J Comp Physiol B (2008) 178:225–233
DOI 10.1007/s00360-007-0216-x

ORIGINAL PAPER

Mating affects reproductive investment into eggs, but not the timing of oogenesis in the flesh fly *Sarcophaga crassipalpis*

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Received: 14 August 2007 / Revised: 28 September 2007 / Accepted: 6 October 2007 / Published online: 24 October 2007
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Abstract We examined the effects of mating on reproductive investment and the timing of oogenesis in the flesh fly *Sarcophaga crassipalpis* by exposing females to males or not. All females exposed to males were mated within a few days and we found that mating affected reproductive investment. Virgin females not exposed to males produced a large clutch of eggs (~91), but females exposed to males and mated produced 10% more. There was no effect of mating on egg length or mass. There was also no effect of mating on the timing of oogenesis. Females in both treatments provisioned their eggs at the same rate with yolk first becoming visible in the oocytes on day three of adulthood and complete provisioning of eggs occurring by the seventh day of adulthood. We examined the biochemical basis of egg provisioning by identifying the yolk proteins and quantifying their blood titer during the oogenic period in both, females exposed to males and mated and those not exposed to males. There was no difference in the timing of the first appearance, peak titer, or disappearance of yolk proteins in the blood between the two treatments. However, consistent with our observation of greater egg production in mated females, these females contained a greater peak yolk protein titer.

Keywords Mating · Oogenesis · Yolk protein · Reproductive investment · Reproductive timing

Communicated by G. Heldmaier.

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Introduction

Mating has been shown to have dramatic effects on female reproductive physiology in many species of animals. In insects, the two most common effects of mating are increasing the rate of oogenesis, therefore increasing short-term reproductive investment, and altering female behavior by making her less likely to remate (Wolfner 2002; Gillott 2003). These effects of mating can be mediated by both the sperm itself and compounds present in the male's seminal accessory fluid. In the fruit fly, *Drosophila melanogaster*, germ cell mutants that do not produce sperm have been developed, but still produce apparently complete seminal accessory fluid. Experiments with these *Drosophila* lines have shown that the presence of sperm in the female storage organs is necessary to maintain the increased rates of oogenesis and egg laying observed in mated females, as well as contributing to the long-term refractoriness to remating by the female (Kalb et al. 1993; Xue and Noll 2000; Heifetz et al. 2001). Therefore sperm are not only important for fertilizing eggs but also play a role in the long-term regulation of female reproductive physiology.

In addition to sperm, the seminal accessory fluid transferred to females during mating contains a complex blend of compounds that can have strong effects on female reproductive physiology, some of which can be beneficial, neutral, or even detrimental to females. Beneficial substances transferred by the male have been shown to increase female fecundity, survival, and offspring quality (Ridley 1988; Boggs 1998). These substances include macronutrients, such as the protein contributions of the spermatophore in the Mormon cricket *Anabrus simplex* (Gwynne 2001), micronutrients like sodium transferred by males of the butterfly *Thymelicus lineola* (Pivnick and McNeil 1987), and defensive compounds, such as pyrrolizidine alkaloids

transferred by the males of the moth *Uthesia ornatrix* (Dussourd et al. 1988). However, males can also transfer compounds that manipulate the female's physiology to change her reproductive tactics in order to increase a particular male's likelihood of siring young. These substances typically act on the female to accelerate the timing of egg production and the number of eggs produced and laid, as well as altering female behavior to decrease the likelihood of remating (Wolfner 2002; Gillott 2003). Male-derived manipulative compounds could have detrimental effects on females by decreasing their longevity and total lifetime fecundity while increasing short-term fecundity, as well as reducing female mate choice and sperm competition.

The best-known group of manipulative substances are the male accessory gland proteins that have been characterized in *Drosophila melanogaster* and mosquitoes (Wolfner 1997, 2002; Klowden 1999). *Drosophila* males produce at least 80 different seminal proteins in their accessory glands, representing a variety of functions including peptides that may act as hormones, protease inhibitors, trehalases, defensive proteins, and proteins that facilitate sperm storage and perhaps sperm competition (Wolfner 2002). Mutant lines that transfer sperm during mating but lack the accessory glands have reduced oogenesis and egg laying and are less refractory to remating by other males than wild-type individuals (Xue and Noll 2000). When considering both lines that do not produce sperm and those that do not produce accessory secretions, it seems that accessory proteins act quickly after mating and are the initial effectors of changes in reproductive physiology whereas storage of sperm is required for the longer-term maintenance of mating-induced changes in female reproductive physiology (Wolfner 2002; Gillott 2003). Interestingly, there is a convincing homology between some of the *Drosophila* accessory gland proteins and seminal accessory proteins produced in the mammalian prostate, suggesting broad-scale conservation of mechanisms of male manipulation of female reproductive physiology (Mueller et al. 2004). In addition to accessory proteins, males of a variety of insect species including bees, grasshoppers and katydids, and moths transfer other manipulative substances including hormones in the seminal fluid (Gwynne 2001; Gillott 2003; Colonello and Hartfelder 2005). For example, males of moth, *Heliothis virescens*, transfer juvenile hormone to females in the male seminal accessory fluid that increases oogenesis by stimulating vitellogenesis, patency, and oviposition and also decreases female remating (Park et al. 1998a, b; Pszczolkowski et al. 2006).

Although mating has strong effects on oogenesis in many insect species, strong effects are not uniformly the case (Papaj 2000; Gillott 2003). Most insects will begin egg provisioning before mating and many will completely provision at least some eggs. Extreme examples of premating oogenesis occur in many Lepidopterans and parasitic wasp species wherein individuals eclose with their full lifetime complement of eggs without mating (Jervis et al. 2007). However, even some species that mature eggs long after eclosion can show little or no effects of mating on oogenesis (Wheeler 1996; Adams 2000). In the flesh fly *Sarcophaga crassipalpis* Macquart, our model for reproductive physiology, females are ovoviviparous wherein eggs are matured synchronously in clutches in the ovaries and passed into a uterus where they are fertilized and hatched before the female deposits mobile first instar larvae. We have observed that females are capable of maturing eggs without mating and holding the unfertilized eggs in the uterus for many days in the laboratory. In the wild, *S. crassipalpis* females deposit their larvae on carrion, which represents a spatially and temporally patchy resource (Kouki and Hanski 1995; Pape 1996). Therefore, the ability to mature a large clutch of eggs prior to mating may be advantageous in a highly heterogeneous environment so that a female is ready to take advantage of larviposition opportunities as soon as possible after mating, decreasing the likelihood of time limitation (Sevenster et al. 1998; Ellers and Jervis 2004).

We expect that exposure to males and mating will have little effect on the timing of oogenesis and the magnitude of egg production in *S. crassipalpis* compared to other model flies, specifically *Drosophila* or the housefly *Musca domestica*, where large effects of mating on female reproductive physiology have previously been found (Wolfner 2002; Gillott 2003). We expect this difference because even though any single oviposition site for *Drosophila* and houseflies may be ephemeral, larvae of both of these species can successfully develop on a wide variety of substrates, from sap fluxes to rotting fruit in *Drosophila* and from rotting vegetable matter to feces in houseflies, making the overall availability of oviposition sites relatively more continuous than in flesh flies which are restricted to ovipositing on carrion (West 1951; Shorrocks 1977; Kouki and Hanski 1995; Pape 1996). We tested this prediction by comparing the timing of oogenesis and the magnitude of reproductive investment into eggs in the first clutch, two critical reproductive parameters, in relation to male exposure and mating in *S. crassipalpis*. To assess effects of male exposure and mating on reproductive timing, we compared the rates of oogenesis, the timing of the appearance and disappearance of yolk proteins in the blood as well as the timing of the peak blood titer of yolk proteins in females that were or were not exposed to males and mated. To assess effects of mating on the magnitude of reproductive investment in the first clutch, we compared the number of mature eggs produced, the average size of eggs produced, and the peak blood titer of yolk proteins in females that were or were not exposed to males and mated. An additional

goal of this work was to quantify the effects of male exposure and mating on oogenesis to determine whether virgin females alone could be used as an assay system to model the physiological events that set reproductive timing in *S. crassipalpis*.

Materials and methods

Insect rearing and experimental protocol

Studies were performed on a laboratory population of the flesh fly *Sarcophaga crassipalpis* reared at the University of Florida. Larvae were fed homogenized beef liver at a density of 80 individuals per 40 g of liver in a 25°C room with a 16L:8D light cycle. Liver was placed in an aluminum foil packet that rested on a bed of dry sawdust in a plastic shoebox (24 × 8 × 6 cm). After feeding, larvae crawled out of the aluminum foil packets and pupariated in the sawdust where they were held until adult eclosion at 25°C. On the day of eclosion, individuals were placed in aluminum screened cages (30 × 30 × 30 cm) and incubated at 22.5°C with a 16L:8D light cycle for the duration of the experiment.

Newly-eclosed flies were sexed and sorted by hand into four cages. Two cages contained 100 females and 100 males representing the male exposure treatment. Only 100 females were placed into each of the other two cages representing the no male exposure treatment. Both treatments received water, sugar, and fresh beef liver, ad libitum throughout the experiment.

Eight females were sampled from each cage daily for the first 10 days after adult eclosion wherein day 0 was the day of emergence and day 9 was the last day of sampling, a period long enough to ensure complete provisioning of the first clutch of eggs. At the time of sampling, 1 μL of blood was drawn from each female by cutting off a leg at the coxa and gently applying pressure to the thorax. The blood was placed in 9 μL of phosphate buffered saline (pH 7.2) containing the Complete Protease Inhibitor Cocktail (Roche, Mannheim, Germany) and frozen at −20°C until analysis. The remaining body was frozen at −20°C prior to dissection.

Frozen individuals were dissected in Ringer's saline and the ovaries, any eggs in the uterus, and spermathecae were removed. The three spermathecae were placed on a glass microscope slide in a drop of saline, crushed with a cover slip to expose their contents, and examined at 100× in phase contrast. Sperm were readily discernable when present and females containing sperm in at least one of the three spermathecae were scored as mated. The progression of oogenesis was quantified by removing the ovaries and opening them to observe the oocytes. The development of oocytes was staged using a modification of the scale of Adams and Reinecke (1979) wherein previtellogenic follicles were staged 1–2, vitellogenic follicles were staged from 3 to 7 progressively based on yolk content and nurse cell morphology, and fully matured, chorionated eggs that had moved from the ovaries into the uterus were assigned an 8. Fully matured stage 8 eggs were removed from the uterus and counted as a measure of fecundity. The length of four stage 8 eggs from each female was measured to the nearest 0.1 mm using a microscope-mounted ocular micrometer as a metric of egg size and the average egg mass for each female was determined from a sample of 20 eggs that were freeze dried to constant mass and weighed to the nearest 0.001 mg.

Biochemical analyses

The total protein content of each blood sample was estimated using a Lowry-type assay, the DC Protein Assay Kit (Bio-Rad, Hercules, CA). A known size aliquot of the blood sample was mixed with 10 μl of Lammeli's sample buffer. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed following Laemmli (1970) with separating gels of 8% 29:1 acrylamide:bis-acrylamide topped by a 4% stacking gel. Gels were run at 150 V constant voltage until the loading dye ran off and stained with 0.1% Coomassie Brilliant Blue R 250 dissolved in a 5:4:1 solution containing methanol, water, and acetic acid. Gels were destained in the same solution without Coomassie. Molecular weights of apoproteins were estimated in SDS-PAGE using standards in the high molecular weight calibration kit (Bio-Rad, Hercules, CA) containing proteins with the following molecular weights: myosin (200,000 kDa), galactosidase (116,250 kDa), phosphorylase B (97,400 kDa), BSA (66,200 kDa), and ovalbumin (45,000 kDa).

Putative *S. crassipalpis* yolk proteins in blood samples were first identified by their migration through gels compared to homogenates of mature eggs. Three bands of appropriate molecular weight were excised from the gels and analyzed at the University of Florida Proteomics Core Facility. Proteins were extracted from gel slices and digested into fragments by trypsin. Capillary rpHPLC separation of protein digests was performed on a 15 cm × 75 μm i.d. PepMap C18 column (LC Packings, San Francisco, CA) in combination with an Ultimate Capillary HPLC System (LC Packings, San Francisco, CA) operated at a flow rate of 200 nL/min. Inline mass spectrometric analysis of the column eluate was accomplished by a hybrid quadrupole time-of-flight instrument (QSTAR, Applied Biosystems, Foster City, CA) equipped with a nanoelectrospray source. Fragment ion data generated by Information Dependent Acquisition (IDA) via the QSTAR-MS were

searched against the NCBI nr sequence database using the Mascot (Matrix Science, Boston, MA) database search engine. Probability-based MOWSE scores were considered for protein identification in addition to validation by manual interpretation of the tandem MS data.

The yolk protein content of each sample was quantified by densitometry of digital images of SDS-PAGE gels ran as above using the program Image J (NIH, Bethesda, MD). Standard curves were generated using known quantities of bovine serum albumin that ranged from 0.2 to 4.0 mg. Internal standards of 0.5 and 3.0 mg bovine serum albumin were included on each gel to correct for gel to gel variation.

Statistical analysis

Effects of male exposure on the timing of oogenesis and yolk protein titer were analyzed using two-way ANOVAs with treatment, age, and age × treatment interaction as explanatory variables. When not significant, the interaction term was dropped from the model. Mean comparisons among groups in the ANOVA were performed by applying an a posterori Tukey's HSD correction for multiple comparisons to the adjusted values from the multivariable ANOVA. The effects of male exposure on mature egg number, length, and mass were assessed using *t*-tests. Because preliminary analysis revealed no differences between individuals sampled from the two replicate cages within each treatment, data from both cages were combined and analyzed together.

Results

Effects of male exposure on mating

In cages that contained both females and males, mating started on the second day of adulthood and the number of pairs in copula was highest on days two and three of adulthood, but pairs were observed in copula throughout the duration of the experiment. Examination of the spermathecae of each female for the presence of sperm showed that for females exposed to males, the majority of females were inseminated between the second and fourth day of adulthood (Fig. 1). Females not exposed to males never contained sperm in the spermathecae, confirming that no mistakes were made in sorting the sexes into their treatments. Within the treatment exposed to males, mating was correlated with age so all data were preliminarily analyzed based on both the treatments (i.e., exposed to males or not exposed to males) and on whether a female was virgin or mated. There was no difference in the results, whether exposure or mating status was used as the main explanatory variable, therefore we present all analyses using treatment

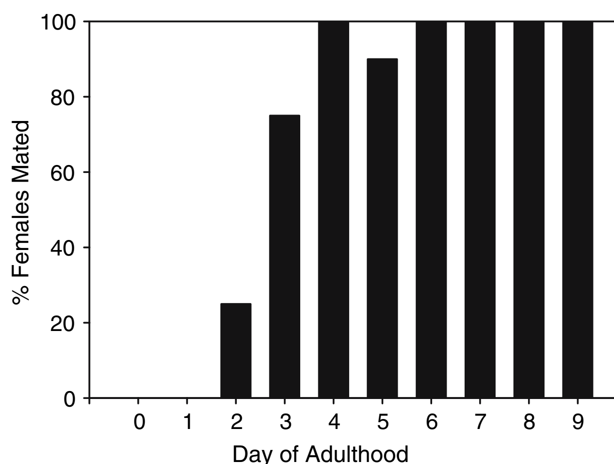

Fig. 1 The majority of mating occurred on days two and three of adulthood so that essentially all females that were exposed to males were mated by the fourth day of adulthood

exposed to males or not as the main explanatory variable and age as a second explanatory variable where applicable.

Effects of mating on the timing of oogenesis and reproductive investment

There was no difference in the timing of the progression of oogenesis in females that were exposed to males and mated or in females which were not exposed to males and remained unmated (Table 1a; Fig. 2). Clear signs of yolk accumulation in oocytes of females in both treatments began on the third day of adulthood and all females in both treatments had fully matured, chorionated eggs that had moved down from their ovaries into the uterus by the seventh day of adulthood.

Table 1 Two-way ANOVA results for the effects of exposure to males and adult age on rates of oogenesis (a) and on yolk protein blood titer (b)

	df	*F*	*P*
(a) Timing of oogenesis			
Whole model	10	1047.78	<0.001
Treatment: male exposure	1	0.03	0.863
Age: day of adulthood	9	1164.19	<0.001
Error	308		
Total	318		
(b) Yolk protein blood titer			
Whole model	19	25.73	<0.001
Treatment: male exposure	1	0.04	0.98
Age: day of adulthood	9	50.06	<0.001
I nteraction: male exposure × age	9	2.19	0.023
Error	264		
Total	283		

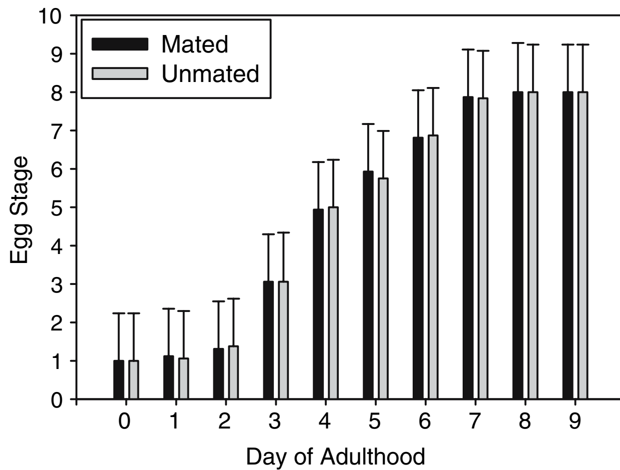

Fig. 2 There was no difference in the timing of the progression of oogenesis in females that were exposed to males and mated or that were not exposed to males and remained unmated. In both treatments yolk began visibly accumulating on day three of adulthood and all females had a full complement of mature, chorionated eggs by the seventh day of adulthood. *Bars* represent one standard error of the mean

There was a clear effect of mating on reproductive investment, wherein females exposed to males produced approximately 10% more eggs than females not exposed to males ($t = 2.31$, $df = 96$, $P = 0.023$, Fig. 3a). Although mated females produced more eggs, neither egg length nor the dry mass of eggs differed between mated females and females which were not exposed to males ($t = -1.02$, $df = 96$, $P = 0.308$, Fig. 3b, and $t = 1.49$, $df = 96$, $P = 0.137$, Fig. 3c, respectively).

Effects of mating on the timing of yolk protein production and peak blood yolk titer

Comparisons of female blood through time with homogenates of mature chorionated eggs revealed three putative yolk protein bands that coincided with the timing of oocyte provisioning (Fig. 4). Mass spectrometric analysis of tryptic fragments from the three putative *S. crassipalpis* yolk protein bands revealed multiple fragments within each band with significant homology to known yolk proteins listed in NCBI from the congeneric flesh fly *S. bullata* [Fig. 5; Hens et al. (2004)]. The blood titer of these three yolk proteins was compared between females exposed to males and females which were not exposed to males to determine whether the timing or magnitude of yolk protein production differed between the treatments. There was a significant interaction between age and exposure to males or not (Table 1b). Females in both treatments had low yolk protein titers, early and late during the oogenic cycle, but females exposed to males had a significantly higher peak yolk protein blood titer only on the

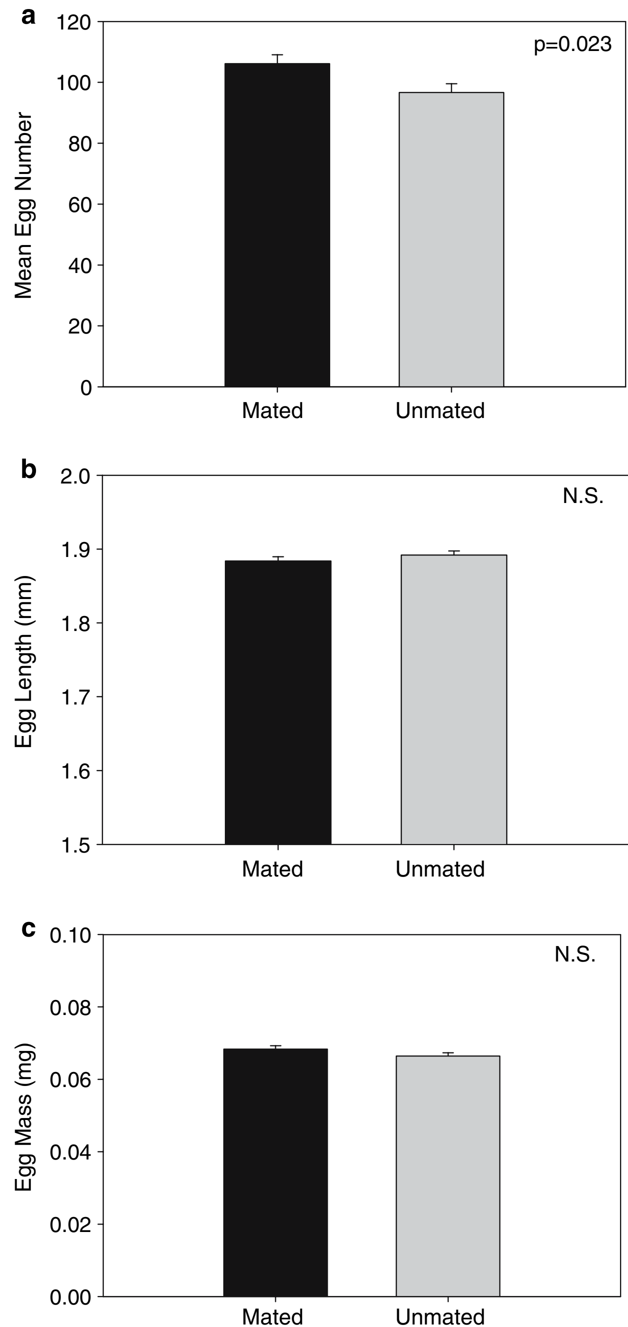

Fig. 3 Mated females produced 10% more eggs than unmated females that were not exposed to males (**a**), but there was no difference in the length (**b**) or mass (**c**) of eggs produced by females in the two treatments. *Bars* represent one standard error of the mean

fourth day of adulthood, which is consistent with the greater reproductive investment of these females into producing more eggs (Fig. 6). Therefore, there was no difference in the timing of the peak titer of yolk proteins in the blood, in the timing of the first appearance of yolk proteins in the blood, or in their disappearance.

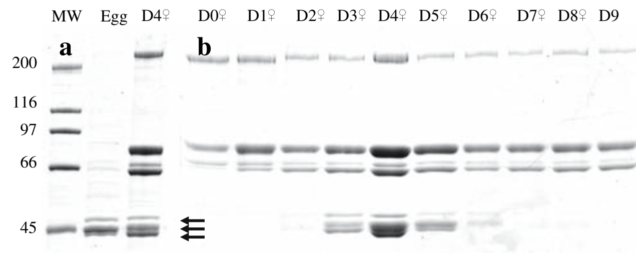

Fig. 4 Eight percent SDS-PAGE gels representing a comparison of homogenized eggs (3 μg protein loaded) and blood from females on the fourth day of adulthood (6 μg protein loaded) (**a**), and a comparison of yolk protein presence in the blood of females from the day of eclosion (day 0) through the ninth day of adulthood (0.2 μl of blood loaded into each lane). Numbers on the *left* denote the mass of the molecular weight markers used and the three *black arrows* on the *right* denote the *Sarcophaga crassipalpis* yolk proteins

Top Yolk Protein Band – 48 kDa
>gi|45685146|gb|AY494776.1| *Sarcophaga bullata* Yolk Protein C –Mowse Score 644
MNPLRTVCLLMGILALASANNPKGPMRWSANSIK**NSLKPTDWLSVSQLESL**
PSMNSVNLKSLEQMPLQEGADLINK**MYHLSQAGEVFQPK**FAPKPRDINCYLI
TPENQKLNFKLNELPRIAEQQNNFGNQEVTIFIAGLPQQTETVKKATR**KLVQA**
YMQRYNGPAPEPLNVKYESASNEQINVGSSEEEWKNGSKKPSGNLVVIEFGN
VLSTANEYTGLDVEQAGIEIGNVLVQLTDKANVPQEIIHVIGSHVGAHVAGAA
GRQFTRQTGHQLRRITGLDPSK**IYAHQSGAVTGLARGDAEFVDAIHTSAYG**
MGTTVRCGDADFYPNGPNEGVPGADNVVEANVRAIRYFAESVVPGNER**NFP**
AVGATSWEEFKQQNGYGKRVYMGINTDYDVEGDFILQVNAKSPFGRSAPVQ
KQQNRRNIHKPWKMSA

Middle Yolk Protein Band – 45 kDa
>gi|45685142|gb|AY494774.1|*Sarcophaga bullata* Yolk Protein A-Mowse Score 374
MTIMNPLRIVCLAALLLVAVNAKQPNSSSQNKLSPSQWLSPSQLQQTPAVDEI
TLQQLENMSMEKGSQLLEQIYHLSQINQDLKPSFVPSPSNVPCYLVKPNGQKV
ATSLDKLASACQQQSNFGNEEVTIFITGMPASQSQSVKKANRKMIEAYLQRYD
NKRQQPQAYEYSGEKMSRTSSEENSNEWQNQQPASGNLVIIDLGDKLNNFKR
FALLDVEQAGAMIGSAIVQMTQKCNVVDETVHVIGQSIAAHVAGAAGNEY
TRQTGRQLRRITALDPSKILAKNAHTLTGLSRGDAEFVDAIHTNVYGMGTIQR
VGDIDFYPNGPSHNLPGAQNVVEASMRATRYFAESVRPGNERNFPAVAANS
LKQYKNNDGLGKRVYMGIDTAYDLEGDYILEVNAKSPFGKRAPVQKQNNYH
GTHNSWKQME

Bottom Yolk Protein Band – 43 kDa
>gi|45685148|gb|AY494777.1|*Sarcophaga bullata* Yolk Protein D- Mowse Score 449
MNPLRILCVVASLLAICAANPTNLHSNAVSGSLKPSNWLTPSELENTPSLDELS
LQKLEQMPLEQGAQLMRKYYHIAQINNDLSPSYVPSPSNIPVYLLQKDGKKQ
AGNLNNLVELAQQQPNFGDEEVTVFITGLPSNTETVKKANRK**LIEAYLQR**NN
HKQQKLQNYKYSEEQTGSRTSSEENSAELKNIK**TNPTSLVIIDLGAALNDVK**
RFAMLNVDETGVMIGKSLVQLSNACDVPQEIIHIVAQGIAAHVAGAAGNEFT
RLTGQKLRRITALDPSKILAKDPKVLSGLSRGDADFVDAIHTSVYGMGTLSRV
GDIDFFPNGPSVGVPGAQNVIEATMRATRYFAESVRPGNEGNFPAVEANSLKQ
YENRDGFGK**RALMGIATDFDLRGDYILQVNPK**SPFGMRTPAMKQNQHNHG
QWPQ

Fig. 5 Peptide fingerprint mapping by QSTAR- tandem MS revealed strong homology to the yolk proteins of the congeneric flesh fly *Sarcophaga bullata*. Peptides within each of the three *S. crassipalpis* yolk proteins that matched the *S. bullata* yolk proteins with significant Mowse Scores are in *bold* and *underlined*

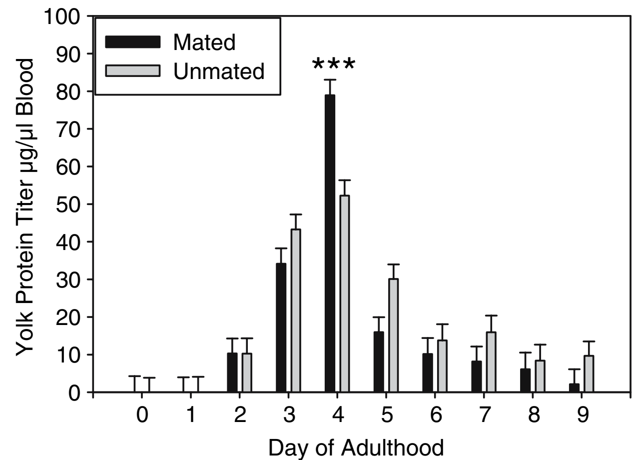

Fig. 6 The blood titer of yolk proteins started almost undetectable, peaked on day four of adulthood, then dropped until they were almost undetectable. The only statistically significant difference in yolk protein blood titer between the two treatments occurred on day four of adulthood when the blood of mated females clearly contained a higher concentration of yolk protein. *Bars* represent one standard error of the mean and the *asterisk* denotes the only day in which yolk protein titer was statistically significantly different between the treatments after applying Tukey's HSD correction for multiple comparisons to the adjusted values from the two-way ANOVA

Discussion

We found that exposure to males and mating did affect the total reproductive investment in the first clutch of eggs in the flesh fly *Sarcophaga crassipalpis*, but there was no effect on the timing of oogenesis. Therefore, virgin females never exposed to males can serve as a model for understanding the physiological and environmental factors that regulate the timing of oogenesis in *S. crassipalpis*.

All females exposed to males in our study were mated between the second and fourth days of adulthood so that exposure to males and mating status were highly temporally correlated, limiting our ability to separate the effects of male exposure, mating, and insemination on female reproductive physiology. Exposure to sexually active males has been shown to have dramatic effects on female physiology and behavior in a variety of vertebrates and invertebrates (Biemont and Jarry 1983; Crews et al. 1986; Bentley et al. 2000; Papaj 2000; Schlupp 2005). This effect of intersexual interaction is particularly apparent in the German cockroach *Blatella germanica* wherein exposure to males or groups of females independent of mating leads to increased juvenile hormone titers promoting vitellogenein production and oogenesis (Schal et al. 1997; Holbrook et al. 2000). However, the vast majority of the effects of mating on female physiology are mediated by substances transferred from the male in both vertebrates and invertebrates (Poiani 2006). Our study does not specifically distinguish which of these factors affect female reproductive physiology in *S. crassipalpis*, only that exposure to males and subsequent mating influences reproductive investment but not timing. Future work will be required to determine the specific mating-associated factors that affect female reproductive investment in *S. crassipalpis*.

Females that were exposed to males and mated produced approximately 10% more mature eggs than virgin females

that were not exposed to males (mean 91 vs. 100 eggs), showing that male exposure and mating plays an important role in a female's reproductive investment strategy. Similar results have been shown in numerous insect species wherein mating with a male or application of male accessory gland substances enhances fecundity. For example, mated females of the tobacco budworm moth, *Heliothis virescens*, produced per day, three times as many eggs as unmated females (~100 vs. 300 eggs, Park and Ramaswamy 1998), mated females of the stink bug *Perillus bioculatus* produced per day, six times as many eggs as unmated females (~20 vs. 130 eggs, Adams 2000), and mated females of *Drosophila melanogaster* produced ten times as many eggs as unmated females (~0.6 vs. 60 eggs, Heifetz et al. 2001). While the ten percent increase in egg production observed in mated *S. crassipalpis* females is not as large as that observed in other insects it is likely a biologically important increase.

While many studies have assessed the effects of male exposure and mating on egg laying and realized fecundity in insects, fewer have directly quantified oocyte development and it is very rare for studies to integrate yolk protein production as a proximate marker of oocyte development in this context. We identified the *S. crassipalpis* yolk proteins by comparing blood samples of oogenic females with the soluble protein component of mature eggs. We identified three predominant proteins ranging approximately 43–48 kDa in molecular weight. Most species of higher Diptera produce three yolk proteins within this size range to provision their eggs. Interestingly, the yolk proteins of higher Diptera are not homologous to the vitellogenins observed in most insect species, which typically include one very large and one smaller subunit as represented in SDS-PAGE, and instead are derived from a group of endogenous lipases (Hagedorn et al. 1998; Hens et al. 2004). Mass spectrometric analyses of tryptic fragments of each of the putative yolk proteins showed that each protein contained three to five fragments with very high identity to the yolk proteins, previously characterized in the congeneric flesh fly *S. bullata* (Fig. 5), and further homology to fragments present in the yolk proteins of other higher flies available in the NCBI database such as the blowfly *Calliphora vicina* and *Drosophila melanogaster* (data not shown). Once we confidently identified the *S. crassipalpis* yolk proteins, we could quantify the blood titer of yolk proteins in females to determine the relationship between organism-level reproductive parameters and their underlying biochemistry.

Increased reproductive allocation in females that were exposed to males and mated requires provisioning of more eggs and increased provisioning was apparent at a biochemical level, wherein females exposed to males had a greater peak blood titer of yolk proteins than females not exposed to males. The titer of egg yolk proteins in the blood is representative of the yolk proteins synthesized in the fat body and transported through the blood to the ovary. Yolk protein blood titer had a clear peak on day four of adulthood in both treatment groups, which agrees well with our data on the progression of oogenesis because the greatest oocyte provisioning occurred between day three and five of adulthood in both treatment groups. Day four of adulthood is the only time when the male-exposed females differed statistically in yolk protein titer from the females not exposed to males, reinforcing that there was no difference in the timing of the occurrence of yolk proteins in the blood between the two treatments. In the salt marsh mosquito *Aedes taeniorhynchus*, mating has been found to affect the production of vitellogenein within a few hours of copulation (Borovsky 1985) however, the total titer of vitellogenin in the blood throughout clutch provisioning was not quantified. In *Drosophila melanogaster*, mating has been shown to increase both the synthesis of yolk proteins and their uptake into the ovary from the blood over many days after mating (Soller et al. 1997, 1999). Because *Drosophila melanogaster* provisions oocytes asynchronously and produces a few eggs every day (i.e., oocytes in different ovarioles are not at the same stage of oogenesis at a given time point), this leads to a prolonged increase in yolk protein titer in the blood for many days after mating despite increased uptake by the ovary. This is in contrast to the discrete difference in yolk protein titer on only a single day of adulthood as observed in *S. crassipalpis*, and is likely due to the contrasting reproductive strategies between the two species (i.e., continuous provisioning of a few oocytes each day in *Drosophila* compared to the highly synchronized provisioning of a large clutch of eggs in *Sarcophaga*).

Although the total number of mature eggs produced was higher for females exposed to males and mated, there was no difference in egg length or dry mass. While egg size responds plastically with respect to maternal environment in some insects, most studies have found that egg size is under strong balancing selection due to effects of offspring size on post-hatching performance and the tradeoff between egg size and egg number (Smith and Fretwell 1974; Capinera 1979; Courtney 1984; Czesak and Fox 2003). Modification of reproductive investment by increasing egg number but not egg size in response to male exposure and mating in *S. crassipalpis* may be indicative of the importance of propagule size in this species. Female flesh flies lay their larvae on carcasses, which represent a spatially and temporally patchy resource that often supports a high density of carrion-feeding insects (Kouki and Hanski 1995). Both intraspecific and interspecific resource competition can be very high within these carcasses and larvae that start out larger may have a competitive advantage (Prinkkila and Hanski 1995). Progeny size has been shown to be

correlated with maternal size in several tropical Sarcophagid species (Tanaka et al. 1990), however females in this study were all approximately the same size.

While mating accelerates oogenesis in many species (Wolfner 2002; Gillott 2003), we found no effect of male exposure and mating on the timing of oogenesis in *S. crassipalpis*. Females that were exposed to males and mated did not display either accelerated egg maturation or any detectable difference in the timing of yolk protein accumulation in the blood. This lack of an effect of mating on timing of oogenesis is consistent with the hypothesis that *S. crassipalpis* females are time limited due to the patchiness of the carrion they oviposit on in the field (Sevenster et al. 1998; Ellers and Jervis 2004). Because carrion is spatially and temporally patchy, and inter and intraspecific competition within a piece of carrion can be intense (Kouki and Hanski 1995; Prinkkila and Hanski 1995), females should provision eggs as quickly as possible so that they are ready to larviposit as soon as they encounter both carrion and mates. Unpredictability of resources for oviposition has been associated with an increased likelihood of ovigeny, eclosing as an adult with all or most of the adult complement of eggs fully provisioned in Lepidoptera and Hymenopteran parasitoids (Jervis et al. 2005; 2007). Testing the hypothesis that the predictability of resource availability for oviposition also plays a role in whether females that mature their eggs after eclosion respond to mating by accelerating egg production will require more comparative work on the effects of mating on the timing of oogenesis across species of insects that differ in the predictability of ovipositional resources.

Acknowledgments This research was supported by funds from USDA-NRI 04-35302-745994 and the Florida State Agricultural Experiment Station to D.H. and a University of Florida Undergraduate Research Scholars Fellowship to M.R. The experiments included in this paper comply with the current laws of the United States of America.

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