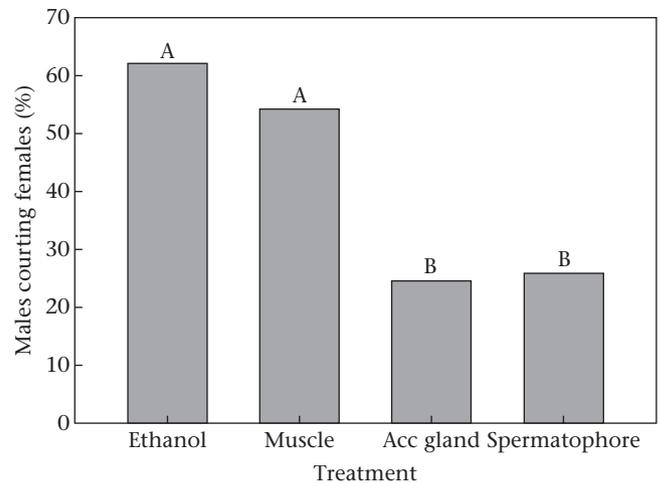
The same behavioural assay as described for the tissue homogenates was used to test the effect of isolated myristyl acetate on courtship by male *L. hesperus*. Female topical treatments included myristyl acetate (Sigma–Aldrich) diluted in ethanol (95%) to two concentrations ( $5 \times 10^{-9}$  and  $5 \times 10^{-7}$   $\mu$ g/ $\mu$ l), male AGs homogenized in ethanol and an ethanol control. A total of 101 trials were conducted, and during each trial all four treatments were run concurrently.

#### Statistical Analysis

The effect of the topical applications on the attractiveness of females was determined by  $2 \times 2$  chi-square tests comparing the percentage of males showing courtship behaviour. Comparisons of myristyl acetate concentration between tissue types, sexes and species were conducted using Kruskal–Wallis one-way ANOVA on ranks to compensate for non-normally distributed data. Dunn's test was used for all pairwise multiple comparison, using  $\alpha = 0.05$ . All analyses were conducted using Sigmaplot 11.0 (Systat Software, Point Richmond, CA, U.S.A.).

## RESULTS

Male courtship propensity was significantly influenced by the composition of the topical application received by virgin females ( $2 \times 2$  chi-square test:  $\chi^2_3 = 40.82$ ,  $P < 0.001$ ; Fig. 1). Compared to females treated with ethanol, those receiving muscle homogenate were equally likely to be courted by males ( $\chi^2_1 = 0.45$ ,  $P = 0.510$ ), but the rate was significantly lower for females treated with either AG ( $\chi^2_1 = 16.453$ ,  $P < 0.001$ ) or spermatophore homogenates ( $\chi^2_1 = 27.54$ ,  $P < 0.001$ ). Males showed clear evidence of being selective. After approaching and antennating females treated with either the AG or spermatophore homogenates, many males would

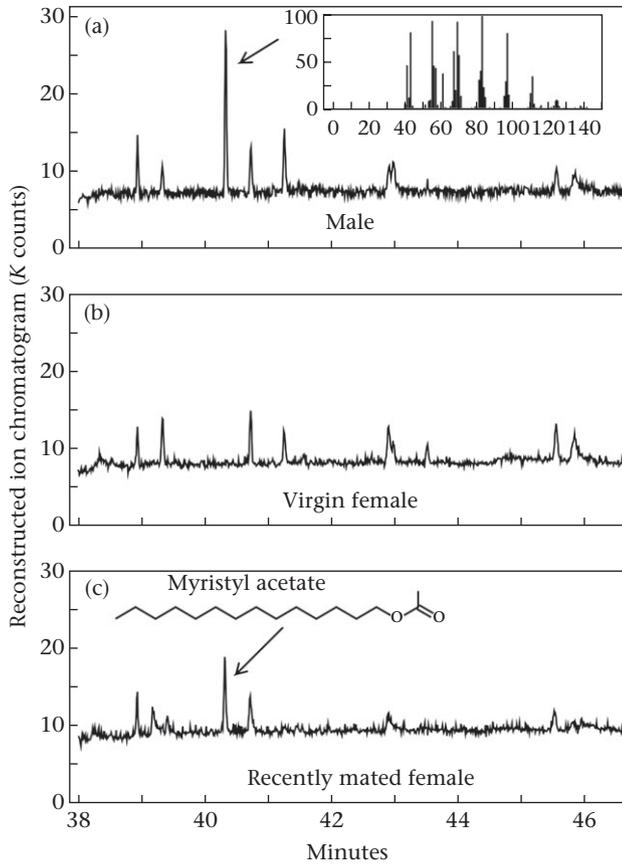


**Figure 1.** Percentage of male plant bugs, *Lygus hesperus*, that courted females typically treated with ethanol (solvent control), or a male tissue homogenate made from muscle, spermatophores or accessory glands. There were 166 samples for each treatment. Different letters above bars denote significant differences between treatments ( $2 \times 2$  chi-square tests:  $P < 0.05$ ).

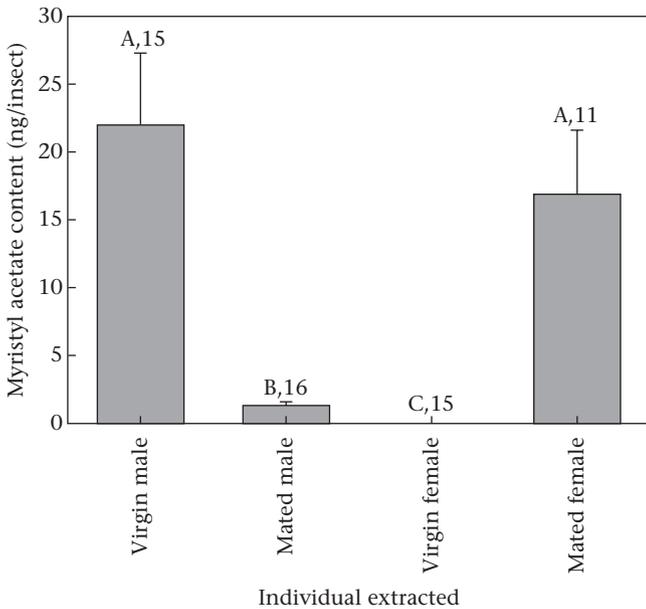
quickly pull back their antennae and retreat away, just as observed when males approach newly mated females (Strong et al. 1970; Brent 2010a). The divergent responses by males to these treatments did not appear to be due to any change in female behaviour. All females showed a similar level of receptivity to courting males regardless of the treatment they received.

Comparisons of whole body extracts from virgin and mated females and males of *L. hesperus* indicated that the primary chemical difference is in the concentration of myristyl acetate (hereafter MA; also named tetradecyl acetate; CAS number 638-59-5; Fig. 2). A male *Lygus* extract with a peak corresponding to MA mixed with a similar amount of MA standard gave peak enhancement on all three GC columns. The compound was absent in virgin females, but present in recently inseminated females of the same age (Figs 2, 3). Newly emerged males, which have generally inactive reproductive organs (Brent 2010a), contained little if any MA ( $N = 12$ ; mean  $\pm$  SE:  $0.12 \pm 0.12$  ng). By 7 days postemergence, virgin males had produced an ample supply (Fig. 3). Newly mated males of the same age had only trace amounts of MA, suggesting that the bulk of the compound is transferred during insemination. Relative to the amounts found with whole body extractions, very little MA was found using body washes of virgin males ( $N = 15$ ; mean  $\pm$  SE:  $0.29 \pm 0.14$  ng) and mated females ( $N = 10$ ; mean  $\pm$  SE:  $0.66 \pm 0.51$  ng), and only occurred in 28% of the individuals tested. This was likely due to leakage of MA from interior organs during the brief solvent wash.

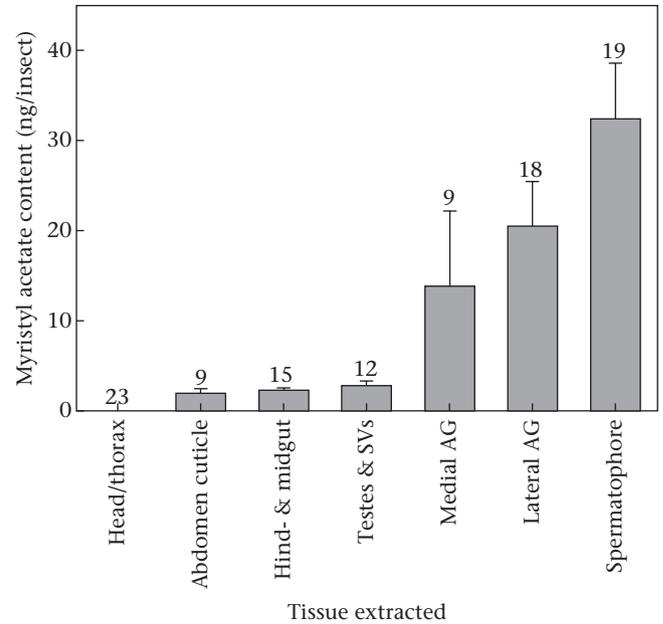
The source of the MA was determined by a comparison of compound content in male tissues (Fig. 4). MA was localized in the abdomen. The highest concentrations were found in the accessory glands, which were cumulatively equivalent in concentration to the spermatophore. This is consistent with the males fully evacuating the contents of their accessory glands when producing a spermatophore (Brent 2010b). Only inconsistent trace amounts were found associated with the cuticle, gut, seminal vesicles and testes. Because the accessory glands are delicate tissues that can easily rupture during dissection, it is likely that these trace amounts are indicative of contamination. Hexane body washes of females 2 h after they had mated revealed that MA from the spermatophores could be externalized ( $N = 10$ ; mean  $\pm$  SE:  $0.66 \pm 0.51$  ng). Similar washes of virgin males also indicated some externalization ( $N = 15$ ; mean  $\pm$  SE:  $0.29 \pm 0.14$  ng).



**Figure 2.** Reconstructed ion chromatograms on chiral GC column of hexane extracts of *L. hesperus* bugs. (a) Virgin male containing 36 ng of myristyl acetate eluting after 40.3 min (1% of extract injected). The compound's mass spectrum is in the inset. (b) Virgin female *L. hesperus* with no detectable myristyl acetate eluting at 40.3 min. (c) Recently mated female (~1 h before extraction) containing 15 ng of myristyl acetate (1% of extract injected).



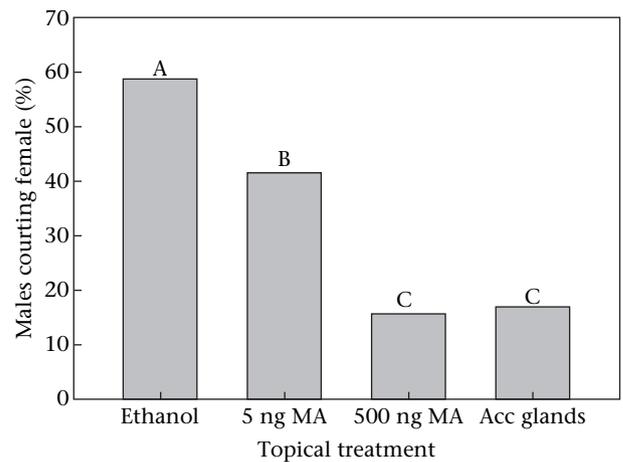
**Figure 3.** Mean + SE concentration of myristyl acetate in virgin and mated female and male plant bugs, *Lygus hesperus*, at 7 days postemergence. Sample sizes are indicated. Different letters above bars denote significant differences between groups (Kruskal–Wallace ANOVA:  $P < 0.05$ ).



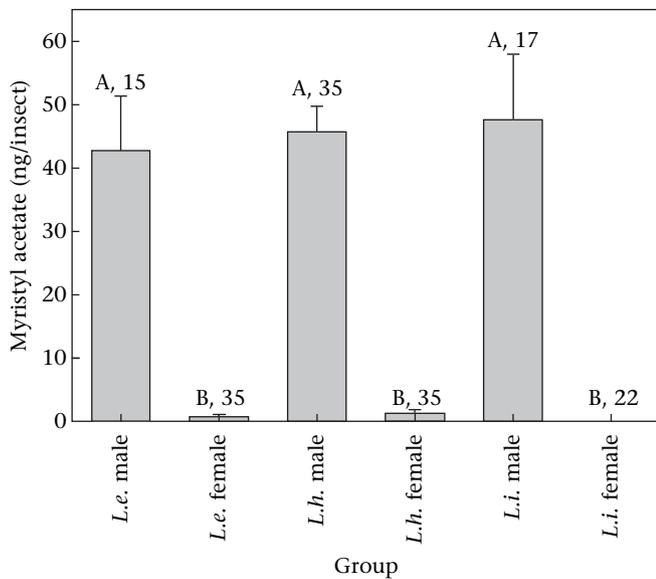
**Figure 4.** Mean + SE concentration of myristyl acetate in male-derived tissues (AG: accessory gland; SVs: seminal vesicles) of plant bugs, *Lygus hesperus*. Sample sizes are indicated.

Topical applications of two dosages of MA to virgin females were found to significantly reduce the likelihood of male courtship relative to controls ( $2 \times 2$  chi-square test:  $\chi^2_3 = 58.11$ ,  $P < 0.001$ ; Fig. 5). The higher dosage of MA evoked an effect comparable to that of the AG homogenate ( $\chi^2_1 < 0.001$ ,  $P = 1.0$ ), and although the inhibition appears dosage dependent, even the lower dosage caused a significant decline in male courtship behaviour relative to the solvent control ( $\chi^2_1 = 37.41$ ,  $P < 0.001$ ).

Examination of males and females from two sympatric *Lygus* species, *elisus* and *lineolaris*, indicated an expression pattern similar to that of *L. hesperus* (Fig. 6). Among field-collected individuals, MA was found in equivalently high concentrations in males but only in very low concentration among the females sampled.



**Figure 5.** Percentage of male plant bugs, *Lygus hesperus*, that courted females topically treated with ethanol, homogenated male accessory glands (Acc), or one of two dosages of myristyl acetate (MA) dissolved in ethanol. Myristyl's effect was dosage dependent. For each treatment there were 101 samples. Different letters above bars denote significant differences between treatments ( $2 \times 2$  chi-square tests:  $P < 0.05$ ).



**Figure 6.** Mean + SE concentration of myristyl acetate in field-collected female and male *Lygus* of three species, *L. elisus* (*L.e.*), *L. hesperus* (*L.h.*) and *L. lineolaris* (*L.i.*). Sample sizes are indicated. Different letters above bars denote significant differences between groups (Kruskal–Wallace ANOVA;  $P < 0.05$ ).

## DISCUSSION

Our results provide the first clear evidence for an antiaphrodisiac in a plant bug. Previous research with *L. hesperus* found that virgin females are much more likely to be courted by males than are recently mated females of the same age (Strong et al. 1970; Brent 2010a). We found that during mating, males transfer myristyl acetate (MA) from their lateral and medial accessory glands into the seminal depositories of females (Figs 2–4), and that this compound immediately renders females unattractive (Fig. 5). Topical application of the compound had no apparent effect on the female's behavioural response to approaching and courting males, indicating that the males were responding solely to the MA and not to a reduction in female receptivity. Male accessory glands are a common source of products transferred during copulation that are capable of manipulating female behaviour, odour and physiology (Gillot 2003; Wolfner et al. 2005). There was no evidence that females are capable of producing this compound. Given the general loss of female sexual receptivity following mating (Brent 2010a, b), this signalling mechanism may have evolved to ensure that males do not waste their time and energy and risk exposure to predators while pursuing females that are uninterested in their advances (Simmons 2001; Gillot 2003).

The response of males to MA appears innate; although the virgin males used in this experiment were isolated from females at adult emergence and had no opportunity to learn to avoid mated females that are likely to repel their advances, they still showed an aversion to MA. Additionally, the body wash results indicate that mature *L. hesperus* males do not externally express MA, so there would be little chance that other males learn an association between the pheromone and the presence of a male. MA also does not appear to act as a general repellent broadcast onto the female cuticle, such as an antisex pheromone (Zhang & Aldrich 2003; Zhang et al. 2007) or a fertility signalling disruptor (Groot et al. 2001), as has been found in other mirids. In contrast, MA is delivered directly into a *L. hesperus* female's reproductive tract along with other male-derived seminal constituents that appear to act in concert to reduce female sexual receptivity (Brent 2010a, b) and increase oviposition rate (Brent et al. 2011; C. S. Brent, unpublished data). Combined,

these effects promote passive mate guarding and an overall increase in male fitness.

Leakage of small amounts of MA from the female seminal depository into the gonopore, which opens externally, is the likeliest mechanism by which males can detect a female's mating status. *Lygus hesperus* males approaching prospective mates always antennate the posterior tip of the female abdomen (Strong et al. 1970; Brent 2010a), where such release would occur. Insects appear to be very sensitive to odour sources that their antennae either touch or approach closely. For example, western subterranean termites, *Reticulitermes hesperus* Banks, have been observed to follow trails with as little as 0.01–0.1 fg dodecatrienol per cm (Saran et al. 2007). This minute level of pheromone detected by termites is  $10^{-5}$  to  $10^{-6}$  times less than the amounts of MA deposited into female *Lygus* (assuming 10 ng). Thus, it is reasonable that male *Lygus* antennating near or upon female genital openings would be able to detect the MA from a recent mating. As the ejaculated MA degrades or diminishes with release, the females should become increasingly attractive. Such a decline has been observed in mated tsetse fly females, in which a similar male-derived signalling alkene diminishes over time (Carlson & Langley 1986). Accordingly, we see female *Lygus* become increasingly likely to be courted on the days following a mating (Brent 2010b). The sensitivity of males to diminishing MA content is also evident in their dosage-dependent responses to topically applied MA (Fig. 5).

MA (tetradecyl acetate) has been reported as a volatile constituent of the female's sex pheromone gland in over 80 species of moths (Lepidoptera), but it has rarely been shown as a major synergistic component of the sex pheromone of these species (El-Sayed, [www.pherobase.com](http://www.pherobase.com)). Several bumblebees (Hymenoptera) appear to use MA as a minor component of their marking pheromone secretion, and MA is found as one constituent of many volatiles in the Dufour's gland of some ant species, but the behavioural role of MA is uncertain in these complex chemical mixtures (El-Sayed, [www.pherobase.com](http://www.pherobase.com)). Several thrips (Thysanoptera) produce MA as a minor component of their anal secretion, which contains several volatiles that can repel ants and may serve a defensive function (Suzuki et al. 2004; Tschuch et al. 2008). MA, with a molecular weight of 256.4, has a moderately low volatility (0.0015 mm/Hg; Hass & Newton 1971) that is appropriate for a short-range marker of mating status. In the cases above, MA functions, if at all, within the context of a blend as a minor component that is probably either a precursor or a by-product of biosynthesis of the major semiochemical components. Although MA appears to account for almost all the effectiveness of a male *Lygus* spermatophore, there may be additional minor components of the spermatophore that enhance or stabilize the signal.

Although this study demonstrates that MA can have at least a short-term effect on mating and persists in the female at a measurable level for at least a few hours, it is unknown for how long the females remain unattractive after being inseminated. The MA may persist and act as an antiaphrodisiac for the several days that the females remain unreceptive to males (Brent 2010b). Alternatively, the loss of attractiveness may be short term, and the mating-induced changes in female behaviour could ensure that subsequent prospective mates are repelled. A third possibility is that the mating induces the female to change her chemical signature in such a way that she is no longer attractive. Reduced production of the female sex pheromone has been observed in a variety of insects (reviewed in Thomas 2010), including other mirids (Fukuyama et al. 2007; Oku & Yasuda 2010). Pheromonostatic peptides from male accessory glands have been implicated in the reduced production of sex pheromones by female *Heliothis zea* (Raina 1989) and *Helicoverpa armigera* (Eliyahou et al. 2003). Although a sex pheromone specific to *L. hesperus* has yet to be

identified (see Ho 2000; Byers 2006), males can differentiate between immature and mature females and adjust their courtship behaviour accordingly (Brent 2010a). This suggests that the male can detect a factor that is correlated with female reproductive status. One possible marker of female maturity may be major defensive volatiles (4-oxo-(E)-2-hexenal, hexyl butyrate and (E)-2-hexenyl butyrate) which are not present in newly emerged females, but which are increasingly expressed by their meta-thoracic glands concurrently with increasing ovarian activity (Byers 2006). A *Lygus* female may modulate the expression of these volatiles or some factor(s) after mating to change her fertility signalling (Aldrich et al. 1988). Graham (1988) found evidence of a sex pheromone expressed near the ovipositor of *L. hesperus* females that might serve this purpose. However, change in the expression profile would take more time to accomplish than the abrupt loss of female attractiveness observed immediately after mating. MA may serve as an interim signal, persisting for the few hours that it might take until the change in the female's purported pheromone signature is completed. We are currently investigating these possible mechanisms.

Because the antiaphrodisiac only conveys information about the mating status of the female, it is not the final determinant of a male's response. Even when presented with the highest dosage of MA, a significant percentage of males were still willing to court females (Fig. 5). We have observed similar percentages of males that seem to be insensitive to female status even after they have mated normally (Brent 2010a). Various external and internal factors could influence the motivational state of males. It has already been established that the activity and preparedness of the male reproductive organs are a key determinant in the males' willingness to court females (Brent 2010a, b). Long-term rearing conditions also appear to affect the propensity of males to copulate with previously mated females (Brent & Spurgeon 2011). In this experiment, the previous experiences of the males could have been a key factor in their response to the antiaphrodisiac. Prior to our behavioural assays, the males had been isolated in single-gender groupings. Similar long-term isolation from females leads to a reduced responsiveness to anticourtship pheromones in *Schistocerca gregaria* males (Seidemann 2006). However, the naïveté of our males with regard to the antiaphrodisiac may have also prevented them from becoming acclimated and less responsive compared to males that regularly encounter mated females. Alternatively, repeated exposure to mated females might help to reinforce the avoidance response as the males learn to associate the odour of MA with females that will be sexually unresponsive, as seems to be the case with *D. melanogaster* (Ejima et al. 2007) and *Lasioglossum zephyrum* (Wcislo 1987). Exploring these possibilities will be a necessary component of developing this pheromone as a future pest control agent.

Although the conclusions that can be drawn from the similar MA expression patterns observed in our comparison of *L. elisus*, *L. hesperus* and *L. lineolaris* (Fig. 6) are necessarily limited until further verification, they are nevertheless intriguing. The data suggest that use of MA as an antiaphrodisiac may be widespread in *Lygus* species. Unlike pheromones used to prevent interspecific matings, antiaphrodisiacs inherently suppress mating and would be under no selective pressure to evolve species specificity. The results may also indicate something about the mating frequency and persistence of the MA in mated females, even though the recent mating history of these field-caught females and males was unknown at the time they were extracted. The low average concentration of MA in females suggest that they were not mating very frequently, and that even for inseminated females, sufficient time had elapsed since mating that the majority of the compound had degraded or been expelled. Of course if the MA does not persist for very long once transferred to the females, this window of time

could be brief. The high concentration of MA among males suggests that males do not mate very frequently, which is consistent with the female data. On the other hand, the high variability in MA content between males suggests that males that have mated are able to rapidly replace the MA, which is consistent with their short refractory period between matings (Brent 2010b).

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