Journal of Insect Physiology 56 (2010) 28–34

Contents lists available at ScienceDirect

Journal of Insect Physiology

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

Reproduction of the western tarnished plant bug, *Lygus hesperus*, in relation to age, gonadal activity and mating status

Colin S. Brent *

USDA Arid Land Agricultural Research Center, 21881 N. Cardon Lane, Maricopa, AZ 85238, USA

ARTICLE INFO

Article history:
Received 7 August 2009
Received in revised form 26 August 2009
Accepted 26 August 2009

Keywords:
Accessory glands
Courtship
Miridae
Ovary
Oviposition
Testis

ABSTRACT

Understanding the basic life history and underlying regulatory mechanisms for a pest insect is essential for developing targeted control strategies, but for many insects relatively little is known. Although the western tarnished plant bug, *Lygus hesperus* Knight (Heteroptera: Miridae) has a substantial negative impact in the western U.S., its basic biology is poorly characterized. To elucidate the regulation of *L. hesperus* reproductive dynamics, the onset times of gonadal activation and mating behavior were examined in young adults. Newly emerged adults reared under laboratory conditions at 25 °C were monitored daily for changes in gamete production and willingness to mate. Males matured more quickly than females. Sperm was present at emergence and a small proportion of males were willing to mate as early as 2 days post-emergence. Females were unwilling to mate until at least 5 days post-emergence, although many produced choriogenic oocytes by 4 days. Males appeared to discriminate female age and were more likely to attempt mating with females >5 days post-emergence than with younger females. Males were also able to detect previous mating and attempted to mount virgins more often than recently inseminated females. Collectively these results indicate that the changes in the mating behaviors of *L. hesperus* are linked to reproductive status, although there is a lag between gamete production and willingness to mate. The results also suggest that interactions of the sexes are chemically mediated.

Published by Elsevier Ltd.

1. Introduction

The regulation of reproduction results from the translation of various exogenous and endogenous stimuli into appropriate physiological and behavioral responses, a process that is mediated by the central nervous system and neuroendocrine organs. The general flexibility of this process promotes the optimal timing of gonadal activity and reproductive behaviors to maximize individual fitness. For many insects, key determinants of reproduction include gonadal and nutritional status, mate availability, and recency of mating (reviewed in Ringo, 1996; Gillot, 2003). Although knowledge of reproductive regulation is crucial for understanding the life history and population dynamics of a given species, and for devising targeted control strategies, there is a dearth of such information for many economically important insects.

One such pest species is the western tarnished plant bug, *Lygus hesperus* Knight (Heteroptera: Miridae), which damages numerous cultivated crops in the western United States (Jackson et al., 1995). Despite its economic importance, relatively little is known about the specific factors influencing the dynamics of *L. hesperus* reproduction. Previous work on reproduction in this species is limited (Leigh, 1963; Beards and Strong, 1966; Strong et al., 1970; Strong and Sheldahl, 1970) and principally descriptive, often relying on modest sample sizes. A more accurate and detailed assessment is necessary to facilitate future studies of the underlying regulatory mechanisms of reproduction in *L. hesperus*, and to ensure appropriate comparisons with data collected from other mirid species.

In *L. hesperus* (Strong et al., 1970), and other mirids (Wheeler, 2001; Castañé et al., 2007), there is a pre-mating period after adult eclosion during which sexual maturation is completed. The first objective of this study was to characterize the progression of gonadal activation in *L. hesperus* and to determine whether male and female reproductive behavior was coordinated with these physiological processes. Beginning at adult emergence, changes in gonad development and sexual attractiveness and receptivity were tracked in both females and males. Because reproductive behavior is also influenced by mating status in many insects (Ringo, 1996; Simmons, 2001; Gillot, 2003), including some mirids (Wheeler, 2001; Gemeno et al., 2007), post-mating changes to female attractiveness and sexual receptivity were also determined.

2. Methods and materials

2.1. Insects

The *L. hesperus* used in this study were obtained from a laboratory colony maintained at the USDA-ARS Arid Land

* Tel.: +1 520 316 6337; fax: +1 520 316 6330.
E-mail address: colin.brent@ars.usda.gov.

0022-1910/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.jinsphys.2009.08.021

Agricultural Research Center (Maricopa, AZ, USA). The individuals in this colony are periodically outbred with locally caught conspecifics. The stock insects were given unrestricted access to a supply of green beans and an artificial diet mix (Debolt, 1982) packaged in Parafilm (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 groups of nymphs reared in 1890-ml waxed chipboard cup (Huhtamaki, De Soto, KS) at a density known to have minimal effect on *L. hesperus* development (≤100 nymphs/container; Brent, in press). 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 adequate 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. Gonadal activity

Gonadal activity was assessed in 25 female and 25 male adults when <24-h-old and on each of the subsequent 10 days. Sampled individuals were preserved in a −80 °C freezer until dissected in Ringers solution under a stereomicroscope. Based on a previously defined pattern of *Lygus* oocyte development (Ma and Ramaswamy, 1987), a three stage scale was used to rate ovarian activity: pre-vitellogenic oocytes only; vitellogenic oocytes present; choriogenic oocytes present. Male gamete production was assessed by homogenizing one testis per male in 20 μl of distilled water. A 10 μl aliquot of the homogenate was placed on a hemocytometer and developing spermatozoa were counted under a compound microscope to calculate sperm number per testis. Because the seminal vesicles rather than the testes of *L. hesperus* store mature sperm (Strong et al., 1970), this measure provides a rough estimate of the relative rates of spermatogenesis among males. It should be noted that all of the testes examined in this experiment were composed of seven lobes, not five as reported by Strong et al. (1970).

In addition to sperm, male *L. hesperus* provide a large volume of other material in the spermatophore transferred to the female (Strong et al., 1970). Spermatophores were dissected from females within 30 min of insemination, and weighed on a microbalance (Sartorius TE153S, Goettingen, Germany). Between 17 and 22 spermatophores were collected for each male age except for males younger than 2 days post-emergence, which did not mate. Although composition of the spermatophore is unknown, this mass originating from the medial and lateral accessory glands and seminal vesicles (Strong et al., 1970) may influence female fecundity, mating receptivity, and ovipositional behavior. Because the ability to produce a spermatophore of a threshold mass may influence male mating behavior, the effect of age on accessory gland condition was determined. At the time of emergence and for 7 days following, the condition of both sets of glands and the seminal vesicles was visually assessed. Because the compounds produced in the lateral accessory glands are often translucent, visual assessment of status was verified by opening the glands. A simple qualitative scale, based on descriptions by Spurgeon (2009), was used to rate condition: *empty*, *filling*, *filled* or *distended*. *Empty* organs were translucent, colorless and smaller than active organs. *Filling* glands were more opaque with white material at either their basal or terminal ends, but not throughout the entire lumen. Filling seminal vesicles had traces of white material throughout, but the lumen was not fully occupied. *Filled* glands and seminal vesicles had material throughout the lumen and tended to have a uniform width, except at their gently tapered ends. *Distended* glands and seminal vesicles had bulbous basal ends that tapered sharply, giving them a club-like shape.

2.3. Reproductive behavior

Mating behaviors were assessed by pairing male and female *L. hesperus* of known age in mating arenas for one hour of observation. All observations were conducted during the morning hours, when mating activity is most commonly observed (Blackmer and Brent, unpublished data). Arenas consisted of glass Petri dishes measuring 1.5 cm × 5.0 cm. Individuals were only used once. Three specific male mating behaviors were recorded. A *Court* was scored when a male moved toward a female and shook his body (Strong et al., 1970). A *Mount* was scored when a male climbed onto a female from behind, curling his abdomen to present his aedeagus. A *Mate* was scored when a male maintained copulation for at least 30 s (less time generally meant failure to achieve full intromission). The duration of each copulatory event was recorded, and mating was confirmed by dissecting the female to ensure a spermatophore was present. The mass of each spermatophore was also recorded. Not all males that courted females attempted to mount, and not all that mounted successfully mated. Female behavior consisted of either rejection (moving away or kicking) or acceptance of males for mating. Although group reared *L. hesperus* females are less likely to mate than those reared individually (Ho, 2000), a clear pattern in changing receptivity was observable. Rarely, females approached males and solicited copulation (brief head bobbing followed by moving in front of the male with raised abdomen), but occurrence of this behavior was too infrequent for analysis. However, it almost always resulted in immediate copulation.

The effect of a male's age on his willingness to mate was assessed by placing individual virgin males of known age (newly eclosed to 7 days post-eclosion) in mating arenas along with a virgin 7-day-old female. For each male age group, 65–81 trials were conducted.

To determine if female age influences male reproductive behavior, individual virgin males that were 7 days post-eclosion were each confined with two virgin females in a mating arena. One female was 7 days old, and the other was younger (1–6 days post-eclosion). Females of each pair were distinguished by marks of enamel paint on the pronotum. Paints were switched between trials to ensure that the males were not attracted by a particular color. For each age combination, there were 37–51 trials.

Male response to female mating status was assessed by confining individual virgin males with two females. One female was a virgin whereas the other was mated 16–18 h before the test. One hour before testing, both females were individually marked with enamel paint, with colors switched between trials to compensate for any potential male color bias. All individuals used were aged 7 days post-eclosion. A total of 95 trials were conducted to determine the frequency with which the males approached, mounted and mated with the females. Following each assay, females were dissected to recover any spermatophores. This was done to verify initial mating status prior to the trial and to confirm mating during the assay.

The influence of male stimuli and spermatophore resources on female ovipositional behavior was determined by placing newly emerged females in 355-ml rearing cups with artificial diet and an oviposition substrate (a Parafilm coated packet containing 15 g/l agar solution). Two groups of ten cups each were monitored daily for 10 days, counting both the number of females alive and the number of eggs oviposited during the previous 24 h. Ten females were placed in each cup of one group, while cups in the other group each contained ten females and ten males. All individuals were

virgins. The population density of adults reared under these conditions was previously shown to have no effect on female oviposition (Brent, in press; but see Ho, 2000), therefore differences in population densities between the two treatments were not expected to influence egg laying. After 10 days post-emergence, all females were dissected to determine if spermatophores were present.

2.4. Statistical analysis

Chi-square tests were used to compare the proportion of males exhibiting each courtship behavior to determine an effect by their age or by the condition (age, mate status) of the females with which they were paired. Effects of male age on estimated number of sperm per testis were examined by one-way ANOVA. Multiple paired comparisons between days were made using the *Holm–Sidak method*. Differences in spermatophore size among male ages were assessed by *Kruskal–Wallis* ANOVA on ranks, followed by individual pairwise comparisons using *Dunn's* method. Effects of male presence on female oviposition were examined using two-way repeated measures ANOVA, followed with multiple paired comparisons between treatments using the *Holm–Sidak method*. All analyses were conducted using Sigmaplot 11.0 (Systat Software).

3. Results

Increasing the age of males had a strong positive effect on the frequencies of reproductive behaviors directed towards 7-day adult females (Fig. 1). Although newly eclosed and day-old males exhibited no mating behavior, some males mated as early as the second day of adulthood. Between days 2 and 7 there were significant increases in the proportion of males that courted ($\chi^2 = 35.6$, d.f. = 1, $P < 0.001$), mounted ($\chi^2 = 35.0$, d.f. = 1, $P < 0.001$) and mated females ($\chi^2 = 19.9$, d.f. = 1, $P < 0.001$). Concurrent with these behavioral shifts were changes in reproductive physiology. Estimated numbers of sperm cells per testis quadrupled between days 0 and 7 (Fig. 2; Holm–Sidak; $t = 6.9$, $P < 0.001$). The seminal vesicles went from being empty on day 0 to being filled by day 2 in a few males and by day 3 in most other males (Fig. 3A). The medial (Fig. 3B) and lateral accessory glands (Fig. 3C) became filled at approximately the same rate as the seminal vesicles. The timing of these changes corresponded with the observed changes in reproductive behavior. By day 7, all three sets of organs were distended. The changing availability of these resources was also reflected by the sizes of the spermatophores

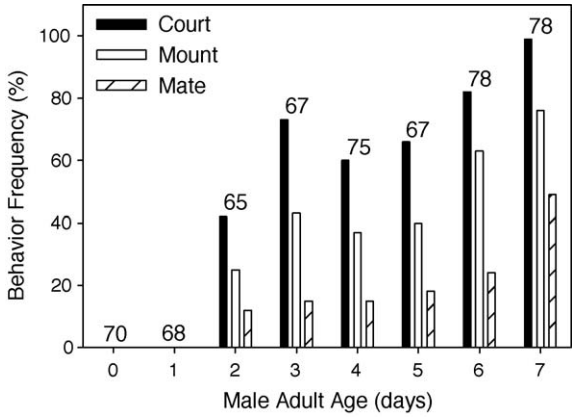

Fig. 1. Effect of male age on respective frequencies of courting, mounting and mating with 7-day virgin females. Frequencies for approaching and mounting increased significantly between days 2 and 7 (Chi-square test, $P < 0.001$). Males younger than 2 days were reproductively inactive. Samples sizes are given.

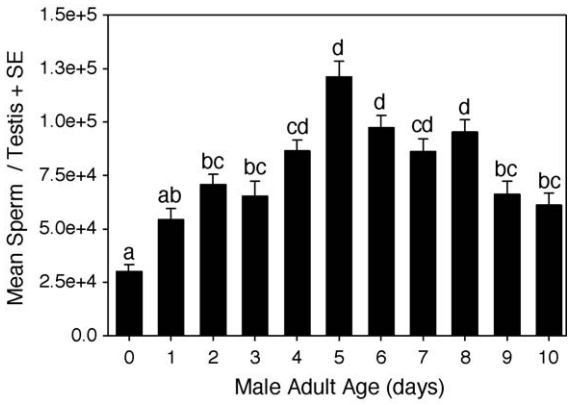

Fig. 2. Effect of male age on the mean (+ se) number of sperm per testis. Significant differences among days are indicated by different letters (Holm–Sidak multiple comparison test after ANOVA, $P < 0.05$). Each mean is calculated from 25 samples.

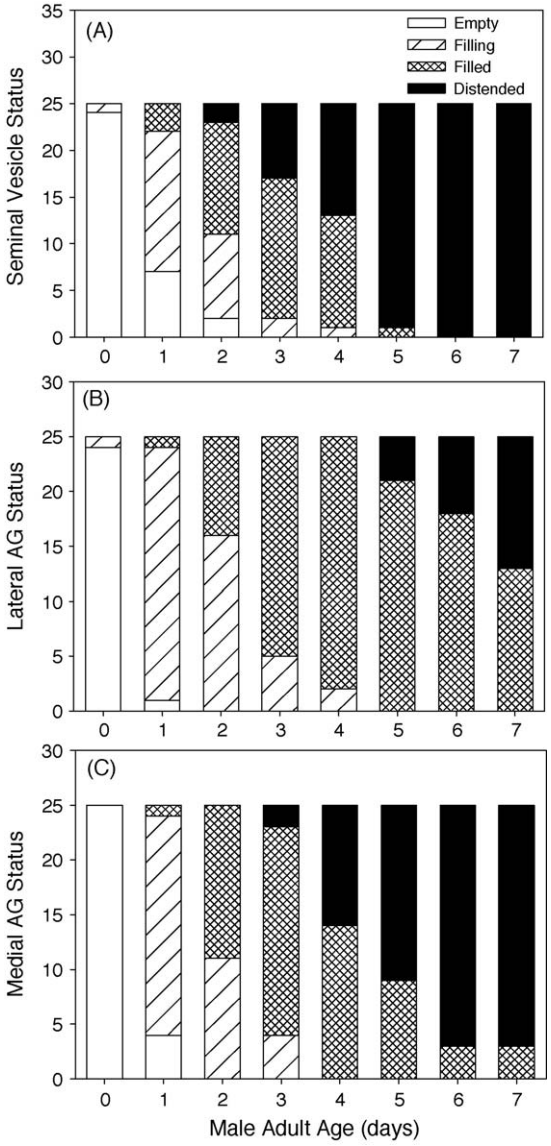

Fig. 3. Effect of male age on the frequency and extent to which the (A) seminal vesicles, and (B) medial and (C) lateral accessory glands are filled with product for transfer to female. Each day consists of 25 samples.

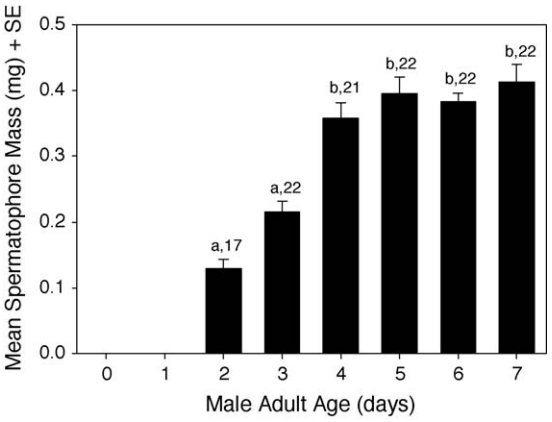

Fig. 4. Effect of male age on the mean (+ se) mass of spermatophores transferred to females. Significant differences between days are indicated by different letters (Dunn's multiple comparison test after ANOVA on ranks, $P < 0.05$). Samples sizes are given.

delivered by males; spermatophore mass tripled between days 2 and 7 (Fig. 4; Dunn's method; $Q = 6.1$, $P < 0.05$).

Male behavior was also affected by the age of the females with which they were paired. Week-old males had a clear preference for females ≥5-day-old compared with younger females. Courting rates (Fig. 5A) for 1- through 4-day-old females were comparable ($\chi^2 = 0.9$, d.f. = 3, $P = 0.833$), and significantly lower than for 7-day-old females with which they were competing for male attention ($\chi^2 = 99.8$, d.f. = 1, $P < 0.001$). Five- and 6-day-old females were courted with frequencies similar to each other ($\chi^2 = 0.1$, d.f. = 1, $P = 0.709$), at a rate that was significantly higher than for younger females ($\chi^2 = 11.0$, d.f. = 1, $P < 0.001$) and comparable to those of 7-day-old females ($\chi^2 = 0.9$, d.f. = 1, $P = 0.333$). Similarly, 1- through 4-day-old females were mounted by males significantly less often than 7-day-old females ($\chi^2 = 120.0$, d.f. = 1, $P < 0.001$). Mount rates for 5 and 6-day-old females were equivalent to each other ($\chi^2 = 0.8$, d.f. = 1, $P = 0.368$), were significantly greater than for younger females ($\chi^2 = 42.0$, d.f. = 1, $P < 0.001$), and were comparable to 7-day-old females ($\chi^2 = 0.02$, d.f. = 1, $P = 0.876$). Males only mated with females that were at least 5-day-old (Fig. 5C). Given that males are willing to court and mount younger females, this delay in mating is likely a consequence of changes in female receptivity. Between the time of eclosion and day 7, female ovarian status changed dramatically (Fig. 6A). Oocyte maturation transitioned from the pre-vitellogenic to the choriogenic stage in as little as 4 days. Several females also exhibited follicular remnants by day 5 (Fig. 6B), indicative of recent egg deposition. The frequency of follicular remnants rose through day 10, as the number females with choriogenic oocytes declined, possibly indicative of the end of an egg production cycle.

In addition to being affected by female age, male reproductive behaviors were also influenced by the mating status of their potential partners (Fig. 7). Compared to previously mated females, virgin females were twice as likely to be courted ($\chi^2 = 49.0$, d.f. = 1, $P < 0.001$), four times more likely to be mounted ($\chi^2 = 50.8$, d.f. = 1, $P < 0.001$) and 17 times more likely to be mated with ($\chi^2 = 35.8$, d.f. = 1, $P < 0.001$) by males. While the difference in mating frequency may be due in part to a change in the behavior of mated females, the males did show clear signs of being selective. While many males would approach and court an inseminated female, most moved away after antennating her abdomen. This is reflected in the low proportion of mated females that males attempted to mount. While 77% of males mounted virgins, only 40% mounted inseminated females. This suggests a decline in male sexual aggression after initial inspection of the female.

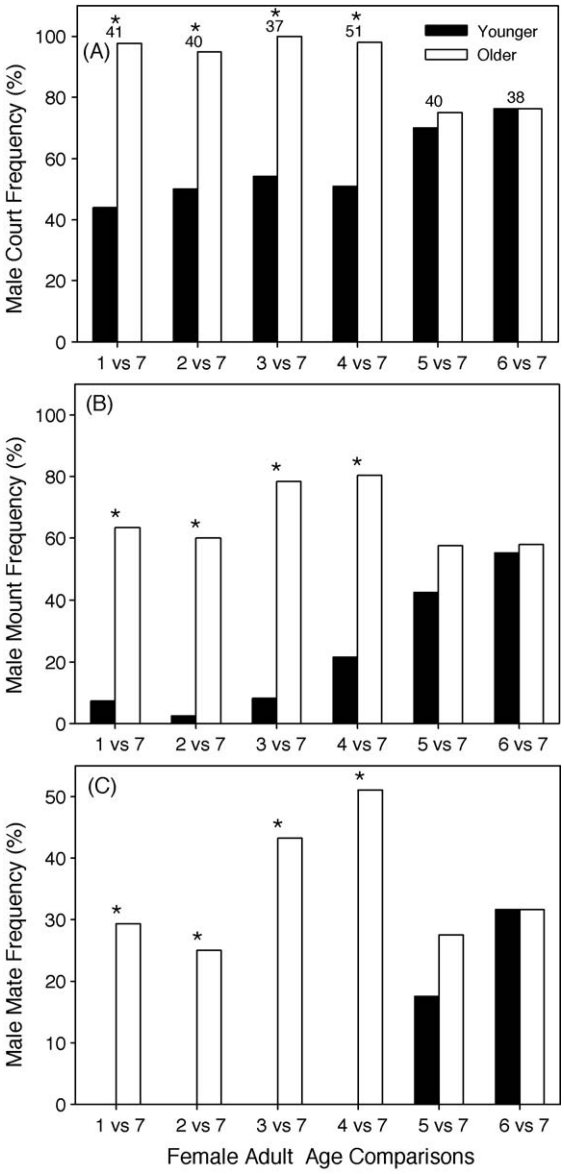

Fig. 5. Effect of increasing female age on the frequency of reproductive behaviors in 7-day males. Males were presented with adult females of two ages, one 7 days old and the other younger. Until females were at least 5 days post-eclosion, males would (A) court, (B) mount or (C) mate significantly more often with the older female (Chi-square test, $P < 0.001$, indicated by *). Samples sizes are given.

Male-invested resources in mating appeared to positively influence oviposition rates (Fig. 8), although there was a significant interaction effect between age and mate availability (two-way RM ANOVA, $F = 15.1$, $P < 0.001$). During this period of 10 days after emergence, egg production rose significantly in females of both groups (two-way RM ANOVA, $F = 98.9$, $P < 0.001$), with oviposition beginning as early as 4 days post-eclosion. Females reared as adults in the presence of similarly aged males oviposited significantly more eggs both overall (two-way RM ANOVA, $F = 42.1$, $P < 0.001$) and for each of days 5 through 10 (Holm–Sidak, $p < 0.05$). Taking into account the number of females in each cup, the average individual daily oviposition rate between days 4 and 10 was 9.7 ± 1.9 and 22.3 ± 2.7 for females nesting in the absence and presence of males, respectively. Dissection showed that after 10 days none of the females reared without males had spermatophores, but 92% of the females from mixed-sex cups had been inseminated.

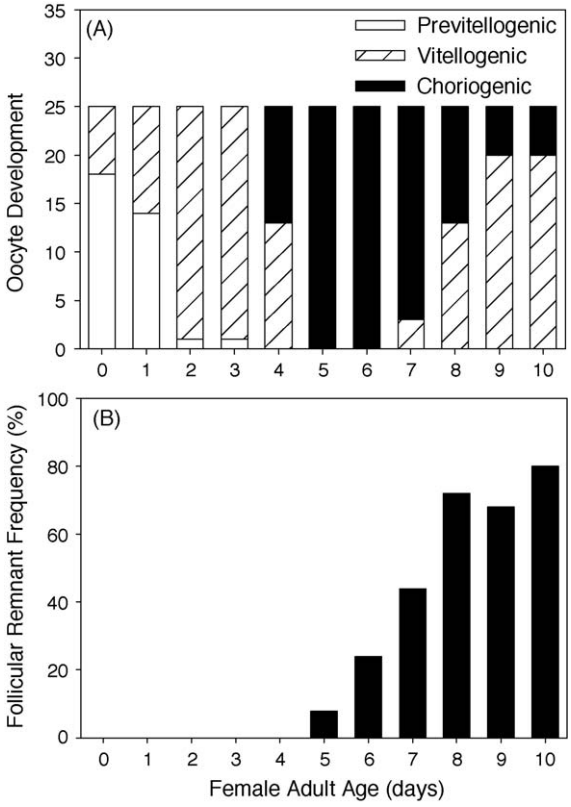

Fig. 6. Effect of increasing female age on frequency measures of (A) oocyte development stage (pre-vitellogenic, vitellogenic, choriogenic) and (B) occurrence of ovarian follicular remnants. Each day consists of 25 samples.

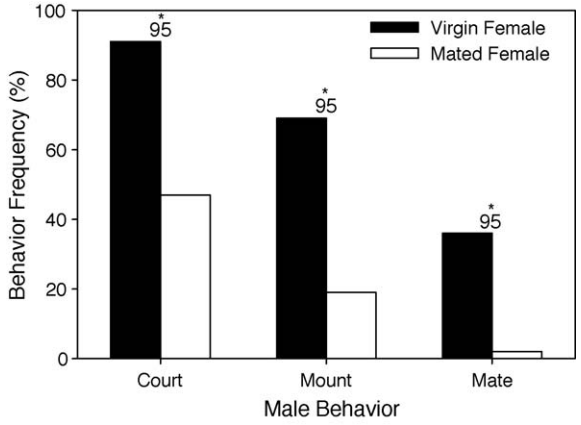

Fig. 7. Effect of female mating status on the reproductive behaviors of 7-day-old males. Seven-day-old virgin females were courted, mounted and mated significantly more often than females mated 16–18 h prior (Chi-square test, $P < 0.001$, indicated by *). Each frequency is calculated from 95 pairings.

4. Discussion

The results demonstrate that the reproductive behavior of *L. hesperus* is strongly associated with changes in the reproductive physiology of both sexes. Interest in mating appears to be primarily constrained by how quickly individuals can produce gametes. Some males were able to deliver spermatophores just 2 days after adult eclosion (Fig. 1), while females required 2–3 additional days to produce eggs (Fig. 6). Until those respective times, neither sex exhibited mating behaviors. Strong et al. (1970) found a similar delay in the onset of female reproductive behaviors. However, they

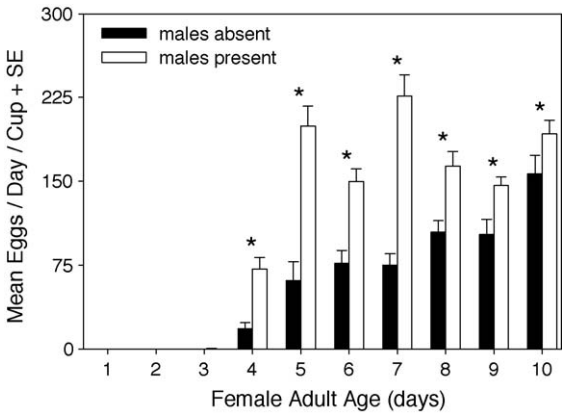

Fig. 8. Effect of increasing female age on the mean (+ se) daily oviposition for groups of 10 females confined with or without males for 10 days. Male presence significantly increased oviposition compared with male absence on days 5–10 (Holm–Sidak multiple comparison test after ANOVA, $P < 0.05$, indicated by *). Each mean was calculated from 10 samples, each composed of 5–10 ovipositing females.

did not observe males mating until 5 days post-emergence. This discrepancy may be due to their small sample sizes ($\leq$4), but they too found that male interest in mating was associated with development of accessory glands. This pattern is also observed in *Lygus lineolaris* and other mirids (Wheeler, 2001; Castañé et al., 2007). Given that the endocrine events necessary to promote gonadal activity (Raikhel et al., 2005) involve the same hormones regulating the activity of the central nervous system (Truman, 2005), it is possible that upregulation of gamete production also primes mating behavior. Additional triggers of sexual behavior can include various endogenous stimuli, such as endocrine feedback from the organs involved in gamete production or the activation of stretch receptors in the common oviduct or seminal vesicles, where readied gametes are stored (Ringo, 1996).

With *L. hesperus* males, the key factor triggering the onset of mating behavior is probably not the availability of sperm, but rather the readiness of other materials that will also be transferred to the female in the spermatophore (Strong et al., 1970). Numerous mature sperm are found in the testes at adult emergence (Fig. 2), and can even be found in 5th instar nymphs (Strong et al., 1970) so they cannot be the primary inducement to mate. Sperm constitute a fairly minor component of the spermatophore, with the rest being comprised of material produced in the medial and lateral accessory glands of mature males. Although the exact composition of the *L. hesperus* spermatophore is unknown, in many insects the mass includes a mixture of carbohydrates, lipids and proteins used to deliver, protect and sustain the sperm (Gillot, 2003). As *L. hesperus* males age, increasing amounts of these materials are sequestered in the glands, which eventually become distended (Strong et al., 1970; Fig. 3). Mating behavior may be triggered once the volume in these organs exceeds a threshold amount. Additional support for this has been found in ongoing studies of *L. hesperus* males in diapause (unpublished). These individuals show a reduced propensity to mate, and while their accessory glands are poorly developed, their testes are still productive and their seminal vesicles become distended with tightly packed sperm.

Once males begin to display mating behaviors, they exhibit selectivity in choosing mates based on female maturity (Fig. 5) and mating status (Fig. 7). The varied responses to female age imply that males are able to directly or indirectly assess the extent of female reproductive development. Given that the loss of antennal flagellum diminishes the likelihood of successful mating in this species (Strong et al., 1970; Graham, 1988), olfactory cues may be important to males in assessing the quality of potential mates. Many insects produce pheromones that convey their reproductive

state (Greenfield, 2002). This linkage can be accomplished when both gamete and odorant molecule production are controlled by the same or closely related endocrine events. In *L. hesperus*, males generally antennated the abdomen of females prior to attempting to mount. Graham (1988) showed that females produce a sex-specific pheromone localized around the ovipositor. Quantitative differences in the expression of the same pheromone may also allow males to discriminate between females of different ages. In fact, age-based increases of several volatile compounds have been recorded for this species (Byers, 2006) and the timing of these increases corresponds to the pace of gonadal activation, perhaps providing an indicator of reproductive readiness.

Once mated, a female undergoes several changes which might be induced by compounds delivered with the spermatophore. Physiologically, the most pronounced effect of mating was an increase in oviposition (Fig. 8). In some insects, the spermatophore provides the female with supplemental macro- or micronutrients, thereby enhancing egg production (reviewed in Vahed, 1998; Wolfner et al., 2005). For females that are not resource limited, enhancement of oviposition may be induced by the physical stimuli associated with copulation and insemination, or by the delivery of male-derived hormones or prohormones (Wolfner et al., 2005). Either of these alternatives or a combination of factors could potentially boost egg production at one or more stages (oogenesis, ovulation or oviposition) to improve female fecundity.

Post-mating behavioral effects were also observed. Female receptiveness to copulation decreased after insemination, corroborating the findings of Strong et al. (1970). Mated females were more likely to evade or kick males attempting to mount and this was reflected in the lower proportion of mounting males that succeeded in mating relative to those courting virgin females (Fig. 7). Similar responses were observed in *Macrolophus caliginosus* (Gemeno et al., 2007). Female refractoriness could be in direct response to the stimuli experienced during copulation and insemination (Ringo, 1996). The activation of pressure or stretch receptors during these activities could trigger a neuronal cascade that dampens further interest in mating (Gillot, 2003). Alternatively, the female refractory period might be induced by chemical cues transferred in the spermatophore (Simmons, 2001; Gillot, 2003), benefiting the male by reducing sperm competition.

Spermatophore compounds may also account for the observed decline in the attractiveness of females after being inseminated. During pairings of one female with two males, male courtship behavior normally ceased shortly after the female mated with another male. Most males nearing a recently mated female were observed to antennate her abdomen then move away, although a small number were still willing to court and mount (Fig. 7). It has been suggested for *Lygus* spp. that the spermatophore may contain factors that change female fertility signaling (Aldrich et al., 1988). If the female signals her fecund state by releasing volatile compounds, their release may be curtailed after mating. In the mirid *Trigonotylus caelestialium*, males are less attracted to the volatile pheromones of mated females than those of virgins (Fukuyama et al., 2007). However, if female fertility signaling utilizes low volatility compounds, such as cuticular hydrocarbons, changes in their expression would occur relatively slowly. Rather than inducing a change in female signaling, factors in the spermatophore may provide signals to dissuade other males from courting (Simmons, 2001; Gillot, 2003; Wolfner et al., 2005). Such compounds could act as a general repellent, or they may simply convey female mating status, saving a potential suitor from pursuing a mate that is unreceptive and reducing the chances of sperm competition. Males might also repel potential suitors by releasing compounds from their metathoracic scent gland while in contact with the female. In other mirids, such secretions have been shown to have negative effects on female fertility signaling (Groot et al., 2001) and male attraction (Zhang and Aldrich, 2003; Zhang et al., 2007).

Collectively, the results of this study enhance our understanding of the onset of reproduction by adult *Lygus hesperus*, clarifying and correcting some of the early physiological and behavioral studies of this economically important pest species. The findings also highlight several research avenues for the potential development of new control methods for this species. Elucidating the underlying hormonal events during this period of maturation would facilitate the use of insect growth regulators to undermine reproduction. Determining how females come to lose their attractiveness to males after copulating may reveal a strong repellant that could be used to disrupt normal mating. Similarly, identifying the factors which induce the refractory period in mated females might provide a means of disrupting reproduction.

Acknowledgements

I gratefully acknowledge Dan Langhorst for his assistance with rearing and dissecting the insects. I also thank John Byers, Steve Naranjo and Dale Spurgeon for their comments on earlier versions of this manuscript.

References

Aldrich, J.R., Lusby, W.R., Kochansky, J.P., Hoffmann, M.P., Wilson, L.T., Zalom, F.G., 1988. Lygus bug pheromones vis-à-vis stink bugs. In: Brown, J.M., Richter, D.A. (Eds.), Proceedings of the Beltwide Cotton Production Conferences. National Cotton Council of America, Memphis, TN, pp. 213–216.

Beards, G.W., Strong, F.E., 1966. Photoperiod in relation to diapause in *Lygus hesperus* Knight. Hilgardia 37, 345–362.

Brent, C.S., in press. Stage-specific effects of population density on the development and fertility of the western tarnished plant bug, *Lygus hesperus*. Journal of Insect Science.

Byers, J.A., 2006. Production and predator-induced release of volatile chemicals by the plant bug *Lygus hesperus*. Journal of Chemical Ecology 32, 2205–2218.

Castañé, C., Alomar, O., Riudavets, J., Gemeno, C., 2007. Reproductive biology of the predator *Macrolophus caliginosus*: effect of age on sexual maturation and mating. Biological Control 43, 278–286.

Debolt, J.W., 1982. Meridic diet for rearing successive generations of *Lygus hesperus*. Annals of the Entomological Society of America 75, 119–122.

Fukuyama, M., Adati, T., Higuchi, H., Tarahashi, A., 2007. Attractiveness of females to males in the rice leaf bug. *Trigonotylus caelestialium* (Kirkaldy)—effect of mating status and age of females. Japanese Journal of Applied Entomology and Zoology 51, 135–137.

Gemeno, C., Alomar, O., Riudavets, J., Castañé, C., 2007. Mating periodicity and post-mating refractory period in the zoophytophagus plant bug *Macrolophus caliginosus* (Heteroptera: Miridae). European Journal of Entomology 104, 715–720.

Gillot, C., 2003. Male accessory gland secretions: modulators of female reproductive physiology and behavior. Annual Review of Entomology 48, 163–184.

Graham, H.M., 1988. Sexual attraction of *Lygus hesperus* Knight. The Southwestern Entomologist 13, 31–37.

Greenfield, M.D., 2002. Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication. Oxford University Press, New York.

Groot, A.T., Drijfhout, F.P., Heijboer, A., van Beek, T.A., Visser, J.H., 2001. Disruption of sexual communication in the mirid bug *Lygocoris pabulinus* by hexyl butanoate. Agricultural and Forest Entomology 3, 49–55.

Ho, H.-Y., 2000. Studies on the sex Pheromones of the Pentatomid Bugs *Chlorochroa ligata*, *Chlorochroa sayi*, and *Chlorochroa uhleri*, and the Mirid Bug *Lygus hesperus* (Hemiptera: Heteroptera). Ph.D. Thesis. University of California Riverside, 236 p.

Jackson, C.G., Debolt, J.W., Ellington, J., 1995. Lygus bugs. In: Nechols, J.R., Andres, L.A., Beardsley, J.W., Goeden, R.D., Jackson, C.G. (Eds.), Biological Control in the Western United States. Division of Agriculture and Natural Resources, University of California, pp. 87–90.

Leigh, T.F., 1963. Life history of *Lygus hesperus* (Hemiptera: Miridae) in the laboratory. Annals of the Entomological Society of America 56, 865–867.

Ma, W.K., Ramaswamy, S.B., 1987. Histological changes during ovarian maturation in the tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae). International Journal of Insect Morphology and Embryology 16, 309–322.

Patana, R., 1982. Disposable diet packet for feeding and oviposition of *Lygus hesperus* (Hemiptera: Miridae). Journal of Economic Entomology 75, 668–669.

Raikhel, A.S., Brown, M.R., Belles, X., 2005. Hormonal control of reproductive processes. In: Gilbert, L.I., Iatrou, K., Gill, S.S. (Eds.), Comprehensive Molecular Insect Science, vol. 3. Elsevier, Boston, pp. 433–491.

Ringo, J., 1996. Sexual receptivity in insects. Annual Review of Entomology 41, 473–494.

Simmons, L.W., 2001. Sperm Competition and its Evolutionary Consequences in the Insects. Princeton University Press, Princeton, NJ.
Spurgeon, D., 2009. Observations of diapause characters in the western tarnished plant bug *Lygus hesperus*. In: Proceedings of the Beltwide Cotton Conference. National Cotton Council, Memphis, TN, pp. 807–812.
Strong, F.E., Sheldahl, J.A., 1970. The influence of temperature on longevity and fecundity in the bug *Lygus hesperus* (Hemiptera: Miridae). Annals of the Entomological Society of America 63, 309–314.
Strong, F.E., Sheldahl, J.A., Hughes, P.R., Hussein, E.M.K., 1970. Reproductive biology of *Lygus hesperus* Knight. Hilgardia 40, 105–147.
Truman, J.W., 2005. Hormonal control of form and function of the nervous system. In: Gilbert, L.I., Iatrou, K., Gill, S.S. (Eds.), Comprehensive Molecular Insect Science, vol. 2. Elsevier, Boston, pp. 135–163.
Vahed, K., 1998. The function of nuptial feeding in insects: a review of empirical studies. Biological Review 73, 43–78.
Wheeler, A.G., 2001. Biology of the Plant Bugs (Hemiptera: Miridae). Cornell University Press, Ithaca, NY.
Wolfner, M.F., Heifetz, Y., Appelbaum, S.W., 2005. Gonadal glands and their gene products. In: Gilbert, L.I., Iatrou, K., Gill, S.S. (Eds.), Comprehensive Molecular Insect Science, vol. 1. Elsevier, Boston, pp. 179–212.
Zhang, Q.-H., Aldrich, J.R., 2003. Male-produced anti-sex pheromone in a plant bug. Naturwissenschaften 90, 505–508.
Zhang, Q.H., Chauhan, K.R., Zhang, A., Snodgrass, G.L., Dickens, J.C., Aldrich, J.R., 2007. Antennal and behavioral responses of *Lygus lineolaris* (Palisot de Beauvois) (Heteroptera: Miridae) to metathoracic scent gland compounds. Journal of Entomological Science 42, 92–104.