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Transfer and fate of seminal fluid molecules in the beetle, *Diaprepes abbreviatus*: Implications for the reproductive biology of a pest species

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Abstract

Molecules transferred from males to females via seminal fluids are important to the study of insect reproduction because they affect female physiology, reproductive behavior, and longevity. These molecules (seminal fluid molecules or SFMs) interest applied entomologists because of their potential use in insect control. SFMs are also interesting because of their relatively rapid evolution and important role in post-mating sexual selection. We studied SFMs in *Diaprepes abbreviatus*, a major pest of numerous plant species of economic importance. Using radiolabeled-methionine (^{35}S), we found that *D. abbreviatus* males synthesized proteins de novo in their reproductive tissues after mating. Males that were fed radiolabeled methionine transferred radioactivity to females beginning within the first 10 min of mating. Male-derived substances are absorbed from the female's reproductive tract into the hemolymph and circulated throughout the body, but are found primarily in the eggs and ovaries. As a result, SFMs may be a useful means of both horizontal (to mates) and vertical transfer (to offspring) of control agents between conspecifics.

Ke words: *Diaprepes abbreviatus*; Seminal fluid molecules; Ejaculate transfer; Coleoptera

1. Introduction

In animals with internal fertilization, sperm are transferred within seminal fluid or a spermatophore matrix. Seminal fluids and spermatophores do not serve simply as media for sperm transport; rather, they also contain molecules (hereafter called "seminal fluid molecules" or SFMs) that mediate fertility of the male donor and the female recipient in a variety of species ranging from arthropods to mammals (Waberski et al., 1999; Ignotz et al., 2001; Gillott, 2003). SFMs have been most thoroughly studied in insects and most studies have focused on those secreted by the male reproductive accessory glands (Chen, 1984, 1996; Wolfner, 1997, 2002; Gillott, 2003), although some SFMs are secreted in other parts of the male reproductive tract (e.g., the ejaculatory duct). SFMs

investigated the transfer and fate of SFMs in the beetle, *Diaprepes abbreviatus* (Coleoptera: Curculionidae).

D. abbreviatus (L.) is a weevil (Curculionidae) that feeds on a wide range of plant species including citrus, sugar cane, and many ornamentals across the Caribbean and in central and southern Florida (Hall, 1995). Despite decades of research on this species, there is no effective control. SFMs are currently being investigated as a tool in the biochemical control of pest species and may prove promising for *D. abbreviatus* (Fernandez and Klowden, 1995). In *D. abbreviatus*, there is evidence that molecules from the ejaculate have the potential to affect female reproductive behavior. Radiolabeled amino acids that were injected into males were found in the hemolymph of female mates as early as 30 min after mating had begun (Harari et al., 1999). In the current study, we investigated the potential role of molecules contained in the male ejaculate (i) in modulating female reproductive behavior and (ii) for developing control strategies for *D. abbreviatus* by studying the transfer and fate of SFMs. Specifically, we addressed the following questions:

1. What is the fate of SFMs within mated females?
2. At what point during mating is ejaculate transferred?
3. Do recently mated males synthesize proteins de novo in their reproductive accessory glands?

2. Methods

2.1. Stud species

D. abbreviatus larvae live in the soil and feed on roots of citrus, ornamental species, and other plants of economic importance (Schroeder et al., 1979; McCoy et al., 2001). Both sexes become reproductively mature 10 to 14 days post-eclosion from the pupal stage in captivity after feeding on new citrus leaves. The adult lifespan of *D. abbreviatus* is up to several months in captivity (L.S., pers. obs.). *D. abbreviatus* is highly promiscuous (Sirot, 2004) and matings are lengthy, sometimes lasting greater than 16 h (Harari et al., 1999). Even though a single mating provides a female with enough sperm to fertilize her eggs for over 2 weeks (on average; Harari et al., 2003), females in some populations mate with up to three different males each day ($\overline{X} = 1$ mating per day; Sirot, 2004). Moreover, females appear to solicit male attention by mounting other individuals (both male and female), which attracts large males (Harari and Brockmann, 1999, 2000). These findings suggest that females may benefit from receiving multiple ejaculates.

2.2. Stud location, time, and subjects

This study was conducted between December 2002 and July 2003 at the United States Horticultural Research Laboratory (USHRL), Ft. Pierce, FL. Different individual insects were used for each step of the study. For all of the experiments except one, laboratory-reared females (to ensure mating status) and field-collected males were used. The female beetles were reared in 30-ml plastic cups containing artificial insect diet (one individual per cup; 26 °C, >95% RH; Lapointe and Shapiro, 1999; Lapointe, 2000). Males collected from naturally occurring populations (citrus groves in St. Lucie County, FL) were used for most steps of our experiments because we wished to test for the transfer and effects of natural levels of SFMs and we do not know how artificial rearing conditions might affect SFM levels. For our experiment on the timing of ejaculate transfer (see Section 2 below), laboratory-reared males were used because wild males were not available.

2.3. Male reproductive organs

SFMs could be produced either in the testes or in the associated ducts and glands. The reproductive organs of male *D. abbreviatus* consist of two testes, each comprised of two lobes enclosed in a common sheath and associated with ducts and glands (Fig. 1). Ducts connect each testis with a lobed structure (Fig. 1). This structure has alternately been called the seminal vesicle (Aslam, 1961) and an accessory gland (Shobha and Pajni, 1994) in Curculionids. In *D. abbreviatus*, this structure is thought to be an accessory gland since it has a secretory epithelium and has not been found to contain any sperm (S.L., unpubl. data). A blind-ended tube-like accessory gland connects to the center of the lobed structure. Hereafter, each testis in association with its tube-like accessory gland and lobed structure is referred to as a testes-accessory gland (TAG) complex.

3. Experimental methods

3.1. Ejaculate transfer and fate of labeled SFMs within mated females

To trace the ejaculate, males were fed radiolabeled methionine (^{35}S; specific activity = 1000 Ci/mmol) and then males and females were tested for radioactivity at different stages of the reproductive process. Before feeding males the radioactive material, they were allowed to mate repeatedly over the course of 3 days to expend stored SFMs. During this preparatory period, the males were food-restricted to induce hunger. At 17:30 (EST) on day 3 of the preparatory period, males were placed individually in plastic round cages (screened lid; diameter: 10 cm; height: 8 cm) containing a plastic water cup with a cotton wick and a piece of mesh screening. We spread ~25 μCi ^{35}S-methionine (diluted with deionized water to make a 2.5 μCi/1 μl solution) onto a 2% agar solution on the surface of small pieces of fresh organically grown carrot. One carrot piece was placed in each male's cage. The cages were placed in an incubator with a 12:12 light: dark cycle (day temp: 30 °C, night temp: 26 °C). On the next morning (Day 4), the males had consumed little of the carrot;

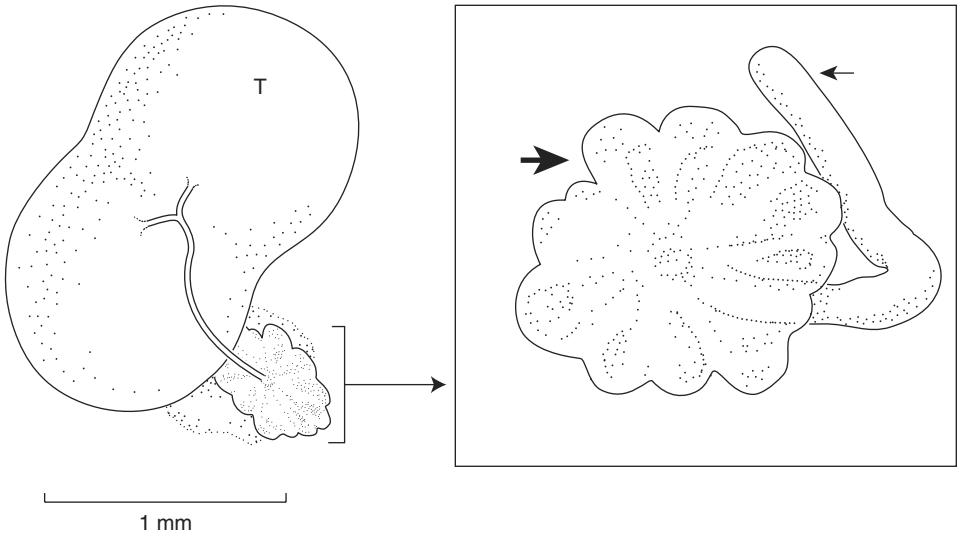

Fig. 1. Internal reproductive structures of male *D. abbreviatus,* with an inset close-up of the lobed structure (thick arrow) and tube-like accessory gland (fine arrow). T: Testes. Drawing by Margaret Nelson.

therefore, a 1 cm^2 piece of young citrus leaf spread with ~10 μCi of ^{35}S-methionine (in the agar solution) was added to each male's cage. On Day 5, fresh untreated citrus leaves were added to each male's cage. Control males were treated identically to the experimental males except that the agar solution on their carrot pieces and citrus leaves contained water rather than ^{35}S-methionine. On the morning of Day 6, the males were randomly assigned to one of three treatments: (i) males were immediately euthanized (by placing them in a −20 °C freezer; $N = 3$ control and 7 experimental males); (ii) males were allowed to mate with a female and were euthanized after mating, and the females were allowed to lay eggs for 24 h and were then euthanized ($N = 3$ control and 12 experimental males); or (iii) males were allowed to mate with a female and were euthanized after mating, and the females were allowed to lay eggs for 3 to 4 days and were then euthanized ($N = 3$ control and 12 experimental males). Both males and females were dissected 2 to 4 days after euthanization.

For the experimental treatments in which males mated ((ii) and (iii) above), unconsumed ^{35}S-methionine-treated food was removed from each cage before females were added. The females used in the experimental treatments were not used during the preparatory period. One female was added to each male's cage, which was then placed in the incubator. Cages were checked hourly to record matings. When each mating ended, the male was removed and a wax paper strip was added to the cage as oviposition substrate (Wolcott, 1933). Eggs were collected each morning and the oviposition substrate was replaced.

Males were dissected in Krebs-ringer bicarbonate buffer. The TAG complexes were removed, rinsed, and placed in a 1.5 ml microcentrifuge tube with 150 μl of tissue solubilizer (Solvable; Perkin-Elmer). The head, thorax, and remainder of the abdomen were each put into separate 1.5 ml microcentrifuge tubes with 150 μl of solubilizer. The tubes were incubated in a 50 °C water bath for 1 h and then at room temperature overnight. The following day, the tubes containing the body sections were vortexed and the supernatant pipetted into new tubes containing 1350 μl of scintillation fluid. The tubes containing the TAG complexes were vortexed and 1350 μl of scintillation fluid was added directly to the tube since the tissue was mostly dissolved. Radioactivity in the body tissues was counted using a Wallac Microbeta liquid scintillation counter. A chemical and color quench curve was created to convert counts per minute to disintegrations per minute (DPMs) for ^{35}S-methionine. Female ovaries were dissected out and their bodies were divided into head, thorax, and abdominal sections for separate analysis. The eggs and female body tissues (including ovaries) were processed and radioactivity was counted as described above for the male tissues.

DPM counts were compared between groups using non-parametric statistics since the data could not be transformed to normality. For these measures, medians and inter-quartile ranges are presented. Radioactivity found in the head, thorax, abdomen (without ovaries), ovaries, and laid eggs of females was compared as percents of the total amount transferred to the female. A repeated measures ANOVA (SPSS 12.0) was used to test for differences in the percent of radioactivity found in the different tissues after arcsine square-root transformation of the data for normalization. The different body tissues were considered the repeated measures. Because the data violated the assumption of sphericity, a Greenhouse-Geisser correction (Zar, 1999), which gives non-integer degrees of freedom, was used. For females euthanized after 3 to 4 days, radioactivity per egg was compared for eggs laid in the first two nights after mating and those laid in the third and fourth night after mating using a paired *t*-test. We calculated radioactivity per

egg by determining the total DPMs in each batch of egg and then dividing by the number of eggs in the batch.

3.2. Timing of ejaculate transfer

To determine the timing of ejaculate transfer, the experiment described above was repeated but the experimental matings were not conducted until Day 8 and pairs were interrupted at different times during mating. Four treatment groups were used: interrupted after 10 min ($N = 5$), 2 h ($N = 4$), 4 h ($N = 5$), or uninterrupted ($N = 3$). Females were allowed to lay eggs for 24 h after mating and were then euthanized. The DPMs in the eggs were counted using the method described above for the TAG complex. The DPMs in their bodies were also determined using the methods described for the female body tissue except that the ovaries were not removed and the three body sections were processed together in the same tube. Therefore, for each female, we have a DPM count for any eggs she laid and for her body tissue. The DPMs for the body tissue and the TAG complexes of the males used in the matings were also counted. For each of the four treatments, the percent of DPMs that were transferred to the female (body tissue and egg counts combined) out of the total DPMs in the female and those remaining in her mate's TAG complexes were compared using a one-way ANOVA. One female was excluded from this analysis because her mate had very low DPMs suggesting that he had not consumed sufficient ^{35}S-methionine. The DPMs in this male's TAG complexes were more than 1000 × less than the average for the other males and more than 400 × less than those for the next lowest male.

3.3. Characteri ation of labeled material in male TAG comple

3.3.1. Percent of labeled material incorporated into proteins

Trichloroacetic acid (TCA) precipitation was used to determine the percent of the labeled materials that were incorporated into proteins in each of the parts of the TAG complex. TCA precipitation precipitates proteins and leaves behind unincorporated amino acids. The experiment described in Section 3.1 was repeated with the following modifications. The males were fed ^{35}S-methionine spread onto a 2% agar gel on 1-cm^2 pieces of young citrus leaves. The males were frozen for only 10 min before dissection and the dissections were conducted using chilled Krebs-ringer bicarbonate buffer with the Petri dish in an ice bath to minimize protein degradation. We had three groups of samples: (i) testes; (ii) lobed structures; and (iii) tube-like accessory glands. As the samples were dissected, they were frozen immediately with liquid nitrogen and then kept frozen at −80 °C until the precipitation. For TCA precipitation, tissues were combined for each tissue type from 6 testes, 4 lobed structures, and 2 tube-like accessory glands. The tissues were homogenized in 100 µl PBS with 1 mM PMSF using a sterilized pestle. The tissues in solution were then centrifuged (14,000g) for 15 min at 4 °C. The pelleted material was resuspended in 100 µl PBS to analyze radioactivity levels in the cell material. The supernatant was transferred to a new 2 ml centrifuge tube and an equal volume of 20% TCA was added. The proteins were precipitated in the TCA on ice for 1 h and then the solution was centrifuged (14,000g) at 4 °C for 20 min. The supernatant was retained to test the radioactivity level. The pellet of precipitated proteins was washed with 200 µl chilled acetone and resuspended in Laemmli buffer. The acetone was retained to test the radioactivity level. Ten microliters of each of the materials (i.e., the resuspended cell material, the TCA supernatant, the acetone, and the precipitated proteins) were vortexed with 1 ml scintillation fluid in separate microcentrifuge tubes and the radioactivity in each sample was counted using a Wallac Microbeta liquid scintillation counter.

3.3.2. Si e of newl s nthesi ed proteins produced in male reproductive tissues

To characterize the approximate size of the proteins in the different male reproductive tissues, SDS polyacrylamide gel electrophoresis (PAGE; 15% acrylamide gel) was conducted on the TCA-precipitated tissue homogenates from experiment 3.3.1 above. Five microliters of the testes homogenate, 10 µl of the lobed structure homogenate, and 20 µl of the tube-like accessory gland homogenate were each run in individual lanes of the gel. Due to limited tissue availability, samples were placed directly in SDS-sample buffer. This precluded protein quantification assays. To visualize the radiolabeled proteins, Kodak BioMax film was exposed to the gel at −80 °C for 1 week. Before exposing the film to the gel, the gel was treated with Autofluor Autoradiographic Image Enhancer (National Diagnostics, Atlanta, GA) to enhance the X-ray film detection of the radiolabeled protein bands.

4. Results

4.1. Ejaculate transfer

The males that were offered radiolabeled ^{35}S-methionine on their food had much higher levels of radioactivity (median: 107,683.5 DPMs; inter-quartile range: 76,146–275,757.5) than the control males (median: 160 DPMs; inter-quartile range: 108.5–576; Mann–Whitney $U = 0$; $N_{\text{experimental}} = 7$; $N_{\text{control}} = 9$; $P < 0.005$). Most of the radioactivity in the males fed ^{35}S-methionine (81 ± 3%) was found in the abdomen and the TAG complex (Friedman's test, $N = 7$, $d.f. = 3$, $X^2 = 15.5$; $P < 0.01$; Fig. 2). Males fed with radiolabeled amino acids transferred radioactive substances to females during mating. Females mated to males who had eaten the ^{35}S-methionine had significantly higher levels of radioactivity (median: 1191 DPMs; inter-quartile range: 667.8–2994.8) than females mated with control males (median: 128 DPMs; inter-quartile range: 78–285.5). Females euthanized 3 to 4 days after mating had higher levels

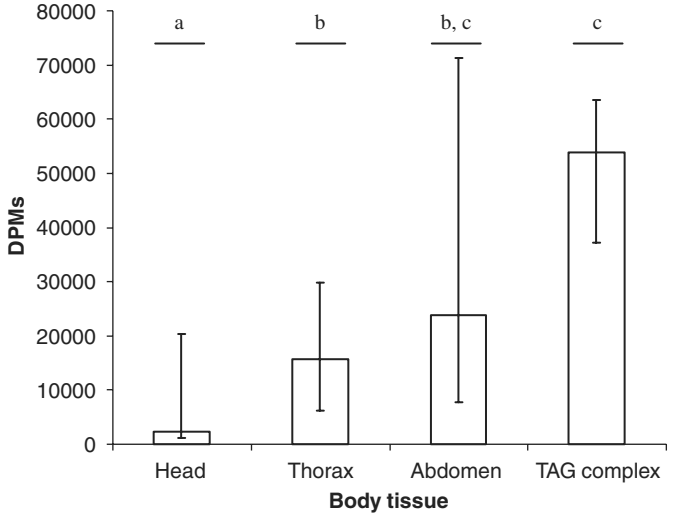

Fig. 2. Results of incorporation of ^{35}S-methionine by feeding into body tissue of males. Radioactivity is presented in disintegrations per minute (DPMs). Medians and inter-quartile ranges are presented. Letters indicate statistically significant differences among body parts. Most radioactivity was found in the abdomen and testes-accessory gland (TAG) complex (Friedman, $N = 7$, $d.f. = 3$, $X^2 = 15.5$, $P < 0.01$). Median radioactivity levels in control males were 0 DPMs for all tissue types except testes which had a median of 93 DPMs.

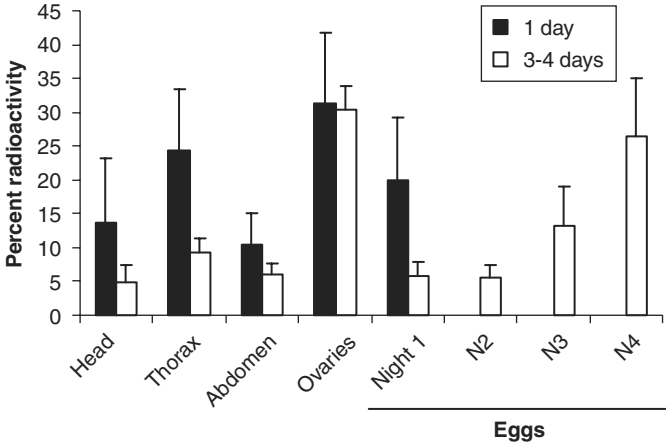

Fig. 3. Percent of total radioactivity found in head, thorax, abdomen, ovaries and eggs (laid on nights 1 to 4 after mating) of females euthanized 1 day or 3 to 4 days after mating with males fed ^{35}S-methionine (mean ± S.E.). The majority of the radioactive material was found in the eggs and ovaries (repeated measures ANOVA of arcsine square-root transformed data: $F_{1.83,\ 34.79} = 26.39$; $P < 0.001$; $N = 8$ for 1-day treatment, $N = 10$ for 3 to 4 day treatment).

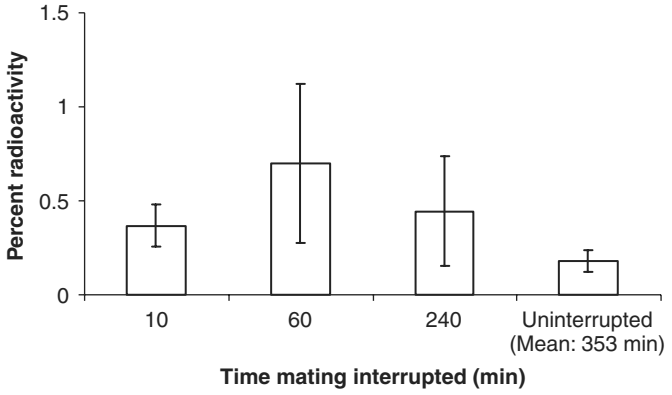

Fig. 4. Percent of radioactivity (mean ± S.E.) in female (body tissue and eggs combined) out of total radioactivity in female and TAG complexes of the female's mate. Matings were interrupted after 10 min ($N = 5$), 60 min ($N = 4$), 240 min ($N = 5$), or were not interrupted ($N = 3$). The percent of radioactive material that was transferred by males was not affected by the mating duration treatments (one-way ANOVA, $F_{4,15} = 0.98$, $P = 0.45$).

of radioactivity (median: 2151.5 DPMs; inter-quartile range: 1387–3920) than females euthanized 1 day after mating (median: 687.5 DPMs; inter-quartile range: 467.5–1041.5; Kruskall–Wallis: $X^2 = 16.6$, $d.f. = 2$, $P < 0.001$; $N_{\text{control}} = 6$, $N_{\text{1-day}} = 12$, $N_{3\ \text{to}\ 4\text{days}} = 12$; post hoc Mann–Whitney U: control vs 1-day females: $U = 4$; $P < 0.01$; control vs. 4-day females: $U = 0$; $P < 0.01$; 1-day vs. 3 to 4-day females: $U = 30$; $P < 0.05$).

Radioactive material was found in all of the female body sections (head, thorax, and abdomen) but the greatest percentage of the material was in the ovaries and the laid eggs (females euthanized 1 day after mating: 51 ± 13%; females euthanized 3 to 4 days after mating: 80 ± 6%; repeated measures ANOVA of arcsine square-root transformed data: $F_{1.83,\ 34.79} = 26.39$; $P < 0.001$; Fig. 3). Two lines of evidence suggested that the amount of male-derived materials incorporated into eggs increased with time. First, there was a trend for a greater percentage of radioactive material found in the eggs and ovaries from females euthanized after 3 to 4 days than from females euthanized after 1 day ($F_{1.83,\ 34.79} = 3.11$; $P = 0.06$). Second, for the females euthanized after 3 to 4 days, the radioactivity per egg laid on the third and fourth night after mating was greater than in those laid on the first and second night (Nights 1–2: $\overline{X}$ ± S.E.: 1.1 ± 0.4 DPMs/egg; Nights 3–4: $\overline{X}$ ± S.E.: 5.5 ± 1.2 DPMs/egg; Paired t-test, $d.f. = 10$, $t = 4.2$, $P = 0.002$).

4.2. Timing of ejaculate transfer

Radioactive substances were transferred from males to females within the first 10 min of mating and there was no apparent increase in the amount of radioactive material that was transferred thereafter (Fig. 4). The percent of radioactive material that was transferred by males was not affected by the mating duration treatments (one-way ANOVA, $F_{4,15} = 0.98$, $P = 0.45$; power: 0.09, 0.40, and 0.83 for small, medium, and large effect sizes, respectively; Cohen, 1988).

4.3. Characterization of labeled material in male TAG complex

4.3.1. Percent of labeled material incorporated into proteins

The ^{35}S-methionine was incorporated into proteins in the testes, the lobed structure, and the tube-like accessory

Table 1
Percent of radioactivity in the male reproductive tissues incorporated into proteins and percent remaining in the cell material, trichloracetic acid (TCA) supernatant using a TCA precipitation, and acetone

	Proteins	Cell material	TCA supernatant	Acetone
Testis	35%	59%	6%	0%
(6 total)	(274,470)	(454,600)	(43,578)	(1,560)
Lobed structure	29%	59%	12%	0%
(4 total)	(650)	(15,490)	(3136)	(0)
Tube-like accessory gland	63%	15%	16%	6%
(2 total)	(18,635)	(4530)	(4608)	(1640)

Total radioactivity levels (in disintegrations per minute) are given in parentheses. Proteins were precipitated out of tissues using a trichloracetic acid precipitation and resuspended. Radioactivity levels of the resuspended proteins were counted in a liquid scintillation counter.

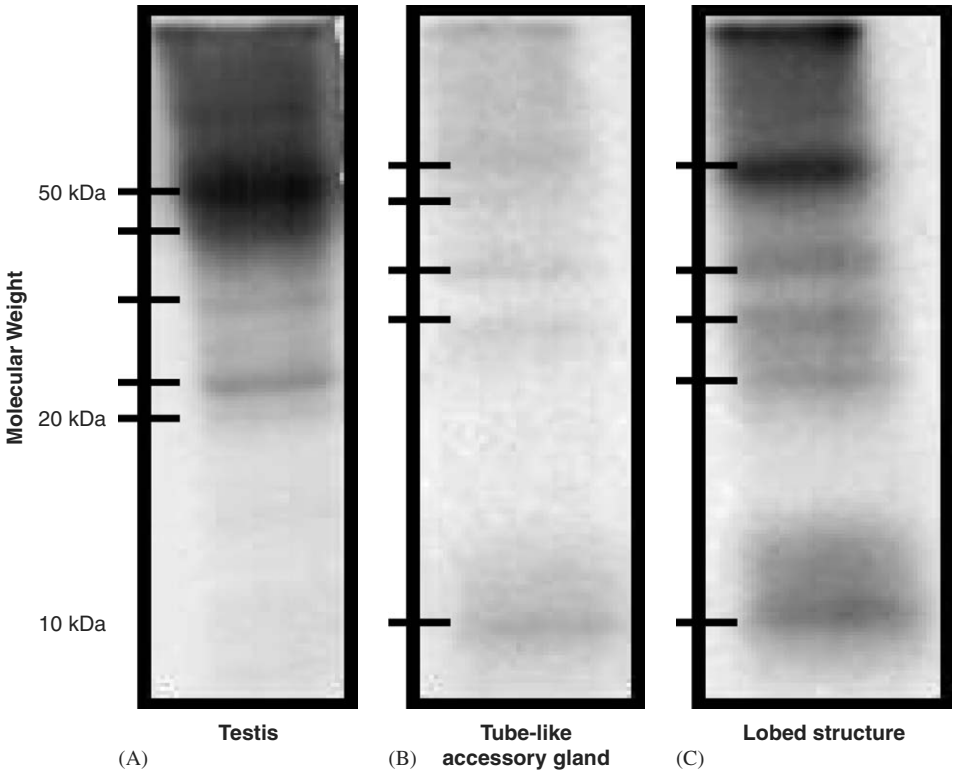

Fig. 5. SDS-PAGE of proteins from reproductive tissues of males fed ^{35}S-methionine. The tissues were exposed to the film for 7 days at −80 °C. Lines mark protein bands emitting the strongest signals for each tissue. Five microliters of the testis homogenate, 20 µl of the tube-like accessory gland homogenate, and 10 µl of the lobed structure homogenate were each run in individual lanes of the gel.

gland (Table 1). The percent of radioactivity that was incorporated into proteins was 35% in the testes, 29% in the lobed structure, and 63% in the tube-like accessory gland.

4.3.2. Si e of newl s nthesi ed proteins produced in male reproductive tissues

The proteins into which the ^{35}S-methionine was incorporated ranged from approximately 10 kDa to over 100 kDa in size (Fig. 5). The radiolabel may have also been incorporated into smaller proteins below the resolution limits of our gel. In the testes alone, the strongest radioactive signals were derived from five bands ca. 20, 22, 27, 38, and 50 kDa, respectively. In the tube-like accessory glands, the strongest signals came from protein bands whose approximate sizes were 10, 25, 30, 48 and 55 kDa, respectively. In the lobed structure, strong signals came from protein bands at ca. 10, 22, 25, 30, and 55 kDa.

5. Discussion

Our results clearly demonstrate that radioactivity derived from fed ^{35}S-methionine moved from the point of delivery of the male ejaculate within the female reproductive tract to all female body sections (i.e., head, thorax, and abdomen) and accumulated primarily in the eggs and ovaries. We found evidence that the amount of male-derived materials incorporated into eggs increased with

time since mating. Furthermore, our results show that ejaculate transfer began within the first 10 min of mating and that recently mated males synthesized proteins de novo in their reproductive tissues after mating.

There are several possible (non-mutually exclusive) means by which the radioactivity contained in the ejaculate of *D. abbreviatus* is transferred to females. The sulfur could be in the seminal fluid either (i) in its elemental form; (ii) incorporated into an amino acid; or (iii) incorporated into a larger molecule (Bownes and Partridge, 1987; Markow et al., 2001). The sulfur could also be incorporated into sperm, which may be subsequently broken down in the female, thus freeing the sulfur. Since *D. abbreviatus* SFMs are found primarily in the eggs and ovaries of mated females, some of these may play a role in the uptake of vitellogenin, as has been proposed for a seminal fluid protein of the Colorado potato beetle (Smid et al., 1997) and *Drosophila* species (Bownes and Partridge, 1987).

The proteins produced de novo in the accessory glands of recently mated male *D. abbreviatus* ranged in size from approximately 10–55 kDa, which is similar to the range of seminal fluid proteins produced by other species of beetles. Proteins produced in the reproductive accessory glands and transferred in the spermatophore of male mealworm beetles (*Tenebrio molitor*) range from 13 to 64 kDa (Happ, 1985; Takiguchi et al., 1992; Paesen and Happ, 1995; Yaginuma et al., 1996) and those transferred in the spermatophore of carrion beetles (*Thanatophilus sinuatus*) range from 14 to 70 kDa (Neuner et al., 1996).

Given the lengthy average mating duration of *D. abbreviatus* ($\overline{X}\pm$ S.E.: 6.5 ± 0.1 h; Sirot, 2004), it is surprising that the amount of radiolabeled material transferred to the female did not increase over the course of mating (Fig. 4). The apparent increase in the amount of radioactivity transferred between the 10- and 60-min time points may have been statistically significant with a larger sample size, but our results clearly demonstrate that the amount of labeled material transferred did not increase after the first hour of mating. These results are supported by the finding that the number of sperm stored by females increases with mating duration up to 1 h but then remains the same for matings lasting between 1 and 2 h (L.S., unpubl. data). Together, these findings suggest that *D. abbreviatus* males transfer their ejaculate at the beginning of mating and that the remainder of the mating may have other functions such as mate guarding (Harari et al., 2003) and/or copulatory courtship (Sirot, 2004). Males are often displaced during mating by other males (Harari et al., 2003) and females often end mating by shaking their body rapidly from side to side (Sirot, 2004). By transferring his ejaculate at the beginning rather than the end of mating, a male ensures that at least some of his sperm are transferred even if the mating is interrupted before sperm transfer is complete. Additionally, if SFMs either stimulate oogenesis, ovulation, and/or oviposition or increase the quality of offspring produced, as suggested by our finding that the majority of labeled material is found in the ovaries and laid eggs, then it would be to a male's advantage to ensure that these proteins are transferred as quickly as possible so that the beneficiary of the effect would be him rather than a female's subsequent mates. It is interesting to note that molecules from one male's ejaculate may be incorporated into eggs fertilized by a different male since male-derived molecules are found in eggs laid 3 to 4 days after mating and females often remate during that time period.

Our finding that male-derived substances transferred in the seminal fluids move into the female head, thorax, and abdomen is important because it implies that mechanisms used in the movement of these substances could be exploited to facilitate the movement of viruses or other control agents from males to females and from females to eggs. Although the mechanisms that underlie this movement are not understood for *D. abbreviatus*, studies of other insects have demonstrated specialized means of SFM transfer from males to females (e.g., Kubli, 2003) and from the reproductive tract to the circulatory system of the female (Lung and Wolfner, 1999). For example, in *Drosophila melanogaster*, an ovulation-stimulating SFM moves from the reproductive tract of the female into her circulatory system through a transiently permeable membrane in the vagina (Lung and Wolfner, 1999). Thus, insects appear to have evolved mechanisms by which SFMs are transported to their sites of action within the female and that are available for use in control strategies of pest insects such as *D. abbreviatus*. Hunter et al., (2003) found that adult *D. abbreviatus* females mated to males infected with the insect iridovirus IIV6 lay infected eggs. Their hypothesis that such transmission might occur via the transfer and absorption of SFMs during mating (Hunter et al., 2003) is supported by our findings. Hunter et al. (2003) suggested that it might be possible to genetically engineer such viruses to deliver lethal or sterilizing agents. One could then introduce "lethal males" into the wild to infect females and their eggs. If one found a virus that was only transmitted through seminal fluid, the lethal male approach would be especially promising for an introduced species with no closely related sympatric species, like *D. abbreviatus* in Florida, because the chance of transmission to non-target species is low. Furthermore, transfer of SFMs into the eggs, hemolymph, the fat body, and other somatic tissue of mated females occurs across many taxa of insects (Rooney and Lewis, 1999), suggesting that the approach of exploiting mechanisms used by SFMs to transfer lethal or sterilizing agents may be widely applicable.

Using PAGE for comparison of radiolabled proteins present in the testes, tube-like accessory glands, and lobed structures, we could not distinguish the presence of radiolabled proteins in the lobed structure that differed in size from those observed in the other structures. Future studies that provide more precise information on protein identification (e.g., 2-D gels, MALDI mass spectrophotometry, Western blots) are necessary to determine if the proteins found in the lobed structure are indeed identical to those found in the other tissues/organs. Aslam's (1961)

careful analysis of the internal structures of Curculionids revealed that the lobed structure is present in some species and absent in others. He called this structure the seminal vesicle but provided no rationale for this assertion and preliminary investigations in *D. abbreviatus* have revealed no sperm in this structure (S. L., unpubl. data). Based on their analysis of 108 Curculionid species, Shobha and Pajni (1994) also argue that the lobed structure is an accessory gland and not a seminal vesicle. The function of this structure deserves more careful attention as it may provide important information for understanding the reproductive biology of Curculionids.

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