Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Insect Physiology 51 (2005) 837–841
Journal of Insect Physiology
www.elsevier.com/locate/jinsphys

Sperm storage and viability in *Photinus* fireflies

Kristian C. Demary*

Biology Department, Tufts University, Medford, MA 02155, USA

Received 27 January 2005; received in revised form 1 April 2005; accepted 2 April 2005

Abstract

In many species females mate with and store sperm from multiple males, and some female insects have evolved multiple compartments for sperm storage. Sperm storage and sperm viability were investigated in two firefly species, *Photinus greeni* and *P. ignitus,* which differ in the morphology of the female reproductive tract. Although the primary spermatheca is similar in both species, *P. greeni* females have an additional, conspicuous outpocketing within the bursa copulatrix whose potential role in sperm storage was investigated in this study. An assay that distinguishes between live and dead sperm was used to examine sperm viability in male seminal vesicles and sperm storage sites within the female reproductive tract. For both *Photinus* species, sperm from male seminal vesicles showed significantly higher viability compared to sperm from the primary spermatheca of single mated females. In single mated *P. greeni* females, sperm taken from the channel outpocketing (secondary spermatheca) showed significantly higher viability compared to sperm from the primary spermatheca. This sperm viability difference was not evident in double mated females. There were no significant differences between *P. greeni* and *P. ignitus* females in the viability of sperm from the primary spermatheca. These studies contribute to our understanding of post-mating processes that may influence paternity success, and suggest that sexual conflict over control of fertilizations may occur in multiply mated firefly females.

Keywords: *Photinus*; Sperm viability; Sperm storage; Sexual conflict

1. Introduction

When females mate with multiple males, differences among mating males in their fertilization success may arise from either post-copulatory female choice (Eberhard, 1996) or through male-mediated sperm competition (Simmons, 2001). Females may control which sperm are used for fertilization by differentially storing or using sperm from different mates, or by creating a competitive environment within their reproductive tract (Hellriegel and Ward, 1998). In a model of sperm storage, Hellriegel and Ward (1998) found that multiple sperm storage compartments within a female's reproductive tract would allow a female to differentially store sperm, and thus bias paternity towards a preferred male. Females potentially can also create a hostile environment for sperm within their reproductive tracts, thus influencing paternity by ensuring that only the most competitive sperm will survive (Simmons, 2001). Bernasconi et al. (2002) found support for this mechanism in *Scathophaga stercoraria,* when they documented lower viability of sperm stored in the females' spermathecae compared to male testes.

Multiple mating may also promote male adaptations to ensure fertilization, including manipulation of sperm viability (Hunter and Birkhead, 2002). Fry and Wilkinson (2004) found in stalk-eye flies, *Cyrtodiopsis whitei,* that seminal fluid and the presence of a selfish genetic element (meiotic drive) influence sperm viability and the number of progeny sired when females mated with two males. Thus, multiple mating can lead to post-copulatory sexual conflict in which different males compete for fertilizations and females attempt to control sperm use for fertilizations (Rice, 1998; Parker and Partridge, 1998).

In many species of *Photinus* fireflies both sexes mate multiply (Lewis and Wang, 1991; Lewis et al., 2004).

*Tel.: +1 617 627 4177; fax: +1 617 627 3805.
E-mail address: kristian.demary@tufts.edu.

0022-1910/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2005.04.001

Photinus males transfer a protein-rich spermatophore to females during mating (van der Reijden et al., 1997) and *Photinus ignitus* females incorporate spermatophore-derived protein into their developing oocytes (Rooney and Lewis, 1999). Because *Photinus* females gain a direct benefit from multiply mating (Rooney and Lewis, 2002), there is a potential for sexual conflict over control of fertilizations. Previous studies of *Photinus* fireflies have examined their courtship and mating behavior (Lloyd, 1966; Rooney and Lewis, 2002; Cratsley and Lewis, 2003), but little attention has been given to how sexual conflict may influence firefly reproductive anatomy and physiology.

This study documents morphological differences in female reproductive tracts between *P. greeni* and *P. ignitus* fireflies and examines how sperm viability differs between male seminal vesicles and various female storage structures. An assay that distinguishes between live and dead sperm was used to examine differences in sperm viability between male seminal vesicles and different sperm storage sites within the female reproductive tract. If female reproductive tracts represent a hostile environment for sperm, sperm viability is predicted to be lower within the female reproductive tract compared to male seminal vesicles. In addition, sperm viability is expected to be lower within double mated compared with single mated females due to inter-male sperm competition.

2. Materials and methods

2.1. Female reproductive morphology

To investigate differences in female reproductive morphology, *P. greeni* ($n = 13$) and *P. ignitus* ($n = 10$) females were collected. Their reproductive tracts were dissected and digitally photographed at 11×. The channel from the primary spermatheca to the common oviduct forms an irregular outpocketing in the bursal wall immediately anterior to the common oviduct. This outpocketing was measured and the size (projected area) of the outpocketing was measured using NIH Image. *P. greeni* and *P. ignitus* are similar in body size. The average elytra length for *P. greeni* is 7.5 ± 0.59 mm and *P. ignitus* is 7.0 ± 0.94 mm; therefore the size of the outpocketing was not scaled to body size. The outpocketing was carefully opened and its contents were examined for the presence or absence of sperm under a dissecting microscope at 11× magnification.

2.2. Sperm viability assay

Sperm viability was measured using the Live/Dead™ sperm viability kit (Molecular Probes), a fluorescence-based assay using two DNA stains that differentiate between live and dead sperm, following methods in Bernasconi et al. (2002). Live sperm are stained green (emission max. 516 nm) with *SYBR-14,* which requires active transport into the nucleus, while dead sperm are stained red by propidium iodide (emission max. 617 nm).

To examine differences in sperm viability between male seminal vesicles and different sperm storage sites within the female reproductive tract, male and female *P. greeni* and *P. ignitus* fireflies were collected at the outset of their respective mating seasons from Lincoln, MA. Females were kept in plastic containers with moist paper towel on a reverse light cycle at 72 °F, and assigned to two mating treatments: *single mated*, mated to a single male, or *double mated,* mated sequentially to two different males with a 24 h intermating interval (females mate only once a night; Lewis and Wang, 1991). Following these assigned matings, females were dissected and sperm viability was measured at 3 days after the first mating, when *P. greeni* females lay the maximum number of eggs (unpublished data). Females were dissected in *Tribolium* saline (Bloch Qazi et al., 1998), and sperm were removed from the primary spermatheca or the channel outpocketing, and placed in 20 μl of saline. This solution was transferred to a slide and stained with the Live/Dead™ sperm viability dyes (5 μl of probidium iodide and 5 μl of 50-fold diluted SYBR-14). Sperm were examined at 40× with an Olympus BX-FLA fluorescent microscope equipped with a triple Chroma 61000, allowing live sperm (green) to be differentiated from dead sperm (red). Because of the small size of the outpocketing in *P. ignitus* females, it was only possible to dissect and assay sperm viability in 5 single mated and 2 double mated *P. ignitus* females.

Inside male seminal vesicles *Photinus* sperm occur in bundles, each containing approximately 100 sperm (van der Reijden et al., 1997). Shortly after transfer to the female, sperm begin to disperse. However, because sperm clump together it was not possible to score individual sperm. Thus, sperm viability was scored as quartiles and analyzed as a categorical variable: category 1 consisted of 0% live sperm, category 2 had 1–25% live sperm, category 3 consisted of 26–50% live sperm, category 4 consisted of 51–75% live sperm, and category 5 consisted of $\geqslant$76% live sperm. To measure sperm viability in the seminal vesicles of 8 *P. greeni* and 7 *P. ignitus* males, sperm viability was estimated as the mid-ranked category for 12–20 bundles.

To compare sperm viability between male seminal vesicles and the female reproductive tract, as well as between species, the number of samples falling into the different sperm viability categories was compared with singly ordered $r \times c$ contingency table analysis using StatXact (Version 4) statistical software.