Journal of Insect Physiology 60 (2014) 104–110

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Characterization of male-derived factors inhibiting female sexual receptivity in *Lygus hesperus*

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ARTICLE INFO

Article history:
Received 24 September 2013
Received in revised form 29 November 2013
Accepted 2 December 2013
Available online 11 December 2013

Keywords:
Accessory glands
Juvenile hormone
Lygus hesperus
Sexual receptivity
Spermatophore

ABSTRACT

Newly mated females of the plant bug, *Lygus hesperus* Knight, enter a refractory period during which their sexual receptivity to courting males is greatly reduced for several days. This behavioral change appears to be induced by male-derived factors delivered in the spermatophore during copulation. To better understand the source of the factor(s) responsible for the inhibition, the homogenates of spermatophores, or of the individual organs that provide the constituents of the spermatophore, were injected directly into the abdomen of virgin females. The contents of the lateral and medial accessory glands both appear to produce inhibitory effects, but those of the seminal vesicle had no effect. Treatment of the homogenate also indicated that the active factor(s) is heat labile and water soluble. Several unique proteins were found in the water soluble fraction of the spermatophore, one of which is similar in size to the *Drosophila melanogaster* sex peptide, a male derived compound known to inhibit receptivity in female flies. In addition, spermatophores contained a substantial quantity of juvenile hormone, a key endocrine regulator of reproductive behavior and physiology in most insects. The results support the hypothesized role of males in manipulating the post-mating behavior of females, and suggest this is achieved through multiple components that act in concert to induce both short- and long-term effects.

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1. Introduction

Like other mirid bugs (Wheeler, 2001), a female of the western tarnished plant bug, *Lygus hesperus* Knight, will mate multiple times during her adult life (Strong et al., 1970). Females first become receptive to the courtship behavior of conspecific males once they reach sexual maturity, which, depending upon rearing environment, occurs 6–10 days after adult eclosion (Brent, 2010a; Strong et al., 1970). The timing of her receptivity corresponds to ovarian readiness and the availability of chorionated eggs for fertilization (Brent, 2010a). Shortly after copulation, a female will normally enter a refractory period during which she avoids contact with other potential mates (Brent, 2010a; Strong et al., 1970) and greatly increases her rate of egg laying (Brent et al., 2011). This period of reduced receptivity lasts for 5 days on average and can extend beyond 2 weeks in some individuals (Brent, 2010b). The delay between matings likely ensures that a male's sperm is given precedence in fertilizing available eggs (Gillot, 2003), and also provides the female the time needed to replenishing resources for producing additional eggs. While ovarian activity has been shown to be an important determinant of female insect sexual receptivity and responsiveness to associated exogenous stimuli (Ringo, 1996), it does not appear to be the only cause for the refractory period of *L. hesperus*.

As with other insect species (Gillot, 2003; Wolfner et al., 2005), male-derived products typically transferred during mating were found to decrease the sexual receptivity of unmated lygus females (Brent, 2010b). An injection of homogenized spermatophores directly into the hemocoel of virgin females, thereby bypassing the reproductive tract, suppressed receptivity to the same extent as mating. This result suggests that one or more components of the seminal fluid may directly modulate female receptivity, and that they need not necessarily be present within the female seminal depository to be effective. The exact nature of these components remains to be determined. Some male insects include prohormones or hormones in their seminal fluid, which could influence mating behavior (Borovsky et al., 1994; Park et al., 1998; Shirk et al., 1980). Juvenile hormone (JH), a key endocrine regulator of reproductive behavior and physiology in most insects, is one such substance. JH plays a key role in stimulating egg maturation in the moth *Heliothis virescens* (Park and Ramaswamy, 1998) and possibly in *Cecropia* silk moths (Shirk et al., 1980). Other male-derived factors can also stimulate JH production in a recipient female (Park et al., 1998). While JH can have important effects on the organization and activity of the ovaries and ancillary tissues that might help regulate the frequency of mating, its impact is

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0022-1910/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.jinsphys.2013.12.001

likely to be slower than the almost immediate cessation of female receptivity after mating in *L. hesperus*. It is also possible that a male-derive peptide, such as the sex peptide of *Drosophila melanogaster*, is involved in regulating mating in *L. hesperus*. Short peptides are sufficiently small to move through the wall of the seminal depository into the hemocoel. They are then carried by the hemolymph to receptor sites in the brain or other parts of the CNS (Chen et al., 1988; Leopold et al., 1971; Lung and Wolfner, 1999; Ringo, 1996; Sirot et al., 2008; Smid, 1998; Yamaoka and Hirao, 1977) where they can effect sensory perception and/or reproductive behavior. Such peptides are usually produced in the male accessory glands, but they can also originate from the testes or ejaculatory duct (Gillot, 2003; Yamane et al., 2008).

Rather than relying on a single mechanism, the male may rely on a combination of factors to achieve long term changes to female mating behavior. Quick acting factors could initially decrease female receptivity, whereas other seminal fluid components acting on the endocrine system and reproductive organs could produce more durable changes (Ringo, 1996). Or all the effects could stem from a cascade of events triggered by short-term exposure to one active factor. The eventual release from the refractory period may occur through decay of these stimuli. As the spermatophore contents are metabolized, competing stimuli such as ovarian stretch receptors activated by the presence of a growing egg clutch may counteract the mating inhibition.

This study was undertaken to elucidate the source and nature of the factors that may be responsible for the loss of mating receptivity during the refractory period of *L. hesperus* females. To achieve this objective, the sexual receptivity of virgin females was assessed after direct abdominal injections of treated seminal materials, and the constituents of the male products were characterized to determine whether hormones or peptides might be present. The results are an important advance toward devising novel control strategies for this major agricultural pest (Jackson et al., 1995).

2. Methods and materials

2.1. Insects

The *L. hesperus* used in this study were obtained from a large, inbred laboratory colony maintained at the US Arid Land Agricultural Research Center (Maricopa, AZ, USA). The individuals in this colony are periodically outbred with locally-caught conspecifics to maintain vigor. The stock insects were given unrestricted access to a supply of green bean (*Phaseolus vulgaris* L.) pods and an artificial diet mix (Debolt, 1982) packaged in Parafilm M (Pechiney Plastic Packaging, Chicago, IL, USA) (Patana, 1982). Both food sources were replenished as needed. Insects were reared at 25 °C, 20% relative humidity, under a L14:D10 photoperiod.

Adults were produced from randomly selected groups of nymphs of varied parentage. The nymphs were reared in 1890-ml waxed chipboard cup (Huhtamaki, De Soto, KS, USA) at a density known to have minimal effect on *L. hesperus* development ($\leqslant$100 nymphs/container; Brent, 2010c). Nymphs in each container were provided approximately 20 g of fresh green beans and 12 g of artificial diet, which was replaced every 48 h. Rearing cups were covered with a nylon mesh to ensure air circulation and light exposure. Daily monitoring allowed adults to be collected within 24 h of emergence. Cohorts of adults of the same age and sex were reared under conditions matching those for nymphs, but with population densities ranging between 50 and 120 adults/container.

2.2. Spermatophore size and refractory period duration

Spermatophore size, a surrogate measure for the relative concentration of the factors transferred from the males, was tested to see if it affected the duration of the refractory period. This was determined by allowing females to mate with males of different ages. Older, previously unmated males accumulate more transferrable resources in the accessory glands and seminal vesicles, and thus produce larger spermatophores than younger males (Brent, 2010a). Two virgin 7 day old females from the same cohort were placed for 1 h in a covered glass Petri dish (60 × 15 mm) with two virgin males, both of which were either 3 or 7 day old. Females observed copulating had their insemination status confirmed by inspecting the dorsal abdomen for a spermatophore lying just underneath the cuticle (Cooper, 2012). One third of the newly mated females were dissected within 30 min of mating, and the spermatophores were weighed on a microbalance (Sartorius TE153S, Goettingen, Germany). The remaining females (n = 53 for 3 day old mates; n = 52 for 7 day old mates) were held individually in Petri dishes along with a section of green bean pod. One day later, the pod section was removed and two virgin males, aged 6–8 days, were introduced for 1 h. The willingness of each female to remate was recorded. Testing was repeated every 24 h on all surviving females for 14 days or until they mated. All females were dissected at the end of the experiment to confirm the presence of a spermatophore from the initial mating. Because spermatophores become depleted over time and shrink, it was readily evident from their size which spermatophores were old or new.

2.3. Origin of inhibitory factors

Microinjections were used to determine the source of the factors in the spermatophore that induce a post-mating refractory period in females (Brent, 2010b). The specific composition of *L. hesperus* spermatophores is unknown, but the mass consists of components from the lateral (LAG) and medial accessory glands (MAG), and the seminal vesicle (SV) (Strong et al., 1970). To determine which, if any, of these sources might influence female mating receptivity, these respective reproductive organs were dissected from virgin males aged 6–8 days and homogenized for injection. The development of these organs is complete at these ages, and each is filled with product when the males are not allowed to mate (Brent, 2010a). For a control homogenate, the midgut was also dissected from the same males. For each sample, five pairs of each organ type were homogenized in 50 μl of insect saline. An additional homogenate (Mix) was created using the combined LAG, MAG and SV from five males. A volume of 0.5 μl of either insect saline or an organ homogenate was injected into the abdominal lumen using a graduated borosilicate glass syringe, as previously described (Brent, 2010b). Injected females were allowed to recover for 1 h, then placed in a covered glass Petri dish (60 × 15 mm) with two virgin males, aged 6–8 days. A 50-mm long section of green bean pod was also placed inside the arena as a source of nourishment. Insects were allowed to interact freely for ~24 h, after which the surviving females were dissected to determine if they had been inseminated. Overnight mortality was $\leqslant$5% across all injected females, which was similar to the rate for the untreated males with which they were housed. Data was not collected from sickly females or those whose mates had died overnight.

2.4. Heat Lability

Microinjections were also used to determine the sensitivity of the male-derived inhibitory factor(s) to heat. For each treatment 79–84 females were injected with either insect saline, the Mix homogenate described above, or a Mix homogenate that had been heated at 95 °C for 10 min then allowed to cool to room temperature prior to injection. As above, surviving females were given access to two males for 24 h in a test arena to determine their propensity to remate.

2.5. Aqueous solubility

To determine whether the inhibition inducing factors within the seminal products were soluble or insoluble in water, females were injected with insect saline, the Mix homogenate described above, or the water soluble or insoluble fractions of a Mix homogenate. For each treatment 62 females were injected. Solubility fractionation was achieved by centrifuging a Mix homogenate at ~14,000×*g* for 10 min at 4 °C. The soluble supernatant layer was removed with a clean glass pipette and the non-soluble fraction was resuspended in a comparable volume of insect saline. Injections and subsequent tests for female receptivity were conducted as described above.

2.6. Protein sizes

Day 7 adult male accessory glands ($n = 10$), day 9 adult virgin female seminal depositories ($n = 20$), and newly transferred spermatophores ($n = 10$) were dissected in insect saline and stored at −80 °C until they were processed. Tissues were homogenized in ice-cold phosphate-buffered saline (PBS) containing 5 mM ethylenediaminetetraacetic acid (EDTA) (Sigma–Aldrich, St. Louis, MO, USA), 0.5% TritonX-100 (Sigma–Aldrich), and 1× Halt Protease Inhibitor cocktail (Thermo Scientific, Waltham, MA, USA). Homogenates were centrifuged at ~14,000×*g* for 15 min at 4 °C with the resulting supernatant transferred to a new tube and re-centrifuged. The protein concentration of each respective supernatant was estimated using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA). After protein quantitation, aliquots of each supernatant were diluted to 1 μg protein/μl. Samples consisting of 5 or 10 μg protein were mixed with 5 μl NuPage LDS loading buffer (Invitrogen Life Technologies, Carlsbad, CA, USA) and incubated for 5 min at 100 °C. Proteins were separated by electrophoresis on a 10% NuPAGE Novex Bis–Tris gel (Invitrogen Life Technologies) for 90 min at 130 V in a MES–SDS running buffer. A 10-μl aliquot of the SeeBlue Plus2 Pre-Stained protein molecular weight marker (Invitrogen Life Technologies) was electrophoresed in parallel with the samples. Proteins were visualized by Coomassie staining using SimplyBlue SafeStain (Invitrogen Life Technologies) according to the manufacturer's instructions. Gel images were processed using Photoshop CS (Adobe, San Jose, CA, USA).

2.7. Hormone content

Reproductive organs were collected from 10 day old adult females and males that were either (1) virgins or (2) had mated within 5 min of collection to prevent sample degradation. Seminal depositories, which were empty in virgin females and contained spermatophores in mated females, were collected. Accessory glands (both lateral and medial) were collected from males. Ten samples were run for each tissue type. For each sample except the seminal depositories, 10 organs were initially dissected in insect saline then stored together in a glass vial containing 200 μl hexane (Sigma–Aldrich). The small size of the empty seminal depositories necessitated pooling 15 organs per sample. Tissue was homogenized by hand and extracted three times with hexane. The collected hexane washes were stored at −80 °C until analysis. To each sample 200 pg of farnesol (Sigma–Aldrich) was added as an internal standard. After purification and derivatization, gas chromatography/mass spectrometry was used to determine juvenile hormone (JH) content as previously described (Brent and Vargo, 2003). JH form was confirmed by first running test samples in SCAN mode for known signatures of JH 0, JH I, JH II, JH III and JH III ethyl; JH III was confirmed as the primary endogenous form in *L. hesperus*. Subsequent samples were analyzed using the MS SIM mode, monitoring at *m*/*z* 76 and 225 to ensure specificity for the d3-methoxyhydrin derivative of JH III. Total abundance was quantified against a standard curve of derivatized JH III and expressed in amounts per organ.

2.8. Statistical analysis

A Mann–Whitney rank sum test was used to determine if there were significant differences in the spermatophore mass produced by either 3 or 7 day old virgin males, and in the refractory period induced in females mating with those males. Frequencies of female insemination for the different injection treatments were compared to the respective saline control values by chi-square tests using the Holm–Bonferroni method to correct for multiple comparisons. For all tests, $\alpha = 0.05$ and adjusted *P*-values are provided. Due to non-normal data, JH content was compared between organs using a Kruskal–Wallis ANOVA on ranks followed by Tukey's test for multiple pairwise comparisons. Analyses were conducted using Sigmaplot 11.0 (Systat Software, Chicago, IL, USA).

3. Results

3.1. Spermatophore size

As previously shown (Brent, 2010a), larger spermatophores were produced by older males than by younger males (Fig. 1). The spermatophore from males 7 day post-emergence were more than twice as large as those produced at 3 day post-emergence (Mann–Whitney rank sum test, $T_{35,35} = 681.5$, $P = <0.001$). Although the effect was not statistically significant, females receiving the larger spermatophores tended to delay remating by 2 day on average compared with females receiving the smaller spermatophores (Fig. 1; Mann–Whitney rank sum test, $T_{27,36} = 728.5$, $P = 0.059$).

3.2. Origin of inhibitory factors

Female mating receptivity varied greatly after homogenate injection (Fig. 2). As anticipated, injection of the homogenate Mix decreased by half the mating propensity of the females for at least 24 h relative to the saline control ($\chi^2 = 19.141$, d.f. = 1, adjusted $P < 0.01$). The inhibition was also observed for spermatophore constituents from the lateral ($\chi^2 = 13.429$, d.f. = 1, adjusted $P < 0.01$) and medial accessory glands ($\chi^2 = 17.382$, d.f. = 1, adjusted $P < 0.01$). In contrast, the gut control ($\chi^2 = 0.720$, d.f. = 1, adjusted

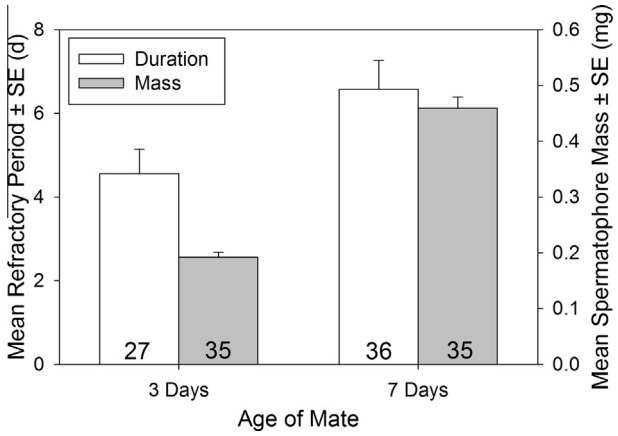

Fig. 1. Mean (±SE) duration of the refractory period of *L. hesperus* females initially mated to males that were either aged 3 or 7 day after adult emergence, and the mean (±SE) sizes of the spermatophore produced by similarly aged males. Sample sizes are indicated.

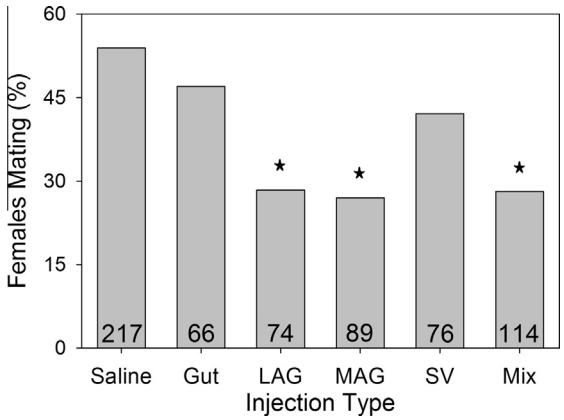

Fig. 2. Percentage of *L. hesperus* virgin females willing to mate after injection with 0.5 μl of either: a solvent control (Saline), or homogenates of male hindgut (Gut), lateral accessory glands (LAG), medial accessory glands (MAG), seminal vesicles (SV), or a mixture of lateral and medial accessory glands with the seminal vesicles (Mix). A star above a treatment indicates a significant difference (Chi-square, adjusted $P < 0.05$) from the saline control. Sample sizes are indicated.

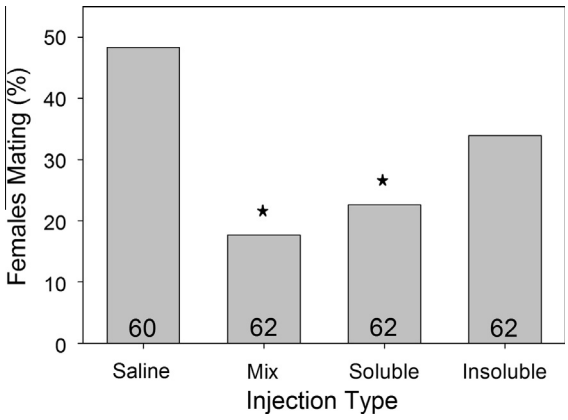

Fig. 4. Differences in sexual receptivity of *L. hesperus* virgin females, as indicated by the percent mating overnight, after an injection of 0.5 μl into their abdominal hemocoel of intact male accessory gland homogenates, or the soluble or insoluble fractions thereof. A star above a treatment indicates a significant difference (Chi-square, adjusted $P < 0.05$) from the saline control. Sample sizes are indicated.

$P = 0.396$) and seminal vesicles ($\chi^2 = 2.687$, d.f. = 1, adjusted $P = 0.202$) had no evident effect on female behavior relative to saline.

3.3. Heat lability

One or more mating inhibition factor in the spermatophore appears to be heat labile (Fig. 3). Compared to females injected with saline, those treated with the denatured Mix mated as frequently ($\chi^2 = 1.084$, d.f. = 1, adjusted $P = 0.594$), while those injected with the untreated Mix were only 40% as likely to mate ($\chi^2 = 13.640$, d.f. = 1, adjusted $P < 0.01$).

3.4. Aqueous solubility

The mating inhibitor(s) appeared to be a water soluble (Fig. 4). Compared to saline, there was a significant reduction in mating frequency for the Mix ($\chi^2 = 11.598$, d.f. = 1, adjusted $P < 0.01$) and soluble fraction ($\chi^2 = 7.767$, d.f. = 1, adjusted $P = 0.01$), but not the insoluble fraction ($\chi^2 = 2.073$, d.f. = 1, adjusted $P = 0.15$).

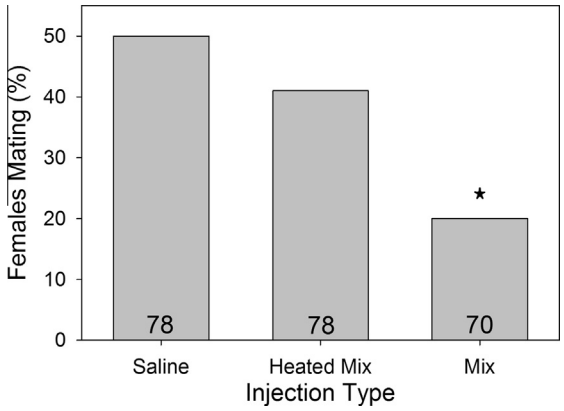

Fig. 3. Differences in sexual receptivity of *L. hesperus* virgin females, as indicated by the percent mating overnight, after injection into the abdominal hemocoel of 0.5 μl of either heated or untreated male accessory gland homogenates, or an insect saline control. A star above a treatment indicates a significant difference (Chi-square, adjusted $P < 0.05$) from the saline control. Sample sizes are indicated.

3.5. Protein sizes

Nine protein bands were identified that appeared in both the accessory gland and spermatophore but were not present in the seminal depositories of virgin females (Fig. 5, see arrows). These were visible in both the 5 and 10 μg runs, suggesting that they are not the product of overloading. The smallest unique band was approximately 4 kDa, comparable in size to that of a small peptide.

3.6. Juvenile hormone

JH III was present in both females and males (Fig. 6). JH content varied significantly with mating status (Fig. 6; Kruskal–Wallis ANOVA $H = 30.96$, d.f. = 3, $P < 0.001$) in both females (Tukey test $q = 6.09$, $P < 0.05$) and males (Tukey test $q = 4.73$, $P < 0.05$). Prior to mating, there was a high concentration of JH within the male

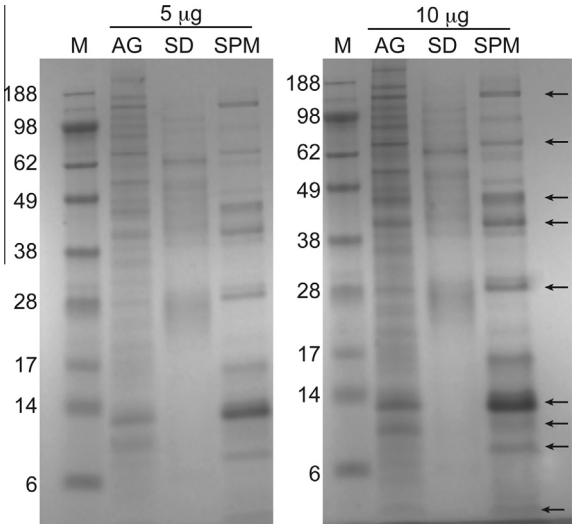

Fig. 5. SDS–PAGE of soluble proteins from *L. hesperus* male accessory glands, virgin female seminal depositories, and spermatophores. Representative image of a Coomassie-stained 10% polyacrylamide Bis–Tris gel electrophoresed in MES–SDS. Protein loading consisted of 5 μg (left panel) and 10 μg (right panel). Arrows indicate protein bands that appear in both the accessory gland (AG) and spermatophore (SPM) but not the seminal depositories (SD). The sizes of the molecular mass marker proteins (M) are shown to the left of each gel.

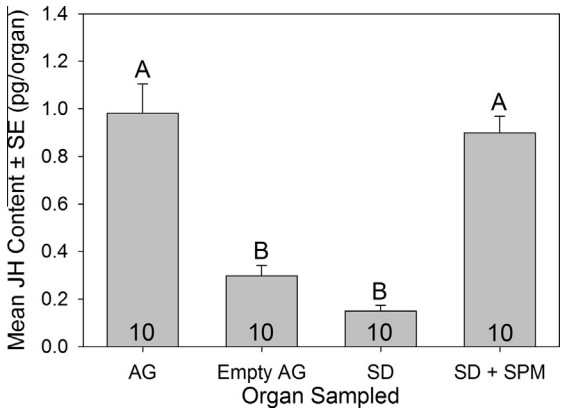

Fig. 6. Mean (± SE) JH content in the accessory glands of virgin (AG) and recently mated (empty AG) *L. hesperus* males, and in the seminal depositories of virgin females (SD) and those recently inseminated with a spermatophore (SD + SPM). Letters indicate significant differences (Tukey's test, $P < 0.05$) between organs. Sample sizes are indicated.

accessory glands, and after mating the amount was reduced by ~70%. Prior to insemination, female seminal depositories contained only trace amounts of JH. Once the spermatophore was deposited, the JH content of the seminal depository was similar to that of filled accessory glands.

4. Discussion

Although it is known that *L. hesperus* males induce a period of reduced sexual receptivity in females following mating (Brent, 2010b; Strong et al., 1970), little is known about the mechanism by which this achieved. The results of the present study confirm the role of male-derived factors in suppressing female mating behavior, and begin to clarify the specific components involved in mediating this effect.

As anticipated based on studies on male maturation (Brent, 2010a), the spermatophores of older males were significantly larger than those of younger males, and when transferred into females produced a refractory period almost 2 day longer (Fig. 1). This suggests that inhibition duration may depend, at least partially, either on the quantity of activating factor transferred to the female or the rate at which spermatophores can be degraded. However the difference in duration may not be due solely to spermatophore size. The final stage of reproductive maturation in adult males starts shortly after emergence and lasts several days. Three days after emergence, most males are still relatively immature and the number willing to mate is quite small compared to the number ready to mate by day 7 (Brent, 2010a). During this period, the base of the accessory glands become activated before the distal portion (Brent and Spurgeon, 2011; Spurgeon and Brent, 2010). Should these portions be responsible for producing different seminal components, then the spermatophore contributions made by the AGs of differently aged males may differ qualitatively not just quantitatively, producing varied behavioral effects.

It was also shown that the inhibitory factor(s) originates from the male accessory glands (Fig. 2). Equivalent suppressive effects on female behavior were observed with injection of homogenate from either the lateral or medial glands. Contents of the seminal vesicles (and therefore the attached testes where the contents are principally produced) do not appear to affect female mating behavior, nor do they have a synergistic effect when combined with contents of the accessory glands. For *L. hesperus*, the primary role of the seminal vesicles appears to be sperm storage. These results are not unusual as the accessory glands are the typical source of refractoriness-inducing products in insects (Wolfner, 2002). However, it is possible that while the primary effector may be derived from the accessory glands, the detection of viable sperm or closely associated factors may contribute towards sustaining the female's refractory period, as has been suggested for *D. melanogaster* (Kalb et al., 1993; Kubli, 1992; Peng et al., 2005) and *Ceratitis capitata* (Miyatake et al., 1999; Mossinson and Yuval, 2003).

The mating inhibitory factor in the accessory glands appears to be heat labile (Fig. 3), and water soluble (Fig. 4). Heating of the male homogenates prior to injection failed to suppress female receptivity to mating. The relatively low temperature at which this was achieved suggests that the compound may be an amino acid polymer that has become denatured, losing its structural functionality. Many of the identified male suppressors of female receptivity are proteins/peptides (Avila et al., 2011). Although the primary inhibitory activity is associated with the water soluble fraction, the insoluble fraction also produced a level of suppression intermediate to that of saline and the untreated homogenate. This could be due to incomplete fractionation, or female receptivity may be inhibited by contributions from both soluble and non-soluble components of the spermatophore.

Accessory gland-derived proteins of varying sizes, from small peptides to large glycoproteins, have been linked to a number of physiological and behavioral changes in females post-mating (Avila et al., 2011; Gillot, 2003). Our SDS–PAGE analysis of water soluble fractions from the male accessory glands and the spermatophore (Fig. 5) shared a number of proteins that were absent from the seminal depositories of virgin females. This observation suggests these proteins/peptides are derived from the male accessory gland and thus constitute a portion of the substances transferred. Intriguingly, among the nine bands identified as accessory gland proteins transferred to females were proteins of 28 and ~130 kDa, which are consistent with the molecular weights of *D. melanogaster* accessory gland proteins (Acp26Aa and Acp36DE). These latter proteins function to promote the release of oocytes by the ovary, and in sperm storage (Heifetz et al., 2000; Neubaum and Wolfner, 1999; Qazi and Wolfner, 2003). Two other *L. hesperus* accessory gland-derived proteins/peptides were comparable in size (3–7 kDa) to that of the pheromone suppressive male factor (6.6 kDa) in *Helicoverpa zea*, the *D. melanogaster* sex peptide (4.3 kDa) and a 7.6 kDa *Aedes aegypti* peptide, all of which have been shown to affect female reproductive behavior (Kingan et al., 1995; Lee and Klowden, 1999; Schmidt et al., 1993). The significance and sequences of these protein/peptides in *L. hesperus*, however, are not known. Based on studies in other species (Wolfner, 2002), we speculate that more than one of these proteins/peptides may be active, either in regulating female receptivity or another facet of reproduction.

It also appears likely that at least one non-soluble component of the spermatophore affects reproduction in *L. hesperus* females. We found that JH III, one of the primary regulators of insect ovarian development and egg production (Flatt et al., 2005; Hartfelder, 2000), was transferred from the *L. hesperus* male accessory glands to the female seminal depository with the spermatophore (Fig. 6). Evidence of similar JH transfers has been found in the moths *Hyalophora cecropia* (Shirk et al., 1980) and *H. virescens* (Pszczolkowski et al., 2006), the longhorned beetle *Apriona germari* (Tian et al., 2010) and possibly some mosquitoes (Borovsky et al., 1994). The source of the JH accumulated in the accessory glands of lygus males is not currently known, but it may result from uptake of circulating hormone originally produced by the corpora allata (Shirk et al., 1976), or from *de novo* production by the accessory glands (Borovsky et al., 1994; Tian et al., 2010). Once transferred to the female, JH may move through the wall of the seminal depository into the hemolymph and onward to various target organs.

This exogenous JH may be the cause of the enhanced ovipositional behavior and egg production rates observed in recently mated *L. hesperus* (Brent et al., 2011). In addition to its effects on the production and uptake of vitellogenin, JH has been implicated in the regulation of female receptivity (Bouletreau-Merle, 1973; Manning, 1966; Ringo et al., 1991, 2005). However, JH normally influences behavior through longer-term organizational effects (Hartfelder, 2000) and is therefore unlikely to produce rapid post-mating reduction of female receptivity. It is possible, however, that JH may also play a role in sustaining mating inhibition over the duration of a lengthy refractory period in *L. hesperus* (Brent, 2010b).

5. Conclusions

Our results support a model of male-induced reduction of female mating receptivity in *L. hesperus*. The behavioral response was independent of either mating stimuli or the presence of a spermatophore in the seminal depository. The behavioral effect appears to be induced, at least in part, by material evacuated from the male accessory glands, and the duration of the response may be influenced by the quantity of material transferred. Potential regulatory factors of female sexual receptivity found within the spermatophore include small proteins or peptides and JH, although their activity is yet to be tested. Whether these factors are produced by the accessory glands or are taken up from the hemolymph is currently being ascertained. Intriguingly, we have recently identified a *L. hesperus* homolog of the sex peptide receptor (GenBank # JF273642), which is a G protein-coupled receptor that mediates the post-mating behavioral switch in *D. melanogaster* (Yapici et al., 2008). Potential roles for this receptor and the accessory gland-derived proteins in mediating reproductive behavior in Lygus females are being investigated.

Acknowledgments

We thank Dan Langhorst for technical assistance, and Dale Spurgeon for valuable input on drafts of the manuscript. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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