Morphological Characters of Diapause in *Lygus hesperus* Knight (Hemiptera: Miridae)[1]

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J. Entomol. Sci. 45(4): 303-316 (October 2010)

Abstract Development of management tactics that reduce reliance on conventional pesticides for control of the western tarnished plant bug, *Lygus hesperus* Knight, will require improved understanding of *Lygus* ecology. One poorly characterized aspect of *Lygus* ecology is the induction of adult diapause. Although photoperiod is a key regulator of diapause, previous research has reported inconsistent results, likely because of the different criteria used to distinguish diapause. To establish standardized criteria, we reared *L. hesperus* under photoperiods of 10- and 14-h and a constant temperature of 26.6°C. Adults were dissected at ages of 3, 7, 10, 14, and 17 days to evaluate associations among characteristics of the fat body and reproductive organs. Whereas most characters examined were at least weakly associated with others, we concluded that seminal vesicle condition, presence of a fatty sheath on the testis, and fat body color were not reliable indicators of *L. hesperus* physiological status. The most appropriate criteria for distinguishing diapause appeared to be hypertrophied fat bodies combined with undeveloped medial accessory glands (for males) or undeveloped ovaries (for females) in adults that were at least 10 days old. We also evaluated less stringent criteria, permitting some development of accessory glands or ovaries to accommodate the delayed reproductive development observed in some specimens reared under the 10-h photoperiod. The descriptions and illustrations of the morphological characters, combined with our suggested sets of criteria, should permit a more standardized assessment of *L. hesperus* physiological status, and thereby facilitate meaningful comparisons of research by different investigators.

Key Words western tarnished plant bug, *Lygus hesperus*, diapause, fat body

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Most efforts to manage the western tarnished plant bug, *Lygus hesperus* Knight, in cotton (*Gossypium hirsutum* L., *G. barbadense* L.) involve conventional pesticides applied according to nominal thresholds. Although cultural methods have been developed to reduce the numbers of *L. hesperus* migrating from alfalfa (*Medicago sativa* L.) to cotton (Stern et al. 1967), those methods have not been widely adopted. Therefore, the need to develop improved, ecologically-based management tactics for *L. hesperus* remains. McGuire et al. (2006) suggested that *L. hesperus* might be better managed by targeting overwintering populations before they become large and disperse to crop hosts. However, development of such tactics requires improved understanding of *L. hesperus* overwintering ecology.

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One key aspect of *Lygus* overwintering ecology that is poorly known is the adult diapause. Although photoperiod is known to induce diapause in *L. hesperus* (Beards and Strong 1966, Leigh 1966, Strong et al. 1970), inconsistencies exist in reports of these photoperiodic responses. Beards and Strong (1966) reported that half of field-collected adult *L. hesperus* were in diapause by mid-September when the photoperiod was about 12.5 h. They also reported that the photoperiodic cue was received during the nymphal stage, and, assuming a development time of 30 days, that all nymphs developing under photoperiods shorter than 13 h would produce diapausing adults. Based on laboratory studies, Beards and Strong (1966) reported a high incidence of diapause under photoperiods between 7 and 11 h at 27°C, but lower incidences of diapause under photoperiods of 6, 12, and 13 h. In contrast, Leigh (1966) reported that most field-collected diapausing *L. hesperus* terminated diapause when exposed to 13-h days at 26.7°C, and Beards and Leigh (1960) reported a laboratory rearing procedure using a photoperiod of 13 h with no mention of diapause or reduced reproduction.

Results of the various experiments on *L. hesperus* diapause cannot be directly compared because of differences in the morphological criteria used to distinguish diapause. Studies by Beards and Strong (1966) focused on females, which were considered reproductive if their ovaries contained at least one chorionated egg and diapausing if the ovaries were undeveloped and the fat body was hypertrophied. Females lacking either set of characters were considered either nonreproductive or in an intermediate condition. In experiments to evaluate the influence of temperature on diapause induction in response to a short (10-h) daylength, Beards and Strong (1966) classified females held at 21°C as diapausing if the ovaries were undeveloped, regardless of fat body condition. Leigh (1966) classified females as diapausing if they exhibited no ovary development and well-developed fat bodies, whereas a reproductive state was indicated by distinct ovary development. Females exhibiting little or no ovary development and poorly developed fat bodies were classified as intermediate. Leigh (1966) also considered atrophy of the seminal vesicles, combined with well-developed fat bodies, as evidence of diapause in males. Strong et al. (1970) reported that male *L. hesperus* collected from the field in October were characterized by slender seminal vesicles, small accessory glands, and hypertrophied fat bodies. In addition, many specimens considered diapausing exhibited atrophied testes.

The criteria used to indicate diapause have also varied among recent reports of a similar species, *Lygus lineolaris* (Palisot de Beauvois). Snodgrass (2003) considered failure of the reproductive organs (ovaries in females, testes and accessory glands in males) to enlarge and presence of a hypertrophied fat body to indicate diapause in *L. lineolaris*. An additional criterion for males was the absence of white fluid in the accessory glands. Snodgrass (2005) used these same criteria, with the additional criterion that recent mating indicated a reproductive state in females. Villavaso and Snodgrass (2004, 2005) reported that diapause in males was indicated by the presence of globular fat combined with accessory glands that were not well developed. Females were classified as diapausing if the fat body was globular and no mature eggs were present. Bugs exhibiting poor development of both the fat body and reproductive organs were classified as "unclear."

A shortcoming of the criteria so far used to study diapause in *L. hesperus* and *L. lineolaris* is that they are subjective and not thoroughly described. Our objectives were to (1) monitor the development of the morphological characters associated with diapause or reproduction in *L. hesperus*, (2) examine associations among these characters, and (3) illustrate these characters to facilitate their more consistent application.

Materials and Methods

Eggs of *L. hesperus* were obtained from a laboratory colony maintained on pods of green bean (*Phaseolus vulgaris* L.) and raw sunflower seeds (*Helianthus annuus* L.) at the WICSRU. Field-collected *L. hesperus* were periodically introduced into the colony to maintain vigor. Fresh green bean pods were placed within each of 3 colony oviposition cages for ~18 h. Beans recovered from the oviposition cages were cut in half, and each half was placed on a filter paper disk within a 100 × 15-mm Petri plate. Petri plates were sealed with Parafilm M (Pechiney Plastic Packaging, Chicago, IL) to prevent desiccation of the bean sections. Petri plates containing the bean sections were assigned to environmental chambers maintained at 26.6 ± 1°C and either a 10:14 or 14:10 h photoperiod. Care was taken to ensure that both halves of a given bean were not assigned to the same chamber. Bean sections were examined daily for newly-emerged nymphs beginning on the 4th day after oviposition. On the 5th day after oviposition, the parafilm was removed and a 4 - 5-cm long section of fresh green bean pod was placed in each plate.

A few eggs hatched as early as the 5th day after oviposition, but most hatched after 6 - 7 d. Because adults held in mixed-sex groups are likely to mate, and mating temporarily changes the appearance of the male accessory glands (Brent 2010), newly-emerged nymphs were transferred individually to 5-dram plastic vials (Thornton Plastics, Salt Lake City, UT). Each vial contained a 4 - 5-cm section of fresh bean pod, and was closed with a snap-cap lid ventilated by a 1.1-cm diam hole covered by nylon organdy. Bean pods were replaced 3 times weekly. Between 50 and 100 nymphs were established in each photoperiod treatment of each of 4 experimental repetitions.

Vials were examined daily for the presence of 5th instars beginning when 4th instars were first observed. The ovipositor is visible beneath the abdominal cuticle of the female 5th instar, and the gender of each 5th instar was determined based on this character. Upon adult eclosion, the date was recorded and bug gender was verified.

Adults of each gender within each photoperiod treatment were randomly assigned to an age at dissection of 3, 7, 10, 14, or 17 d. Because we hypothesized that oviposition may be a reliable nondestructive indicator of physiological status, the bean section of each adult female was checked daily for the presence of eggs until and including the day of dissection.

On the assigned day, each bug was pinned through the prothorax to a plate of paraffin and dissected in saline (0.7% NaCl [w/v]) at 15 - 30 × under a dissecting microscope. Dissections were performed by first removing the dorsal plate of the abdomen, taking care to minimize the loss or displacement of adhering fat body. Then the alimentary canal was removed, and the terminal segments of the abdomen were pulled free to expose the reproductive organs.

Characters recorded during dissection were selected based on observations during preliminary experiments (Spurgeon 2009). Fat body quantity was classified as (1) sparse, (2) abundant, or (3) hypertrophied. Sparse fat bodies were characterized by absent or inconspicuous fat deposits (Fig. 1a) or fat present in diffuse sheets only (Fig. 1b). Fat bodies classified as abundant exhibited conspicuous fat deposits that did not largely obscure other internal organs (Fig. 1c) or that were mostly confined to the dorsal portion of the abdomen. Hypertrophied fat bodies obscured most or all of the

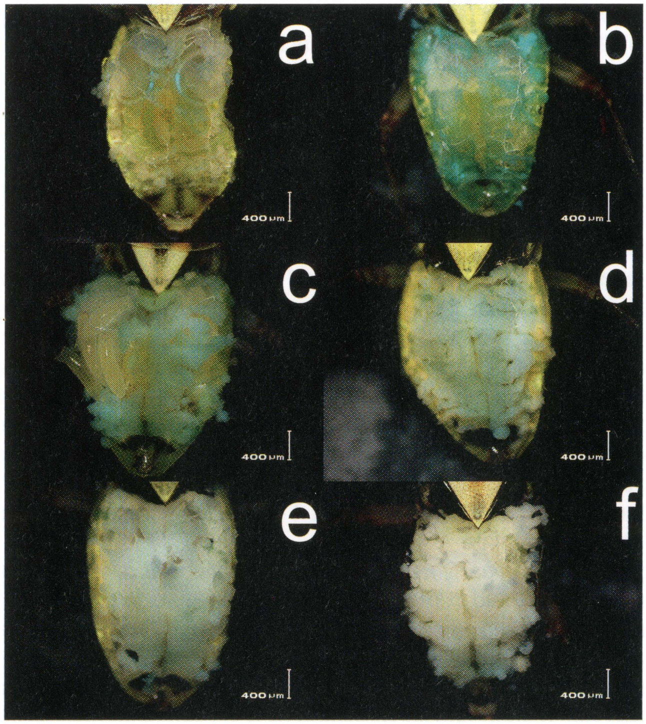

Fig. 1. Fat body characteristics of adult *Lygus hesperus*. (a) Sparse fat body in a 3-d-old male; (b) sparse fat body in a diffuse sheet in a 7-d-old female; (c) abundant fat body in a 3-d-old female; and hypertrophied fat bodies in (d) a 3-d-old female, (e) a 7-d-old female, and (f) a 10-d-old male. Hypertrophied fat bodies (d-f) are arranged in columns adjacent to the dorsal vessel and often feature globules of fat on the anterior and lateral margins of the abdomen (e, f).

other organs (Fig. 1d-f). In addition, in bugs with a hypertrophied fat body, large globules of fat adhered to the alimentary canal during its removal, and the ventral surface of the abdomen remained obscured by fat after the alimentary canal and reproductive organs were removed. Fat body color was recorded as (1) aqua, (2) light aqua, or (3) white. More than 1 fat body color was often recorded for an individual bug. Fat body configuration was recorded as combinations of (1) sheet-like, (2) globular, (3) in columns adjacent to the dorsal vessel, or (4) in columns along the dorsal vessel and with globules of fat along the anterior and lateral margins of the abdomen. An uncommonly observed fat body condition, in which the fat appeared as small transparent beads (Fig. 2), was recorded separately from other fat body characteristics.

Male characters recorded included the respective conditions of the seminal vesicles, medial and lateral accessory glands, and testes. Because preliminary observations failed to suggest any association between testes size and diapause status (Spurgeon 2009), no effort was made to measure the testes. Seminal vesicle condition

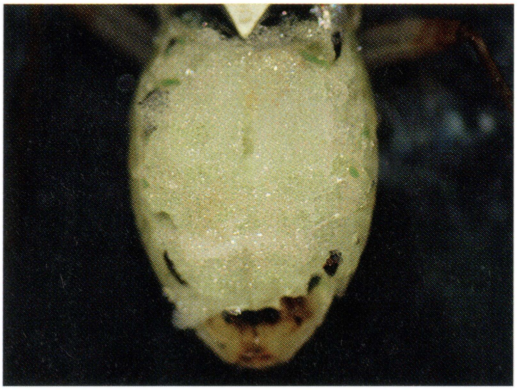

Fig. 2. Hypertrophied fat body composed of the glass bead fat type in a 43-d-old diapausing female *Lygus hesperus* adult.

was recorded as (1) empty, (2) filling (characterized by a filament of white sperm in the center of the lumen of the seminal vesicles, Fig. 3a), (3) filled (lumen of the seminal vesicle is full but not distended), and (4) distended (distal half of the vesicle is bulbous or club-shaped, Fig. 3c). The sizes of medial and lateral accessory glands were characterized as large (Fig. 3a-c) or small (Fig. 3d). Because these size ratings were completely subjective, we also characterized the contents of the accessory glands. Most contents of the lateral accessory glands are translucent. However, the bases, and the distal sections encircling the seminal vesicles, become opaque with increased development. Therefore, the lateral glands were characterized as being empty (Fig. 3d), or as having opaque material in their basal and distal sections (Fig. 3a-c). Basal and distal sections of medial accessory glands were rated separately. The distal sections of the medial accessory glands were rated as containing a colored (white) material (Fig. 3a-c) or not (Fig. 3d). In addition, the basal section of the medial gland was recorded as being empty or nearly so (Fig. 3d), or as filling (Fig. 3a), filled (Fig. 3b), or distended (Fig. 3c). Medial glands that were conspicuously small and that contained little or no opaque material in the basal section, and no visible contents in the distal section, were referred to as not developed (Fig. 3d). Testes were characterized based on the absence (Fig. 3a-c) or presence of a fatty sheath (Fig. 3d).

Only ovary condition was recorded in females in addition to the fat body characters. Ovary condition was recorded as (1) no development (Fig. 4a), (2) presence of vitellogenic oocytes (Fig. 4b), (3) presence of eggs (distinguished by their glossy surface and presence of an operculum; Fig. 4c), and (4) presence of follicular relics (Fig. 4d), which resulted from previous oviposition.

Statistical analyses. All association analyses were conducted separately for males and females. Associations between fat body quantity classes and fat body color classes, and between fat body quantities and fat body configurations, were examined in contingency tables using PROC FREQ of SAS (SAS Institute 2008). Fat body color or configuration classes that occurred ≤3 times were pooled with the most similar classification available that had a frequency larger than 10. For example, the single insect exhibiting both aqua and white fat was pooled with insects exhibiting light aqua

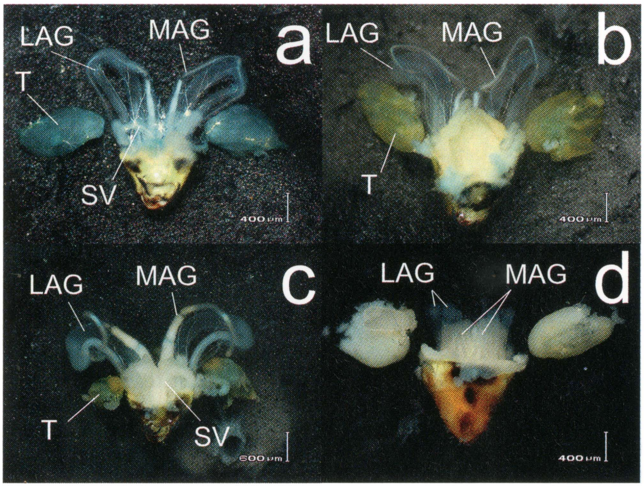

Fig. 3. Male reproductive organs of *Lygus hesperus* adults exhibiting (a-c) reproductive and (d) diapause characters. (a) Filling medial accessory glands and seminal vesicles in a 2-d-old male; (b) medial accessory glands with filled bases in a 3-d-old male; (c) medial accessory glands with slightly distended bases in a 7-d-old male; (d) undeveloped accessory glands and testes enclosed in a fatty sheath in a 10-d-old male. LAG, lateral accessory gland; MAG, medial accessory gland; SV, seminal vesicle; T, testis.

and white fat. For males, characteristics of the reproductive organs (presence of a fatty sheath on the testis, seminal vesicle rating, and ratings of medial and lateral accessory glands) were examined for association with fat body quantities, and for association with each other. For females, ovary condition was examined for association with fat body quantity classes. For both sexes, fat body, reproductive characteristics, and diapause status were also examined for association with adult age and/or photoperiod. The intent of these analyses was to assess how consistently the various characters were associated to gain insight into whether their use as criteria of diapause may result in classification errors.

Mantel-Haenszel statistics were used for the analyses of associations because those methods are subject to less restrictive sample size requirements than the Pearson or likelihood ratio chi-square tests (Stokes et al. 2000). Also, the Mantel-Haenszel approach provides greater statistical power than conventional chi-square statistics when categorical classes could reasonably be ordered (for example, sparse, abundant, and hypertrophied fat bodies). When both row and column variables in the contingency table could be ordered, and a linear trend could reasonably be expected based on preliminary data (Spurgeon 2009), association was evaluated based on the correlation statistic (Q_{CS}, Stokes et al. 2000). When only the row variable could be ordered, or a nonlinear trend was anticipated, the mean score test was used (Q_{SMH}, Stokes et al. 2000).

Because the 2 photoperiod treatments could not be applied within the same environmental chamber, we were concerned that subtle differences in temperature between the

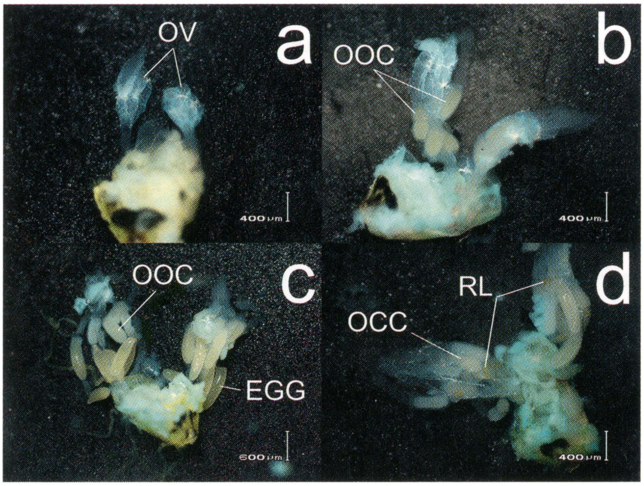

Fig. 4. Female reproductive organs of *Lygus hesperus* adults exhibiting (a) diapause and (b-d) reproductive characters. (a) Undeveloped ovaries of a 3-d-old female; (b) ovaries with oocytes only in a 2-d-old reproductive female; (c) complement of oocytes and eggs in a reproductive 3-d-old female; (d) reduced egg complement and follicular relics in a reproductive 10-d-old female. EGG, chorionated egg; OCC, vitellogenic oocyte; OV, ovary; RL, follicular relic.

2 chambers would result in differences in the development rates of adult reproductive organs. In the absence of other evidence, these differences might be interpreted as legitimate effects of photoperiod. To assess the potential for such confounding effects, nymphal development time was compared between the photoperiod treatments using mixed-model ANOVA using the GLIMMIX procedure of SAS (SAS Institute 2008). The ANOVA model contained fixed effects of photoperiod, sex, and their interaction, and the random effect of experimental repetition.

Results

Associations among fat body characters. A total of 211 female and 224 male *L. hesperus* were dissected. These insects were approximately evenly distributed among combinations of photoperiod and adult age. Examination of the relationships between fat body quantity classes and colors, controlling for age and photoperiod, indicated significant association for both *L. hesperus* sexes (females, $Q_{SMH} = 78.7$, df = 2, $P < 0.01$; males, $Q_{SMH} = 64.3$, df = 2, $P < 0.01$). No females containing fat bodies classified as sparse ($n = 33$) contained only white fat, although 12% exhibited a combination of light aqua and white fat. Most females with sparse fat bodies (88%) exhibited aqua or light aqua fat. Most (78%) females with abundant fat bodies ($n = 94$) exhibited aqua or light aqua fat, whereas 22% exhibited fat that was white, or white and light aqua. Only 11% of females with fat bodies classed as hypertrophied ($n = 84$) lacked white fat, and 58% of females exhibiting hypertrophied fat bodies contained only white fat. The remainder (31%) contained a mixture of light aqua and white fat.

Trends for males were roughly similar to those for females except that white fat was more frequently observed in males without hypertrophied fat bodies. Males with sparse fat bodies (n = 112) most often exhibited aqua or light aqua fat (89%). Only 7 and 4% contained light aqua and white, or white fat, respectively. Only 24% of males with fat bodies rated as abundant (n = 54) contained aqua fat, whereas 57% contained either light aqua, or light aqua and white fat. The remainder (19%) contained white fat only. No males with hypertrophied fat bodies (n = 58) contained aqua fat, and 97% contained fat that was either light aqua and white, or white.

Significant associations between fat body quantity classes and fat configuration were also observed (females, Q_{SMH} = 106.1, df = 2, $P < 0.01$; males, Q_{SMH} = 104.2, df = 2, $P < 0.01$). In females with sparse fat bodies, 76% had fat present in diffuse sheets whereas 24% exhibited sheet-like fat in columns adjacent to the dorsal vessel (Fig. 1b). In males with sparse fat bodies, 97% exhibited fat in diffuse sheets. Fat bodies classified as abundant usually occurred in discernable columns (females, 79%; males, 89%), whereas the remainder were classified as being sheet-like. Ninety-four percent of females and 97% of males with hypertrophied fat bodies exhibited the fat in columns with globules of fat near the anterior and lateral margins of the abdomen (Fig. 1e,f).

Fat body quantity classes were not associated with adult age for females (Q_{CS} = 0.16, df = 1, P = 0.69) or males (Q_{CS} = 2.93, df = 1, P = 0.09). Over all age groups, fat body classifications of sparse, abundant, or hypertrophied occurred in 16, 44, and 40% of females, and in 50, 24, and 26% of males, respectively. However, when fat body quantity classes were compared between photoperiods, controlling for adult age, there were significant associations with photoperiod for both sexes (females, Q_{CS} = 44.51, df = 1, $P < 0.01$; males, Q_{CS} = 79.42, df = 1, $P < 0.01$). Most females reared under the 10-h photoperiod exhibited hypertrophied fat bodies (61%) whereas only 8% had fat bodies rated as sparse. In contrast, only 12% of females reared under 14-h daylengths exhibited hypertrophied fat bodies and 25% were rated as sparse. Trends for males were similar to those for females; 50% of males reared under 10-h days contained hypertrophied fat bodies, and 26% exhibited fat bodies rated as sparse. None of the males reared under 14-h days contained hypertrophied fat bodies, and 76% contained fat bodies rated as sparse.

The "glass bead" type of fat (Fig. 2) occurred in only 0.9% of males and 1.4% of females. Therefore, its low frequency of occurrence precluded any statistical analysis. This unique type of fat was observed in adults of 3 ages (3, 14, and 17 d), in both sexes exhibiting well-developed reproductive organs, in a female exhibiting no ovary development, and under both photoperiods. However, all adults exhibiting the glass bead fat type contained fat bodies rated as abundant or hypertrophied.

Associations among characters of males. The presence of a fatty sheath enclosing the male testis was strongly associated with fat body quantity (Q_{SMH} = 81.1, df = 2, $P < 0.01$). The fatty sheath was present in 98% of males with hypertrophied fat bodies, but this character was present in only 17% of males with fat bodies rated as abundant. A single male with a fat body rating of sparse (0.9%) was recorded as having the fatty sheath. That male was 3 d old at the time of dissection, and was the only male reared under 14-h days to exhibit the sheath. Although we did not record the color of the testicular sheaths, they varied from light aqua to yellowish white and tended to reflect the color of the surrounding fat body.

Seminal vesicle condition was associated with both photoperiod (controlling for age; Q_{CS} = 10.37, df = 1, $P < 0.01$) and adult age (controlling for photoperiod; Q_{CS} = 142.42, df = 1, $P < 0.01$). The influence of photoperiod appeared to be caused by reduced development of seminal vesicles of 3-d-old males reared under 10-h days compared with those reared under 14-h days. At 3 d of age, 9% and 70% of males reared under 10-h days had seminal vesicles rated as empty and filling, respectively. No males reared under 14-h days were observed with empty seminal vesicles at any age, and 61% of the 3-d-old males had seminal vesicles rated as filled. By 7 days of age, most males had seminal vesicles rated as filled (10 h, 86%; 14 h, 77%), and by 10 days most seminal vesicles were distended with sperm (10 h, 59%; 14 h, 76%). All males contained seminal vesicles distended with sperm by 17 days of adult age. Despite the association between seminal vesicle condition and photoperiod, no relationship between seminal vesicle condition and fat body quantity was observed ($Q_{CS} = 0.13$, df = 1, $P = 0.72$).

An association was detected between medial accessory gland condition and fat body quantity, controlling for age and photoperiod ($Q_{CS} = 65.55$, df = 1, $P < 0.01$). The basal section of the medial gland was either filled or distended in 98% of males with sparse fat bodies. These classifications were observed in only 9% of males with hypertrophied fat bodies. However, at least some development of the medial accessory glands was observed for 47% of males with hypertrophied fat bodies. Medial gland conditions were more evenly distributed among males with fat bodies rated as abundant; 87% of those males had glands exhibiting at least some development, and 85% had medial glands with opaque material in both the basal and distal sections of the gland. There was also a negative association between medial gland condition and presence of the testicular sheath ($Q_{CS} = 48.88$, df = 1, $P < 0.01$). However, only 45% of males with the fatty sheath also had medial glands with no development, and the testes of 5% of males with undeveloped medial glands lacked the fatty sheath. Finally, a distinct association was detected between the respective conditions of medial and lateral accessory glands ($Q_{CS} = 85.09$, df = 1, $P < 0.01$). All males with undeveloped medial glands also contained undeveloped lateral glands, and all males exhibiting medial glands with filled or distended bases also possessed completely developed lateral glands. Most males (62%) with opaque material only in the bases of the medial glands also had undeveloped lateral glands, whereas most males (70%) with opaque material in both sections of the medial gland also contained fully developed lateral glands.

Associations among characters of females. Ovary condition in females was associated with age (controlling for photoperiod; $Q_{CS} = 23.66$, df = 1, $P < 0.01$) and with photoperiod (controlling for age; $Q_{CS} = 82.59$, df = 1, $P < 0.01$). Most females reared under 10-h daylengths (91%) had ovaries exhibiting no signs of development at day 3 of adulthood whereas the remaining 9% contained chorionated eggs or follicular relics. The percentage of females exhibiting no ovary development declined to 61% by day 7, and then fluctuated between 40 and 48% at subsequent ages. A total of 8% of females reared under 10-h days exhibited only vitellogenic oocytes. This classification was observed at ages of 7, 10, and 17 d (3 females at each age). In contrast, no females reared under 14-h days had undeveloped ovaries at any age. Only 45% of females reared under 14-h days had eggs or relics at 3 days of age, but all females had either eggs or relics by day 7. The slow decline under 10-h days in the proportion of females that contained undeveloped ovaries, combined with the continued occurrence of ovaries with vitellogenic oocytes only, suggested exposure to the short photoperiod delayed but did not prevent ovary development in some individuals.

Ovary condition was also associated with fat body quantity class for females reared under 10-h daylengths ($Q_{CS} = 43.14$, df = 1, $P < 0.01$) but not for females reared under

14-h days (Q_{CS} = 0.25, df = 1, P = 0.62). Among females reared under 10-h days, 81% of undeveloped ovaries were associated with hypertrophied fat bodies, and 79% of ovaries with eggs or relics were associated with sparse or abundant fat bodies. However, 25% of females with hypertrophied fat bodies also had ovaries that exhibited vitellogenic oocytes, eggs, or relics. All females reared under 14-h days had ovaries with at least vitellogenic oocytes, regardless of age or fat body condition, and all females with fat bodies rated as sparse had ovaries with eggs or relics.

Although the females in this study were unmated, many of them oviposited before they were dissected. There was a significant association between oviposition and female age, controlling for photoperiod (Q_{CS} = 50.57, df = 1, $P < 0.01$). No females reared under 10-h daylengths oviposited by day 3 of adulthood, but 26% oviposited by day 7. The percentage of females ovipositing increased at later ages from 39% at day 10 to 48% by day 17. A single female reared under 14-h days oviposited by day 3, and the percentage of females that oviposited subsequently increased from 80% at day 7 to 100% by day 14. However, 15 - 25% of females reared under the 10-h photoperiod to ages ≥10 d exhibited developed ovaries but did not oviposit. Therefore, the occurrence of oviposition was not a reliable indicator of ovary status for females reared under short daylengths.

Associations between adult age and diapause status. When the diapause condition in females was defined as the presence of a hypertrophied fat body and the absence of ovary development, no association between the frequency of diapause occurrence and female age was detected for females reared under 10-h days (Q_{SMH} = 0.84, df = 4, P = 0.93). The corresponding percentages of females classified as diapausing ranged from 52% at 7 days to 40% at 17 days of age. No females reared under the 14-h days met these criteria for diapause. When less stringent criteria (hypertrophied fat body and absence of eggs or relics) were used to indicate diapause, a single 3-d-old female (1% of the total) reared under the 14-h photoperiod was classified as diapausing. Relaxing the criteria to indicate diapause did not result in an association between female age and diapause status for bugs reared under 10-h days (Q_{SMH} = 3.00, df = 4, P = 0.56), and only slightly increased the percentage of females classed as diapausing (ranging from 43% at day 3 to 61% at day 10).

Whether diapause in males was determined using stringent criteria (presence of hypertrophied fat body, undeveloped medial accessory glands), or relaxed criteria (hypertrophied fat body, medial accessory glands undeveloped or with bases filling), no diapausing males were observed in association with the 14-h photoperiod. When the stringent criteria were applied to males reared under 10-h daylengths, an association between adult age and the occurrence of diapause was observed (Q_{SMH} = 11.33, df = 4, P = 0.02). The incidence of diapause was 17% in 3-d-old males, increased to 45 and 41% at 7 and 10 days, respectively, and then declined to 12% (14 d) and 17% (17 d). When the relaxed diapause criteria were applied there was no statistical association between age and diapause status (Q_{SMH} = 8.61, df = 4, P = 0.07), and the percentage of males classed as diapausing ranged from 22% at day 3 to 59% at day 10. The inconsistencies in the respective relationships between the two sets of criteria with male age were caused by the apparent initiation of medial accessory gland development in many males older than 7 days of age.

Nymphal development time. No difference in nymphal development time was observed between *L. hesperus* sexes (F = 0.00; df = 1, 464.1; P = 0.96) and no photoperiod by sex interaction was demonstrated (F = 0.32; df = 1, 464.6; P = 0.57). However, a difference in development time between photoperiods was indicated (F = 5.09; df = 1, 464.3; $P = 0.02$). Development time from egg to adulthood was shorter under the 14-h photoperiod (14.3 ± 0.22 d) than under 10-h days (14.6 ± 0.22 d). It seems likely that this small difference in development time was caused by slight differences in the temperatures maintained in the respective environmental chambers rather than by photoperiod.

Discussion

The observed associations among fat body quantity classes and fat colors indicate that most *L. hesperus* adults with hypertrophied fat bodies exhibit fat that is predominantly white, or white and light aqua. When both white and light aqua fat was observed in hypertrophied fat bodies, the light aqua fat usually occurred in the posterior portion of the abdomen (Fig. 1e,f). However, in some individuals, especially at earlier ages, the light aqua fat was more generally distributed (Fig. 1d). There was a more consistent association between fat body quantity class and fat configuration; 94% of females and 97% of males exhibiting hypertrophied fat bodies had fat present in columns adjacent to the abdominal midline with globules of fat anteriorly and laterally. Therefore, in assessing fat body hypertrophy of *L. hesperus* adults it appears more appropriate to emphasize the configuration of the fat than to emphasize its color. Although we did not detect a statistical association between fat body quantity class and adult age, differences in the appearance of hypertrophied fat bodies among adult ages were apparent (Fig. 1d-e). Based on these differences, it seems prudent to defer assessments of diapause status until at least 10 days of adult age under the temperature conditions of our study.

The unique glass bead fat type (Fig. 2) was not observed in sufficient frequency to permit analyses of association, and we have found no previous report of this fat body type. However, this fat type was observed in both sexes, under both photoperiods, and in both the youngest and oldest adults examined. Therefore, the glass bead fat does not appear to be indicative of physiological status. Although this fat type occurred infrequently during our study, ongoing studies of diapause characters in *L. hesperus* reared from eggs deposited by field-collected bugs indicate the glass bead fat type is very common in field populations (D.W.S, unpubl. data). In addition, the glass bead type does not appear to be associated with a pathological condition, because it was first observed during preliminary studies in a female held as a diapausing adult for 44 d at 28°C.

Although the presence of an opaque sheath of fat on the testes of some males was associated with fat body quantity and medial accessory gland condition, most males exhibiting the fatty sheath also exhibited developed accessory glands. Furthermore, the appearance of the fatty sheath tended to mimic the appearance of surrounding fat body. Therefore, presence of the fatty testicular sheath seemed a simple consequence of fat accumulation irrespective of physiological condition. In addition, seminal vesicle condition was not related to fat body quantity, and therefore should be ignored in assessments of diapause. The observed association between seminal vesicle condition and photoperiod was caused primarily by slightly delayed development of these organs in 3-d-old males held under the 10-h photoperiod compared with those held under the 14-h daylength. This delay may be explained by slightly lower temperatures in the 10-h environmental chamber implied by the slower nymphal development in that chamber compared with the 14-h chamber. Because recent mating results in at least a temporary reduction in the contents of the seminal vesicles (Brent 2010), the previously reported associations with diapause of slender (Strong et al. 1970) or atrophied (Leigh 1966) seminal vesicles likely resulted from recent mating since these earlier studies used field-collected or mixed-sex groups of *L. hesperus* adults, respectively.

Medial accessory gland condition was also associated with fat body quantity, but many males with hypertrophied fat bodies possessed medial glands with obvious development. Small, cone-shaped deposits of opaque white material at the base of even the smallest medial accessory glands were common (Fig. 3d). Although these were classified as undeveloped, it appears that at least slight development of these glands is appropriately associated with diapause. The bases of these glands are obscured by a fatty shield in males with substantial fat bodies (Fig. 3b), and thus the small deposits of white material were only visible from the ventral aspect. However, medial glands with more extensive development were often observed in males with hypertrophied fat bodies, and presence of this development was responsible for the observed reduction in the proportion of males classed as diapausing at ages >10 d. The delay in medial accessory gland development observed in many 14- and 17-d-old males held under 10-h daylengths, compared with males held under 14-h days, was too great to be explained by the small difference in temperature implied by observed differences in nymphal development. The endocrinology of diapause, or that of accessory gland development, is not understood for *L. hesperus*. Therefore, we could not determine whether the delayed development of the medial glands in males held under 10-h days was a photoperiodic response unrelated to diapause, or an indication of premature termination of a low-intensity diapause. This uncertainty provided the rationale for the application of both stringent and relaxed diapause criteria. Therefore, future studies of *L. hesperus* diapause may benefit from the application of both sets of criteria so the results will remain interpretable when the endocrinology of accessory gland development becomes better understood. Because mating at least temporarily causes the medial glands to resemble glands at an earlier stage of development (Brent 2010), it is not clear that the relaxed criteria are appropriate for diapause assessment in studies where adults are held in mixed-sex groups. Medial accessory gland condition was also closely associated with lateral gland condition. However, ratings of the medial glands are preferred because their earlier stages of development are more clearly discernable, and their uniformly opaque contents allow more objective assessment, compared with the lateral glands.

Ovary classes of females were also associated with fat body quantity, but only for females held under 10-h photoperiods. As in the case of males, some delayed development (presence of ovaries with oocytes only) was observed at ages too late to be explained by small temperature differences between the 2 environmental chambers. This delayed ovarian development occurred less frequently than the delayed development observed for male accessory glands. The production and uptake of egg yolk is metabolically expensive and therefore seems incompatible with the diapause state. However, we assessed diapause status of females using both stringent and relaxed criteria in case the late appearance of vitellogenic oocytes marks the termination of diapause. Regardless, application of the relaxed criteria did not substantially change estimates of the proportions of diapausing females. Although prior oviposition was associated with both female age and photoperiod, the number of reproductive females that failed to oviposit under either photoperiod was too great to permit use of this character as a nondestructive indicator of diapause.

Relationships between the occurrence of diapause and adult age were not observed for females regardless of the criteria used to indicate diapause, and were observed for males only when the stringent criteria were applied. Overall, our observations were not consistent with the assertion of Leigh (1966) that reproductively immature females lacking ovary development will also lack substantial fat bodies. Although we could not devise an objective method for judging fat body maturity, it was obvious that the overall appearance of hypertrophied fat bodies continued to change until at least 10 d of age. This adult age seems an appropriate time for assessment of diapause because it provides ample time for development of fat body and reproductive characters. Also, egg hatch, the development of both male and female reproductive organs, and the occurrence of oviposition, all occurred substantially earlier than was reported by Leigh (1963) or Strong et al. (1970). These observations suggest the need to reexamine the relationship between temperature and development of *L. hesperus*.

The most surprising result of our study was the low incidence of diapause observed in response to the 10-h photoperiod, compared with observations reported by Beards and Strong (1966), Leigh (1966), and Strong et al. (1970). A part of these discrepancies may be explained by the different criteria used to distinguish diapause in the various studies. However, it may also be relevant that the reports of Leigh (1966) and Strong et al. (1970), and a portion of the report by Beards and Strong (1966), were based on dissections of field-collected *L. hesperus.* In fact, we have initiated studies to compare the diapause response of adults derived from eggs deposited by field-collected bugs to that of adults originating from a laboratory colony. Preliminary results (D.W.S., unpubl. data) suggest the insects originating from the field exhibit a higher and more consistent (but not complete) diapause response to a 10-h photoperiod than do insects originating from the laboratory colony. Therefore, it seems appropriate to evaluate how quickly and to what extent the diapause response is modified by laboratory selection before other aspects of the diapause phenomenon are investigated using laboratory-reared insects.

The validity of the two sets of diapause criteria we propose cannot be unambiguously assessed without additional knowledge of the endocrinological or molecular mechanisms controlling reproductive development and diapause in *L. hesperus.* However, these criteria provide a biologically-reasonable means of standardizing and preserving the integrity of diapause-related data until additional information becomes available. Consistent application of these criteria should facilitate direct comparisons of studies conducted by different investigators, and reduce the opportunity for differences in interpretation of similar data.

Acknowledgments

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.

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