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Authors: Cock, RaphaΛl De, Faust, Lynn, and Lewis, Sara

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COURTSHIP AND MATING IN *PHAUSIS RETICULATA* (COLEOPTERA: LAMPYRIDAE): MALE FLIGHT BEHAVIORS, FEMALE GLOW DISPLAYS, AND MALE ATTRACTION TO LIGHT TRAPS

RAPHAËL DE COCK[1*], LYNN FAUST[2] AND SARA LEWIS[3]
[1]Evolutionary Ecology Group, University of Antwerp, B-2610 Antwerp, Belgium

[2]11828 Couch Mill Road, Knoxville TN 37932 USA

[3]Department of Biology, Tufts University, Medford MA 02155 USA

*Corresponding author; E-mail: rdecock@hotmail.com

Supplementary material for this article in Florida Entomologist 97(4) (December 2014), including a video of *P. reticulata* mating behavior, is online at http://purl.fcla.edu/fcla/entomologist/browse

ABSTRACT

In contrast to most other North American fireflies that use flash dialogs for courtship, males of *Phausis reticulata* Say (Coleoptera: Lampyridae) (Fender 1966), often called blue ghost fireflies, glow as they fly slowly over the forest floor searching for flightless, neotenic females that are likewise bioluminescent. Recently, these Blue Ghost firefly displays have become increasingly popular as ecotourist attractions. Nevertheless, surprisingly little work has been done on *P. reticulata* courtship and mating behavior, and little is known of female oviposition patterns. Extensive field observations were conducted at 2 locations in Tennessee USA, leading to the description of new categories of male mate-searching search behaviors and nightly display activities. Spectrophotometric measures of bioluminescence were similar in both sexes (λ_{max} = 552 nm). There was a 3-fold variation in female body size, and size was correlated to the number of dorsal photic organs (3 to 9). Field experiments suggested that, in addition to their courtship glows, *P. reticulata* females might also emit a sex pheromone. Males were more likely to approach artificial lures that had a greater number of female-like light spots. Mean copulation duration was 7.9 ± SE 1.3 min and females oviposited an average clutch size of 31 eggs (n = 3; range 27-37). Females guard their eggs and this is described for the first time in any lampyrid. We present photographs of eggs and first instar larvae. These results indicate that the courtship signaling and mating biology of *P. reticulata* is more complex than previously thought, and we suggest future research.

Key Words: reproductive ecology, display activity, bioluminescence, sex pheromones, oviposition, larva

RESUMEN

En contraste con la mayoría de otras luciérnagas norteamericanas que usan diálogos de flash (destellos) para el cortejo, los machos de la luciérnaga fantasma azul, *Phausis reticulata* Say (Fender 1966) (Coleoptera: Lampyridae), brillan mientras que vuelan lentamente sobre el piso del bosque en busca de hembras no voladoras y neotenicas, que son igualmente bioluminiscentes. Recientemente, estas exhibiciones de la luciérnaga fantasma azul se han vuelto cada vez más populares como atracciones ecoturísticas. Sin embargo, muy poco se ha trabajado en el cortejo y el comportamiento del apareamiento de la *Ph. reticulata*, y se sabe poco del patrón de oviposición de las hembras. Varios autores han sugerido que las feromonas podrían jugar un papel en el cortejo, pero no se han realizado estudios experimentales hasta ahora. El objetivo de este estudio fue proveer información adicional sobre la ecología reproductiva de esta especie de luciérnaga enigmática y bastante atípica. Este estudio provee varios nuevos conocimientos sobre el comportamiento del cortejo y de la ecología reproductiva de la luciérnaga fantasma azul norteamericana, *Ph. reticulata*. En base a las observaciones extensas de campo en 2 sitios en Tennessee, EE.UU., describimos varias nuevas categorías del comportamiento de búsqueda en los machos, la actividad noctura de exhibición de los machos y proveemos las primeras mediciones espectrofotométricas de las señales de cortejo bioluminiscentes emitidas por cada sexo (λmax = 552 nm). Se describe una variación de 3 veces en el tamaño del cuerpo de las hembras, que parece estar asociada con las variaciones en el número de puntos luminosos de las hembras (que van de 3 a 9 puntos de luz) y sus patrones. Los resultados de los experimentos de campo que evalúan las señales

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del cortejo de las hembras sugieren la posibilidad de que, además de sus brillos de cortejo, las hembras *Ph. reticulata* también podrían utilizar las feromonas como una señal de cortejo. Además, nuestros experimentos usando señuelos brillantes como los de las hembras sugieren que los machos se acercan preferentemente a los señuelos con más puntos de luz. Por último, proveemos observaciones detalladas de la cópula (el promedio de la duración de la cópula: 7.9 ± 1.3 min SE) y el comportamiento de oviposición de la hembra (el promedio del tamaño del grupo de huevos: 31; n = 3, rango 27-37), que incluye la primera descripción de la hembra de cualquier especie de luciérnaga que vigila los huevos. También fotografiamos los huevos y por primera vez las larvas de primer estadio. Estos resultados indican que la señalización del cortejo y la biología del apareamiento de la *Ph. reticulata* es mucho más enigmática de lo que se pensaba anteriormente y sugieren nuevos caminos para futuras investigaciones.

Palabras Clave: ecología reproductiva, actividad de exhibición, bioluminiscencia, feromonas sexuales, oviposición, larva

Male *Phausis reticulata* Say (Coleoptera: Lampyridae) (Fender 1966) glow as they fly slowly over the forest floor searching for females (Frick-Ruppert & Rosen 2008). In contrast to most other North American fireflies that use precisely-timed flash dialogs for courtship, both sexes of *P. reticulata* emit long-lasting, near continuous glows. In forested regions of the southern Appalachian Mountains, large numbers of males create an eerie glowing display, which gave rise to the widely used nickname of "blue ghost firefly". Watching these blue ghost fireflies during the dual-peak flight seasons, i.e., Apr-May for "early season" and Jun-Jul for "late season (Faust & Weston 2009), has become increasingly popular, with annual tours held at conservationist Don Lewis's "Firefly Forest" in South Carolina (http://upstateforever.org/portfolios/firefly-forest/) and DuPont State Forest in North Carolina (http://www.dupontforest.com) and other sites attracting ecotourists (http://www.yoursmokies.com/firefliesinthesmokies.html).

Previous work described *P. reticulata* morphology (Fender 1966), geographic distribution (Lloyd 2004; Frick-Ruppert & Rosen 2008), and seasonal and nightly activity patterns (Frick-Ruppert & Rosen 2008). *Phausis reticulata* exhibits strong sexual dimorphism: while males have normal wings and can fly, the neotenic females lack both elytra and wings (Lloyd 1971, 1983, 1997a, 1997b, 2004; Cicero 1988; Branham & Wenzel 2003). Because of the specific geographic range and the female's larviform appearance, the species has also been referred to as the "Appalachian glow-worm" (Lloyd 1971; Branham & Wenzel 2003). These flightless females have several spot-like light organs whose glow is visible dorsally through the transparent cuticle (Lloyd 1965; Frick-Ruppert & Rosen 2008). Although such "glow-worm" females are common in Europe, flightless neotenic females are rare among North American fireflies. Another unusual feature of *P. reticulata* biology is that their males produce light, while the males of most other glow-worm fireflies are not luminescent (De Cock 2009).

Surprisingly little work has been done on *P. reticulata* courtship and mating behavior. Frick-Ruppert & Rosen (2008) described courtship behaviors of both sexes and observed a few successful matings. While some authors have suggested that pheromones might play a role in *P. reticulata* courtship (Lloyd 1965, 1997a, 1997b, 2004; Frick-Ruppert & Rosen 2008), no relevant experimental studies have been conducted. In addition, little is known about female oviposition patterns (but see Wing 1988).

The goal of this study was to provide additional insight into the reproductive ecology of this enigmatic and rather atypical firefly species. Working with field populations in Tennessee, USA, we observed *P. reticulata* courtship and copulation behaviors, as well as female oviposition behavior. We also performed controlled field experiments to investigate courtship signals used by *P. reticulata* females, including the relative importance of pheromones and bioluminescent glows. Additionally, we report here for the first time spectral measurements of *P. reticulata* bioluminescent signals, intraspecific variation in female light organ patterns, and detailed descriptions of copulation and oviposition, including female egg guarding.

Materials and Methods

Distribution, Seasonality and Courtship Activity

This study is based on fieldwork conducted during 4-19 Jun 2011 and 15-26 Jun 2013 on *P. reticulata* populations within the Great Smoky Mountains National Park (GSMNP). Co-author Lynn Faust made additional observations over 22 years (1991-2013) in the GSMNP in Sevier and Blount counties and additionally in Morgan, Jefferson and Knox counties, Tennessee, USA. The geographic distribution of *P. reticulata* is centered in the mountains and valleys of southern Appalachia, although small populations have been reported as far west as Texas and Oklahoma (Fender 1966; Lloyd 2004; Frick-Ruppert & Rosen 2008). This species typically displays in

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moist forested habitats, often with small streams or wetlands nearby, and soils overlain by a thick layer of leaf litter (Frick-Ruppert & Rosen 2008; Faust & Weston 2009; Faust 2010). The GSMNP populations that we studied occurred in Appalachian secondary and old-growth oak-hickory forest (475-950 m asl), where they often occur sympatrically with the synchronous firefly, *Photinus carolinus* (Green) (Faust & Weston 2009). We and others have seen *P. reticulata* displays at some high elevation sites within GSMNP (1280-2025 m), where they inhabit open areas with grasses and sedges bounded by dominant trees of red spruce, yellow birch, and rhododendron (Frick-Ruppert & Rosen 2008; A. Mayor, personal communication; A. Bedinger, personal communication). Some lowland populations of *P. reticulata* can also be found on dry forested hilltops and woods and open field margins (LF, personal observation), as well as dry habitats on Chilhowee Mountain in the Appalachian foothills (A. C. Cole Collection, Univ. Tennessee-Knoxville).

Within GSMNP, we focused our studies on the *P. reticulata* mating peak that occurs between mid-Jun and mid-Jul (Lloyd 1965; Frick-Ruppert & Rosen 2008). This is the second of 2 seasonal peaks that have been observed in both lowland (Knoxville area) and GSMNP *P. reticulata* populations (Faust & Weston 2009): an early-season, high-density peak occurs in April-May, followed by a lower density peak that occurs Jun-Jul. It is not currently known whether these constitute a single species or distinct subspecies. Displays begin when *P. reticulata* males start flying and glowing in full darkness at 20:30 h (~40 min after sunset), and courtship activity has ceased by midnight. Male flight activity depends on ambient light levels (fewer males display at full moon), and is curtailed by heavy rainfall or very dry conditions.

Field observations were made nightly from 20:00-24:00 by patrolling ~4 km tracks through *P. reticulata* habitat at several locations in GSMNP: on the west side of Burnt Mountain (at 730 m elevation), near Park Headquarters (475 m), on the north side of Sugarlands Mountain (845 m), and on the NW flank of Mount LeConte (946 m). Civil twilight was between 21:15-21:23 pm EDT, and full moon fell on 15 Jun 2011 and 23 Jun 2013. Air temperatures during male flight periods ranged between 16-25 °C. Additional observations on *P. reticulata* reproductive behavior were made by co-author Lynn Faust during the early and late peaks in Knoxville and the Cumberland mountains, and are also reported here.

The flightless, tiny *P. reticulata* females are difficult to find (Frick-Ruppert & Rosen 2008), but we located glowing females by searching after sunset in shaded areas with thick, moist leaf litter. Females were often found nestled into the leaf litter, although some displaying females were found exposed on top of the leaf litter or up to 20 cm on low vegetation, stumps or fallen branches (Lynn Faust, pers. obs.). We photographed each displaying female (n = 14) with a Sony Cybershot DSC T20 to determine the number, size, and distribution of light organ spots; spot patterns were later transferred to schematized drawing of a *P. reticulata* female. These light spots and their position within the female body can only be determined in live glowing females (Frick-Ruppert & Rosen 2008). It was sometimes difficult to determine light spot position exactly, as some were located at the borders between body segments, and internal organs shift their relation to the exoskeleton during movement. We also estimated female body length, which we categorized as small (≤ 6 mm), medium-large (between 6 and 8 mm) or large (> 8 mm). In 2013, we were able to take photomicrographs that enabled us to measure body size more precisely as the area of each female's pronotum (head covering). We examined whether the number of light spots on females covaried with their body size using Pearson's correlation test.

We conducted our behavioral observations in the laboratory and field using blue-filtered headlamps, as some but not all other firefly species show low sensitivity to blue wavelengths (Buck 1937; Lall & Worthy 2000). Additional behavioral observations in the lab were video recorded under infrared illumination using a Sony Nightshot video camera.

Field Observations of Courtship Behaviors and Tests of Courtship Signals with Live Females

In Jun 2011, we observed *P. reticulata* courtship behavior in the field by placing glowing females into an area where courting males flew in high density. Three females (2 large and 1 small, see Table 1) were individually placed in 9 cm diam petri dishes furnished with moist paper and native leaf litter for shelter. Each petri dish was sealed with fine-mesh fabric to prevent males from reaching and copulating with the females, and each was placed on a 1 m^2 square of dark fabric to facilitate behavioral observations once males had landed.

In Jun 2013, we conducted a field experiment to investigate the courtship signals, including possible pheromones, emitted by *P. reticulata* females. We compared the number of attracted to live field-collected females assigned to 3 treatments: emitting Glow-only (n = 5), emitting Glow + possible Pheromone (n = 6) and emitting possible Pheromone-only (n = 7). In each treatment females were placed individually into cylindrical cardboard containers (6 cm high, 9 cm diam) with moist paper for humidity and local leaf litter for shelter. For the Glow-only treatment (Glow), each container was sealed with an airtight transparent cover that allowed males to see the fe-

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TABLE 1. DETAILS ON *PHAUSIS RETICULATA* FEMALES FOUND IN 2011 AND 2013; INITIAL DATE FOUND, DATE OF DEATH OR RELEASE (25.VI.13), LIGHT SPOT NUMBER, BODY LENGTH AND SIZE CLASS, OVIPOSITION DATE AND CLUTCH SIZE. (/ = INAPPLICABLE OR NO DATA).

Date/time found	Date of death	# Light spots	Length – size class	Oviposition date	Clutch size
04.VI.11 >23:00	14.VI.11	4	6mm - small	/	/
04.VI.11 >23:00	13.VI.11	7	8mm - medium-large	11.VI.2011	infertile (?) clump
06.VI.11 22.30	14.VI.11	3	5mm - small	/	/
06.VI.11 21:30	18.VI.11	7	8mm - medium-large	17.VI.2011	37[#]
07.VI.11 ~22:00	15.VI.11	7	9mm - large	11.VI.2011	infertile (?) clump
09.VI.11 ~22:00	16.VI.11	6	10mm - large	/	/
10.V.13 >22:00*	25.V.11	4	7mm - medium	18.V.2011	27[#]
18.VI.13 >22:00	Released	4	small	/	/
18.VI.13 >22:00	Released	7	medium-large	/	/
18.VI.13 >22:00	Released	9	large	/	/
19.VI.13 >23:20	Released	8	very large	/	/
21.VI.13 23:15	Released	6	medium-large	/	/
21.VI.13 23:15	Released	7	large	/	/
21.VI.13 21:39**	Released with eggs	9	medium-large	24.VI.2013	30[#]
22.VI.13 >23:20	Released	7	large	/	/

*"Early season, Knoxville low-land" female, mating when collected, eggs hatch 24-25 VI.13: 4 larvae died 26.VI.11.
**Mating when collected, remated 21.VI.13 and 23.VI.13.
[#]Showed egg-guarding behavior.

male's glow. For the Pheromone-only treatment (Pheromone), each container was covered with mesh that allowed any possible sex pheromones to disperse yet prevented males from contacting females; these containers were also fitted with cardboard shields that prevented males from seeing the female's glow. For the glow + pheromone treatment (Glow + Pheromone), containers were only covered with mesh, which allowed males to see the female's glow and allowed any possible sex pheromones to disperse. Females were acclimatized to their containers for at least 12 h before the start of experiment. Because female availability was limited (National Park Service regulations strongly limit collection within GSMNP), females were re-assigned to different treatments across several nights.

Tests were conducted over 4 nights. Each night, the females were set out in their natural habitat ~20-30 min before the male flight period began, with each container centered on a 30 × 30 cm dark fabric to facilitate counts of any males that landed near the container (a minimum 2 m distance between containers was used). Based on previous observations of male approach behaviors in 2011, we also marked out an area 1 m in radius around each container that we defined as the "airspace" from which a flying male would be able to see a glowing female.

Each female's container was observed continuously from the beginning of the male flight period at 21:30 until male flight activity ended. Every 10 min during this observation period, we counted the number of *P. reticulata* males in the following categories:

1) airspace males, defined as any male that passed through the 1 m radius around each female, 2) influenced males, defined as any male that altered their flight behavior near females, including direct approaches, sudden turns towards or within the 1 m radius, circling around the female's container, and spotlight behavior (defined below), 3) males that landed on the ground within 1 m radius of the female's container, and 4) males that landed on the female's container. We also recorded the sequence of behaviors shown by males, recording which airspace males landed within 1m, and whether they subsequently landed on the container. We compared the number of males observed within the airspace of females from the 3 experimental treatments and early (< 22:10) vs. late (>22:10) in the flight period using two-way ANOVA.

We considered a female to have successfully attracted a male if any males landed within 1 m or on her container. We compared females' success in attracting males (yes/no) between the 3 treatments using a Freeman-Halton exact contingency table test. For females in the Glow and Glow + Pheromone treatments, we also tested whether a female's attractiveness (measured as the proportion of airspace males that landed) was related to how many light spots she showed using Pearson's correlation test.

During these experiments, we also estimated male flight activity by periodically counting the

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number of males flying (and glowing) every 10 min. We used these data to divide the number of males observed at each 10 min interval by the maximum number counted in any interval on that particular night and location to yield male activity at each time point as a proportion. In this way, we were able to account for variations in male density between nights and locations to estimate temporal patterns of male activity over the entire flight period.

Testing Male Attraction to Glow Signals using Artificial Lures

We conducted field experiments using artificial light lures and traps to investigate whether males would be differentially attracted to different female glow patterns of female light organs. Using such glow lure-trap combinations provides an effective method to study which characteristics of female glow signals are attractive to males (Schwalb 1961). Lure glow patterns can easily be modified to test natural variants of the female signal (e.g. changing number and patterns of light spots etc.), or to test male preferences for different glow colors. When mounted in an appropriate trap design that collects males, the number of trapped males can be used as an estimate for the attractiveness of the glow lure.

Glow lures consisted of reusable lures using Betalights (SRB Technologies, Pembroke Ontario: www.betalight.com), glass tubes in which tritium activates a pigment that provides a constant dim glow closely resembling the light emitted by a *P. reticulata* female. Betalight lures have many advantages. They glow for decades, do not require electricity or batteries, and are weatherproof. We made preliminary tests of Betalights in 2 different colors, yellow (λ_{max} = 574 nm) and green (λ_{max} = 530 nm) but employed the green for further experiments.

Each glow lure was made by inserting a 2 × 25 mm cylindrical Betalight into a 40 mm-long section cut from an opaque black plastic straw that had been pierced with several small holes. We positioned these holes so that the light shining through the straw from the glowing Betalight inside created a pattern that closely resembled the light spot pattern seen in *P. reticulata* females. These lures were mounted inside funnel bottle traps made by cutting off the neck of a 2 liter soda bottle and inverting the upper tapering section onto the base of the bottle, effectively forming a funnel trap (Ineichen 2004; Ineichen & Rüttiman 2012). *Phausis reticulata* seem to avoid landing on shiny surfaces, so we painted both the interior and exterior of the funnels matte dark green. To prevent males from flying out of our funnel traps, we coated the interior of the trap and funnel with Fluon®, an aqueous dispersion of polytetrafluoroethylene (AGC Chemicals, Exton, Pennsylvania: www.agcchem.com), which makes surfaces too slippery for insects to climb. We also added ~1 cm of water to the bottom of each trap. To complete each trap, a green Betalight glow lure was suspended on a thread just under the opening of the funnel.

We used these traps to test 2 specific hypotheses about what characteristics of female glow signals are attractive to *P. reticulata* males. The first hypothesis we tested was whether males were more strongly attracted to lures with a greater number of glow spots. We compared the number of males attracted to traps containing lures with 4-spots versus 8-spots. We added a realistic female body outline by applying a 3 × 10 mm piece of whitish masking tape surrounding the spots. These tests were performed on 22 and 23 Jun 2013 with 12 replicates per treatment.

The second hypothesis we tested was whether males need the pale body outline of females to identify an appropriate mate, in addition to the glow spots. To do this, we compared the number of males attracted to traps containing 8-spotted lures that had a pale, female-like body outline created with masking tape as above vs. control 8-spotted lures with dark body outline. Tests were performed on 24 and 25 Jun 2013 at Elkmont in GSMNP with 12 replicates per treatment. Lure data were analyzed using exact binomial tests and Fisher's exact tests on 2 × 2 contingency tables.

Measuring Induced Glow Behavior and Bioluminescent Emission Spectrum

Many lampyrid larvae and adults glow when they are disturbed, which is thought to serve as an aposematic signal (Sivinski 1981; Underwood et al. 1997; De Cock & Matthysen 1999, 2003; De Cock 2009). We investigated this aspect of *P. reticulata* biology in laboratory studies conducted during Jun 2011 and 2013. Field-collected adults (10 males and 14 females) were separated by sex and kept in small containers provided with moist paper and local leaf litter. To test induced glowing, the fireflies were left covered and undisturbed in a dark room for 10-15 min. We then presented them with stimuli designed to mimic a sequence of increasing threat intensity: nearby noise (rustling of plastic bag), substrate vibration (knocking on table), air currents (opening containers and blowing on them), touching their bodies gently (with a feather), and gently grasping their bodies (with soft forceps). We also made note of any induced glowing behavior that we observed in the field or as a result of handling in the lab.

We measured the bioluminescent emission spectrum of both sexes using a RedTide USB 650 spectrophotometer with a QP600-2-VIS-BX optic probe (Ocean Optics, Dunedin, Florida USA). *P. reticulata* males light organs are located on the

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fifth and sixth ventral sternites; these appear as a whitish-yellow, kidney-shaped area occupying nearly the entire segment (drawing in Lloyd 1965; Frick-Ruppert & Rosen 2008). Males can control their luminescence by glowing from one or both light segments (Frick-Ruppert & Rosen 2008).

Working in a darkened room, males were immobilized by holding them ventral side up on cotton batting then covering each male with fine mesh. This disturbance generally induced glows lasting several s, and the optic probe could be held directly against the glowing light organ. We obtained replicate scans with high signal strength and low noise for 2 males using an integration time of 2 sec and boxcar width of 10: 3 scans (each over 700 counts) were obtained from one male and 2 scans (each over 600 counts) were obtained from the other male. We combined these by standardizing each scan to minimum intensity = 0 and maximum = 1 to obtain an average bioluminescence spectral curve for *P. reticulata* males.

We used similar methods to measure the bioluminescence spectrum of 2 *P. reticulata* females. During the nightly display period when females were glowing, we took measurements by holding the optic probe directly against the female's abdomen. However, because the female glow is quite faint and the light spots are quite small, it was difficult to properly position the 600 µm probe opening over the glow spot(s). To obtain readings we needed to use longer integration times, and there was considerable noise in the measurements. We obtained replicate scans (each over 700 counts) from 2 females: 4 scans were obtained from one female using an integration time of 10 sec and boxcar width of 10, and 2 scans were obtained from another female using an integration time of 20 sec and boxcar width of 10. Again, we combined these female scans by standardizing each (to minimum intensity = 0 and maximum = 1) to obtain an average bioluminescence spectral curve for *P. reticulata* females.

Mating and Oviposition Behaviors

Because so little is known concerning the mating behavior of *P. reticulata* fireflies, we conducted observations in the lab on mating interactions and investigated the possibility of female remating. Mating observations were initially performed in darkness, but we found that males and females would also readily mate under daylight conditions. In Jun 2013, 7 *P. reticulata* females were each placed in a petri dish with wild-caught males. Behavioral interactions were video-recorded, and copulation durations measured with a stopwatch. Mating observations were made on large vs. small *P. reticulata* females (2 each) in Jun 2011; females were acclimatized for 30 min in a dark room before males were introduced. We checked every 5 min to determine when copulation occurred.

To check whether females would remate, a female that we found mating in the field on 23 Jun 2013 was placed in a container with a different wild-caught male. We also recorded mating and egg-laying behaviors exhibited by early-season females from Knoxville (early Jun 2011 and May 2013). Lastly, a late-season *P. reticulata* female from GSMNP was mated in captivity with a Knoxville male.

Some of the data on female glow activity during glow and pheromone experiments in the field, as well as colored versions of the Figs. 2, 3, 4, 8 and 9 can be seen online in Florida Entomologist 97(3) (September 2014) at http://purl.fcla.edu/fcla/entomologist/browse.

Results

Field Observations of Courtship Behaviors

Male Search Behaviors

In the late-season *P. reticulata* populations that we studied in GSMNP, estimated peak male densities were ~10 males per 100 m^2. Males began flying and glowing in search of females at 30 min after sunset, near the end of civil twilight (21:30 EDT, Fig. 1). Male display activity quickly rose to a maximum by 21:30-21:50, after which activity declined sharply. We saw a small but noticeable secondary rise in male display activity near 23:00 (Fig. 1). After 23:10, only a few males were displaying, and almost none were seen flying by midnight. At the beginning of their flight period, *P. reticulata* males flew only a few centimeters above the forest floor, rising to fly 20-50 cm above the ground later at night. Males typically flew slowly in meandering paths, but sometimes males flew higher (50 cm-1m) and more quickly (10 cm per sec).

Based on our observations of hundreds of male search flights, we described the following categories of male search behavior: *Patrol flight* – groups of 2-5 males travel in parallel flight paths, moving a single direction (often through open areas). *Zooming* – males fly high and fast in a straight path. Late-flying males (after 22:00) often showed this behavior when flying along open roads or paths. *Spotlighting* – when males fly low (< 20 cm above the substrate), their glowing light organ casts a greenish circle onto the ground. These eerie dim lights are most apparent to human observers when males fly over level roads or paths. Low-flying males may use this reflected light for altitude control, or to avoid crashing into understory vegetation. *Jitterbugging* – males hover low over a single spot and fly within a single horizontal plane using small, jerky movements. After jitterbugging, males sometimes land and then

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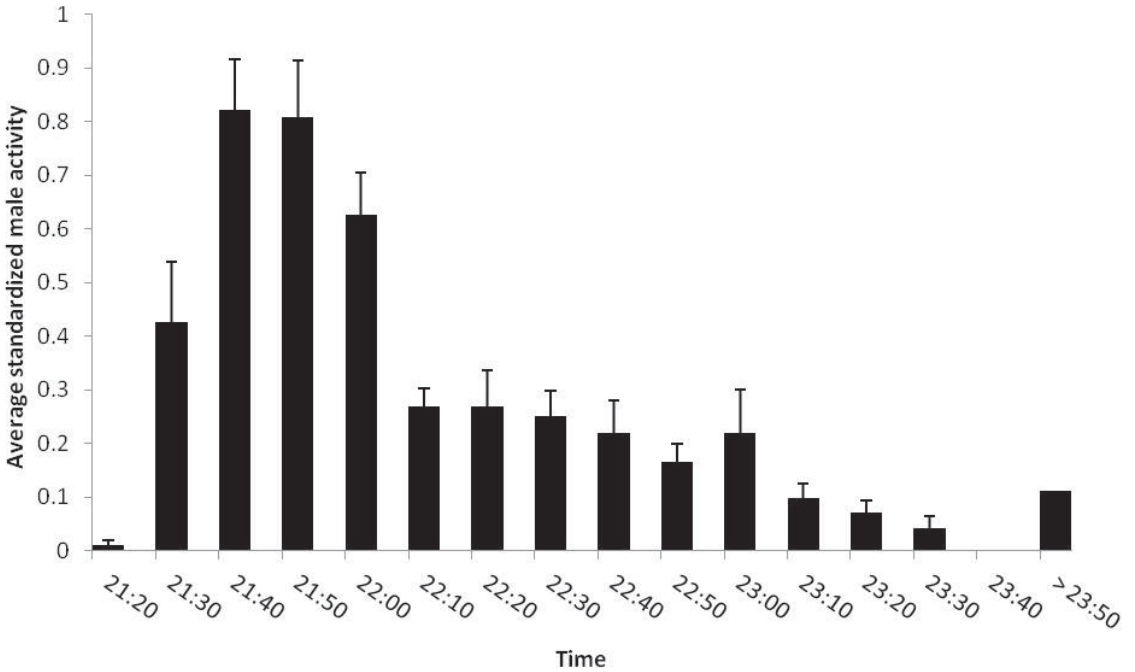


Fig. 1. Nightly flight activity of *Phausis reticulata* males, measured at 10 min intervals by counting glows from flying males. Counts were standardized to maximum interval count observed at each night and location, then averaged (n = 7, bars show mean + 1 SE).

take flight again. This behavior was often observed near our experimental females, and may allow males to locate partially hidden females by changing their visual perspective. *Elevator flight* – males fly up and down in a vertical plane, covering up to 60 cm. We often saw groups of 2 to 5 males exhibiting this behavior. Again, this may allow males to locate females by changing their perspective. *Hopping* – males fly in short (< 20 cm) hops along the ground or through vegetation, repeatedly landing and taking flight again. Males were often observed hopping in dense vegetation or as they approached a glowing female. *Magnet flight* – males fly directly toward a female from far away (3 to 6 m). We observed this behavior among late-flying males in response to experimental females. This could represent male response to a pheromonal signal released by females, as female glows do not appear to be detected by males at these distances.

We observed *P. reticulata* males in the field that were clearly attracted to dimly glowing light sources, such as small dots of phosphorescent paint, and small Betalights, but not to stronger light sources, such as LEDs or 9V lightbulbs. As males flew over, they would often circle back to inspect these fainter light sources. Males flew around these glows for up to 15 sec at ~ 20 cm height, moving in small circles or spirals ~10 to 80 cm in diameter, often while jitterbugging and spotlighting, often spending up to 10 or 15 s in close vicinity. Some males eventually approached quite closely, and then dropped directly onto or within 2 cm of the glowing source. In the course of our study we had opportunities to observe males' reactions to many different types of glow sources and males appeared more likely to inspect and land on lures that more closely resembled *P. reticulata* females (e.g. female-sized pattern of several light spots). These observations suggested that males could not detect female-like glows beyond 1 m distance. On one occasion we noticed that males would only approach and inspect but not land at a female-like glow pattern made with phosphorescent paint. However once the glow lure was partly covered with some leaves, 4 males landed directly on the light source within 2 min. These approaches and landings only occurred during peak male activity (21:45-22:00); later males seemed to ignore this glow lure.

We observed many instances of spider predation on *P. reticulata* males by orb weavers, sheet web weavers and even a black widow (*Latrodectus mactans* Fabricius 1775). On one occasion, 5 males were trapped in a single web. During one night of peak display in the early season Knox County population (7th May 2013), 13 of 143 *P. reticulata* censused by their glows turned out to be males caught in spider webs. Glows emitted by trapped males seemed to attract flying males, which sometimes also become ensnared.

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Female Display Behavior

Phausis reticulata females displayed by glowing from on top of leaf litter or soil, and some were found up to 20 cm on low vegetation. Most females commenced their display near the beginning of the males' flight period, though some females began later. When they failed to mate, females continued glowing after peak male flight activity. Females that had already mated in the lab left their shelters after sunset, climbing to a fixed position and assumed the typical display glow posture on subsequent nights, suggesting that females will mate more than once. Based on our observations of the same experimental females over several nights, it appears that females initially began displaying around the time of peak male activity or later, but on subsequent nights these unmated females often began displaying even before males had started flying. At one field site, we found a glowing female (with 7 light spots) positioned at the sloped edge of a forest path; although she appeared highly visible to us, she failed to attract any males even though she displayed continuously from 22:00 until 23:15 when we left the site.

Female body size was highly variable, and we found a 3-fold difference in size measured as pronotal area (Fig. 2a, b). Larger females often showed highly distended abdomens that were filled with white or yellowish eggs clearly visible through the dorsal cuticle (Fig. 2c).

Previous studies have reported 4 to 6 light spots in *P. reticulata* females (Lloyd 1965; Frick-Ruppert & Rosen 2008), but our study found this number to range between 3 and 9 spots (Fig. 3), and these were visible both dorsally and ventrally. We monitored spot patterns for females kept in captivity, and these maintained the same light spot pattern throughout our study. However, light spot position was highly variable among females (Fig. 4); only 3 of 14 females showed fully symmetrical arrangements (2 with 4 spots, one with 6 spots; Fig. 4a, f), while the remainder were asymmetrical. Such asymmetry manifests either as different numbers of spots on each side or as differences in light spot positions within a body segment.

Female light spot pattern varied with body size. The smallest females usually showed a pattern of 4 light spots (e.g. female shown in Fig. 4a, b, c): 2 spots were located on abdominal segment 1, and 2 spots located on either segment 6 or 7. The general pattern in larger females was a pair of spots in the first 2 abdominal segments, with more (usually smaller) spots positioned in other abdominal segments (e.g. females shown in Figs. 3 and 4). In 2013 we measured female body size as pronotal area, and found this was positively correlated with the number of light spots (Fig. 5).

Tests of Courtship Signals with Live Females

We conducted a field experiment to investigate the courtship signals used by *P. reticulata* females by comparing how many males were attracted to live *P. reticulata* females assigned to 3 treatments: emitting Glow-only (n = 5), emitting Glow + possible Pheromone (n = 6) and emitting possible Pheromone-only (n = 7). In this experiment, the number of males we observed flying through the 1 m radius airspace around each female on a given night (Fig. 6a) did not differ significantly among treatments (two-way ANOVA, treatment $F_{(2, 32)} = 1.92$, $P = 0.164$). There was a marginally significant decline in the number of airspace males during the late (> 22:10 h) vs. early (< 22:10 h) flight period (time $F_{(1,32)} = 4.00$, $P = 0.054$), which was concordant with declining male activity during the late flight period. There was no significant treatment * time interaction ($F_{(2, 32)} = 1.50$, $P = 0.239$). For this experiment, we considered a female to be successful in attracting a male passing through her airspace if he subsequently landed either directly on or within a 1 m radius of her container (Fig. 6b). The proportion of airspace males that landed was highest for females in the Glow + Pheromone treatment, followed by Glow females and then Pheromone females. Glow and Glow + Pheromone treatments show about similar proportions if early and late airspace males are combined, while the Pheromone treatment clearly shows lowest proportions in such a consideration.

Across all treatments, female success increased over the flight period. As indicated by the wide error bars in Fig. 6b, we saw considerable variation among females within treatments. For example, the most successful female was a large individual with 7 light spots (Glow treatment): she attracted 28 males into her airspace, and 20 of these males subsequently landed within 1 m of her, and 16 of these (thus 57% of airspace males) eventually landed on her container. In another trial she attracted 1 male that landed directly on her container. Yet other brightly glowing females in the Glow treatment only had a single airspace male landing within 1m, and no other females attracted males directly to their containers.

Although relatively few males landed near females in the Pheromone (no glow visible) treatment, we observed 3 males that had been flying as high as 1 m above the female drop directly down onto these females' containers, and 3 additional males landed within the 1m perimeter. Additional males were observed approaching these Pheromone females from up to 3 m away, some showing magnet flight and others approaching by jitterbugging and eventually landing nearby.

We also considered the attraction success for each test female, i.e. whether any males landed near her on a given night. We observed that 57%

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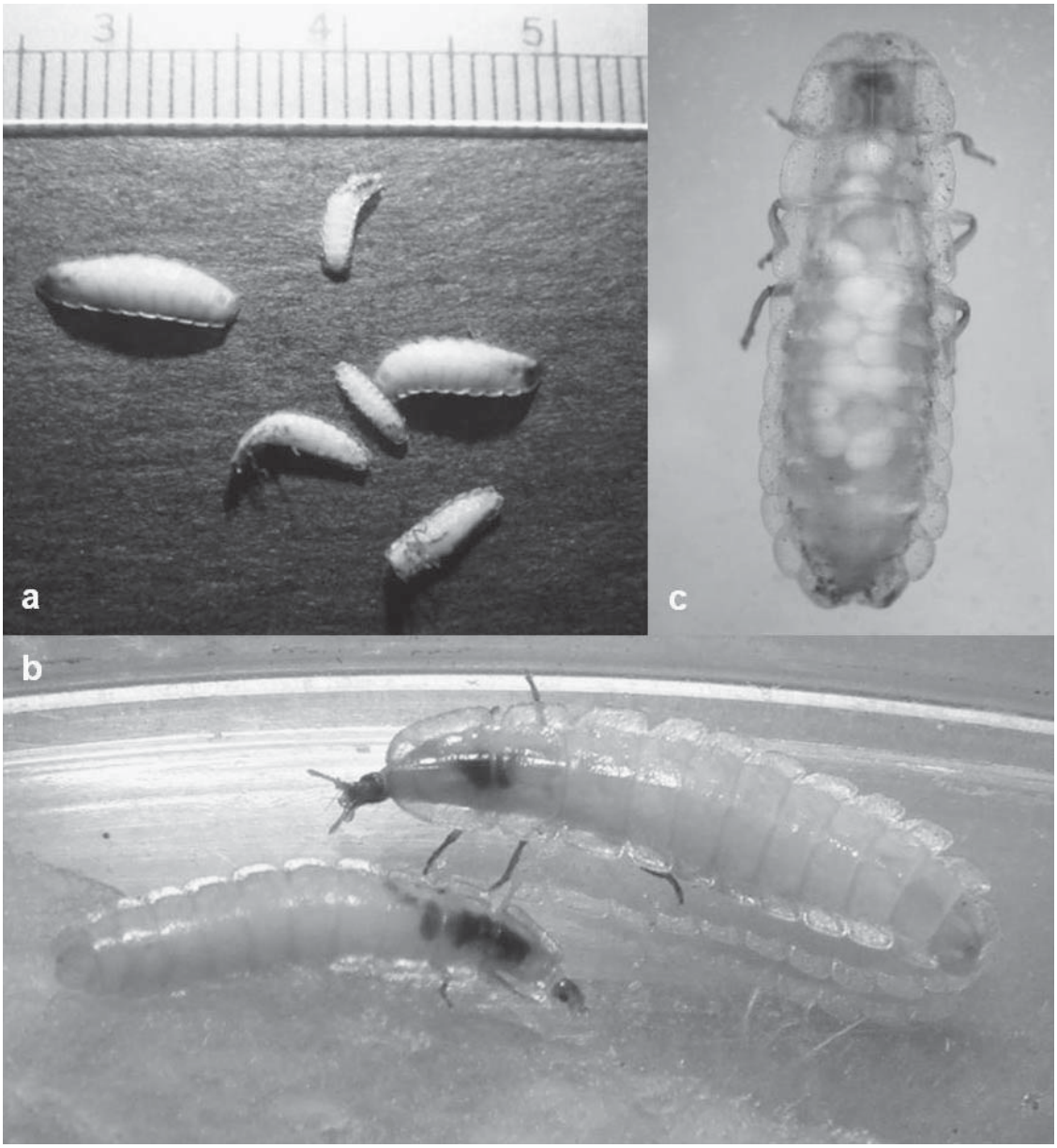


Fig. 2. Size variation among *Phausis reticulata* females (a, b). Female with eggs visible through the transparent dorsal cuticle (c). (Photos a, b: R. De Cock; photo c: L. Faust). A colored version of this figure can be seen online in Florida Entomologist 97(4) (December 2014) at http://purl.fcla.edu/fcla/entomologist/browse .

of females in the 7 Pheromone trials successfully attracted males, compared to 60% of those in the 5 Glow trials, and 100% of females in Glow + Pheromone treatment. However, these proportions were not significantly different across the 3 treatments (Freeman-Halton exact test of 2 × 3 contingency, two-tailed $P = 0.2$). Additionally, we noticed only 2 out of 5 females in the Glow treatment attracted any males after 22:10, whereas 5 out of 6 females in the Glow + Pheromone treatment still successfully attracted males after 22:10, thus after peak male activity. In the Pheromone treatment, 2 out of 7 females attracted males during peak male activity (before 22:10), and 2 other females were successful after 22:10, thus after peak male activity.

We tested whether a female's attractiveness (measured as the proportion of airspace males that landed) was related to how many light spots she showed, using only data from treatments

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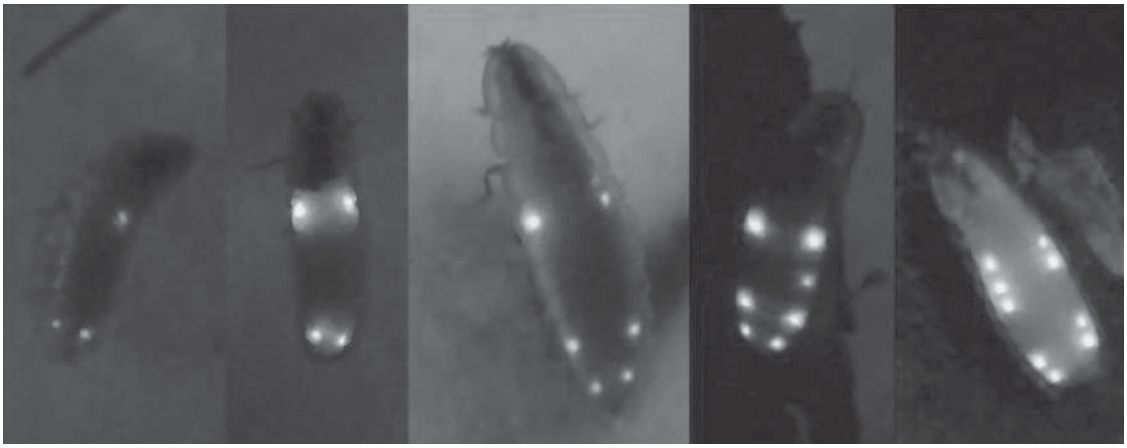


Fig. 3. Variation among *Phausis reticulata* females in the number, size and position of light spots used in courtship displays. (Photos: R. De Cock.). A colored version of this figure can be seen online in Florida Entomologist 97(4) (December 2014) at http://purl.fcla.edu/fcla/entomologist/browse .

with visible glow signals (thus Glow and Glow + Pheromone). We found only a weak correlation between the number of female light spots and her attractiveness (n = 10, Pearson r = 0.39, P = 0.3) that explained about 15% of the variation.

During many hours of observation, we made additional notes on male flight behaviors outside and inside the female perimeter which give extra anecdotal clues about the possible presence of female pheromones compared to the importance of female glows (supplied in Supplementary Results).

Testing Male Attraction to Glow Signals Using Glow Lures

The first hypothesis we tested using the glow lure trap method was whether *P. reticulata* males would be more strongly attracted to lures with a greater number of glow spots. A total of 20 males were attracted to and captured in traps baited with an 8-spot lure, while only 5 males were captured with 4-spot lures. Treating each of these 25 males captured as an independent trial, males appear to be preferentially attracted to 8-spot rather than 4-spot lures (binomial test of equal probability that a male will enter either trap type, P = 0.002). However, the assumption of independent trials may be violated if the glows emitted by trapped males attracted additional males into traps. Thus, we re-analyzed these data considering only whether or not each trap had captured any males (rather than the number of males). This more conservative approach showed that 8 out of 12 8-spot lures captured males (66% capture success) compared to 4 out of 12 4-spot lures (33% capture success); although the difference in capture success was in the predicted direction, this was not significant (Fisher's exact test of 2 × 2 contingency table, P = 0.2).

The second hypothesis we tested using this method was whether the pale body color of *P. reticulata* females, in addition to their glow, might help males to locate mates. Males hovering above females and spotlighting might be able to use the reflection from the whitish body to distinguish females from other glowing objects, such as other males trapped in spider webs, fungus gnats or other firefly species. We found that 13 males were attracted to and captured in traps baited with pale-colored 8-spot lures resembling females, while 11 males were caught with dark-colored 8-spot lures (n = 12 traps each). Treating each of the 24 males captured as an independent trial, males were not preferentially attracted to the pale lures (exact binomial test of no preference, P = 0.27). Similarly, we found no significant difference in capture success between pale-colored lures (75%) and dark-colored lures (34%: Fisher's exact test of 2 × 2 contingency table, P = 0.2).

Bioluminescent Emission Spectrum

Although flying *P. reticulata* males appear to emit a bluish glow when viewed from a distance or from above (hence the name "blue ghosts"), when viewed directly and close-by, the light from their lantern appears yellowish-green. When viewed from certain angles, flying males give the impression of emitting a slightly modulated flickering glow. When flying males are viewed from below however, they emit a steady glow.

Spectrophotometer measurements confirm the lime-green color of male *P. reticulata* bioluminescence (Fig. 7), as the emission spectrum shows a maximum at 552 nm. The 50% spectral

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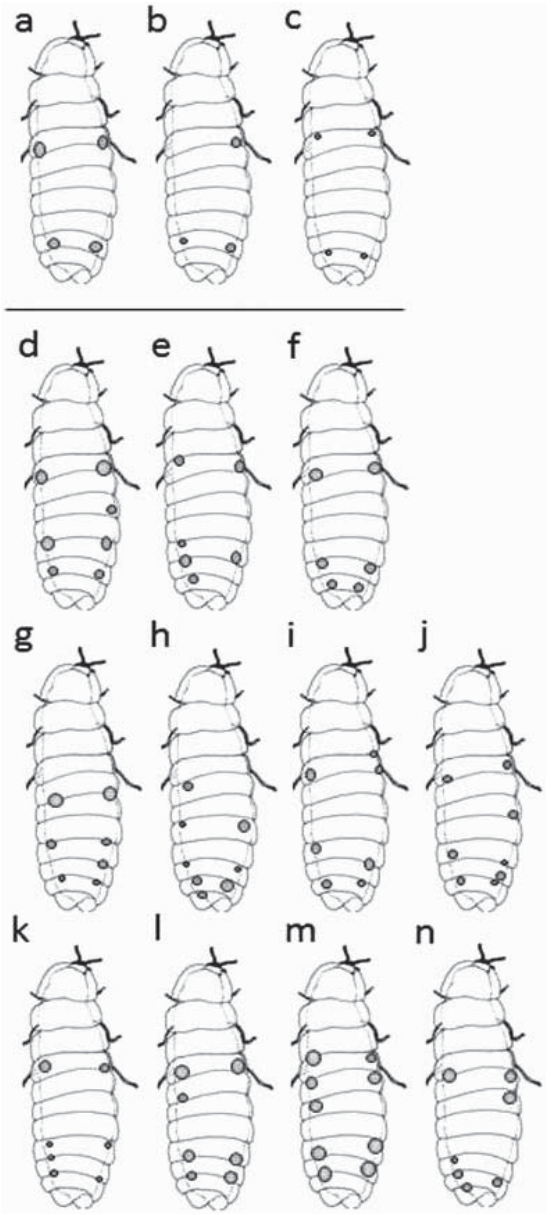


Fig. 4. Schematics showing variation and asymmetry in light spot patterns among 14 "late season" female *Phausis reticulata* observed in Jun 2011 and Jun 2013from the GSMNP. Female body margins visible through transparent broader tergites are indicated by a dotted line. Small type females 5-6mm (a- c) bear 3 to 4 light organs, while large type females 8-12mm (d-n) showed 6 up to 9 lanterns. A colored version of this figure can be seen online in Florida Entomologist 97(4) (December 2014) at http://purl.fcla.edu/fcla/entomologist/browse .

bandwidth spans 61 nm and lies between 525 nm (blue-green) and 586 nm (yellow-orange). The 10% spectral bandwidth (or the base of the spectral curve) spans 154 nm and lies between 500 nm (turquoise green) and 654 nm (bright poppy red). Although females were difficult to measure due to their much weaker glows and tiny light spots, the overall shape of the female spectrum closely resembles that of males (Fig. 7). The maximal wavelength of female bioluminescence lies at 547 nm, close to the male maximum. The 50% spectral bandwidth spans 63 nm and lies between 523 nm and 586 nm, which closely matches that of males. The 10% spectral bandwidth spans 237 nm and lies between 497 and 736 nm, although female spectra were quite noisy at wavelengths above 620 nm.

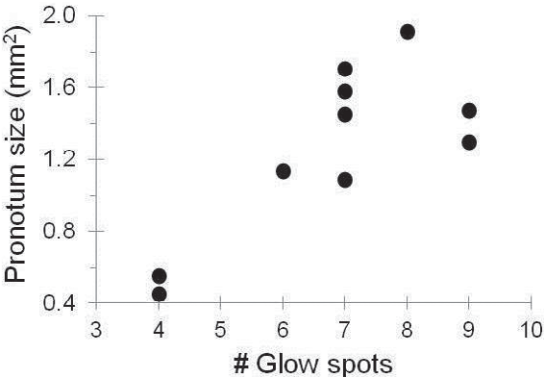


Fig. 5. The relationship between female body size measured as pronotal area and the number of glow spots observed in *Phausis reticulata* females in Jun 2013. Female body size, and number of light spots show a positive Pearson correlation (n = 10, Pearson r = 0.78, P = 0.007).

Induced Glow Behavior

When disturbed or threatened, in 20% of cases (50 trials: 10 males tested 5 times) *P. reticulata* males exhibited induced glow behavior. When lightly touched most of the responsive males lit both photic organs, though others glowed only from one of both. The response did not seem to be shown consistently within the same individual but happened more randomly. When presented with threatening stimuli, males often feigned death (thanatosis) by becoming immobile and depressing their antennae. One male showed thanatosis at first touch, but glowed in reaction to subsequent touch.

Induced glow in response to disturbance was observed in 59% of 14 *P. reticulata* females tested: 31% of the females glowed in response to substrate vibration and 28% glowed only when touched. These induced glow responses varied between differently sized females. Compared to small females, large females responded more frequently (41% vs. 18% of 22 trials), responded

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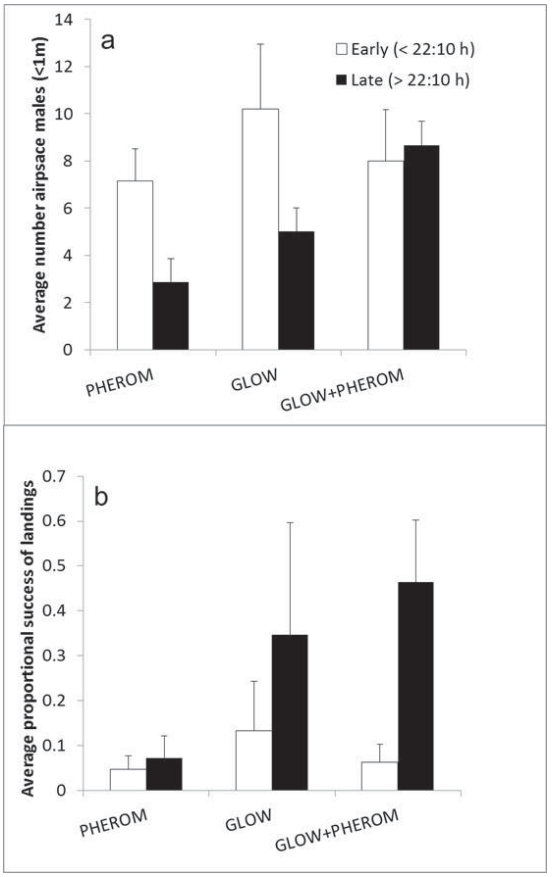


Fig. 6. a. Number of *Phausis reticulata* males that flew through the airspace (within 1 m radius) of experimental females assigned to 3 treatments (mean + 1 SE); PHEROM (n = 7; female emitting possible pheromone, no glow), GLOW (n = 5; female emitting glow, no pheromone), and GLOW+PHEROM (n = 6; female emitting glow and possible pheromone). Counts are shown divided between early flight period (before 22:10) and late flight period (after 22:10). b. Proportion of these airspace males that landed directly on or within 1 m of females (mean + 1 SE) in the same 3 treatments and time periods.

more quickly (<1 sec vs. 4-5 sec), and had longer glow durations (5 – 30 sec vs. < 5 sec.). Yet when they were disturbed repeatedly, the smaller females responded more quickly and with longer glow durations. Large females glowed more frequently in response to substrate vibrations: this included one ovipositing female and one female that was guarding eggs. Both large and small females glowed in response to touch (14%). Females that were glowing gradually stopped when they were disturbed by bright light or threatening touch. We did not observe any females turning on their glow in response to males flying overhead, a behavior reported by Lloyd (1965).

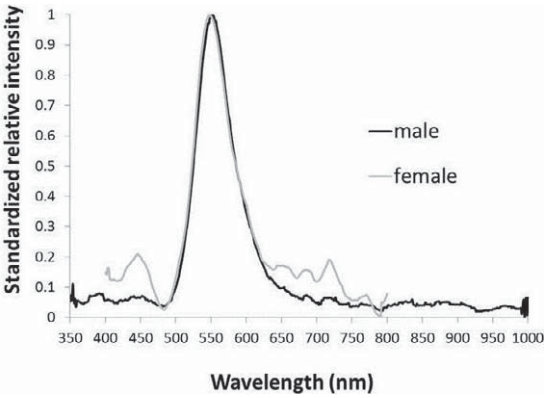


Fig. 7. Bioluminescence spectra for adult *Phausis reticulata.*

Mating and Oviposition Behavioral Observations

We observed *P. reticulata* mating behavior both in the wild and in the laboratory. When placed together with a female, males typically approached, antennated, and climbed onto the female's back. When multiple males were placed together with a single female, males sometimes competed by butting into one another with their pronotum and flipping other males away. Females seemed to be able to exert some choice over their mating partners by tucking under their terminal segments and moving away from certain males. Intromission began in the dorsal mount position (Fig. 8), which lasted ~ 1 min before the male rotated 180 degrees to continue copulation in the tail-to-tail position (see video in Florida Entomologist 97(4) (December 2014) online at http://purl.fcla.edu/fcla/entomologist/browse). Copulation durations ranged from 5 min 30 s to 10 min, with a mean (± 1 SE) duration of 7.9 ± 1.3 min (n = 5 pairs). Males did not glow during copulation, and under natural conditions female glows were gradually extinguished. Copulations ended subtly; as the male disengaged, he often remained near the female for several min. Two females that had already mated in the wild were observed to remate with new males in the lab.

In 13 mating trials conducted in captivity, 7 females were ignored by males even after they contacted and antennated the female. This occurred in all trials with small females that had 4 or less glow spots. In these trials the females kept crawling and males showed no particular interest in them, even up to 47 min after introduction. Also during a trial with a large female and 2 males, the female kept crawling slowly but elicited no response from either male even after antennal contact. None of the females glowed during mating experiments conducted in captivity. For comparison, Frick-Ruppert & Rosen (2008) observed

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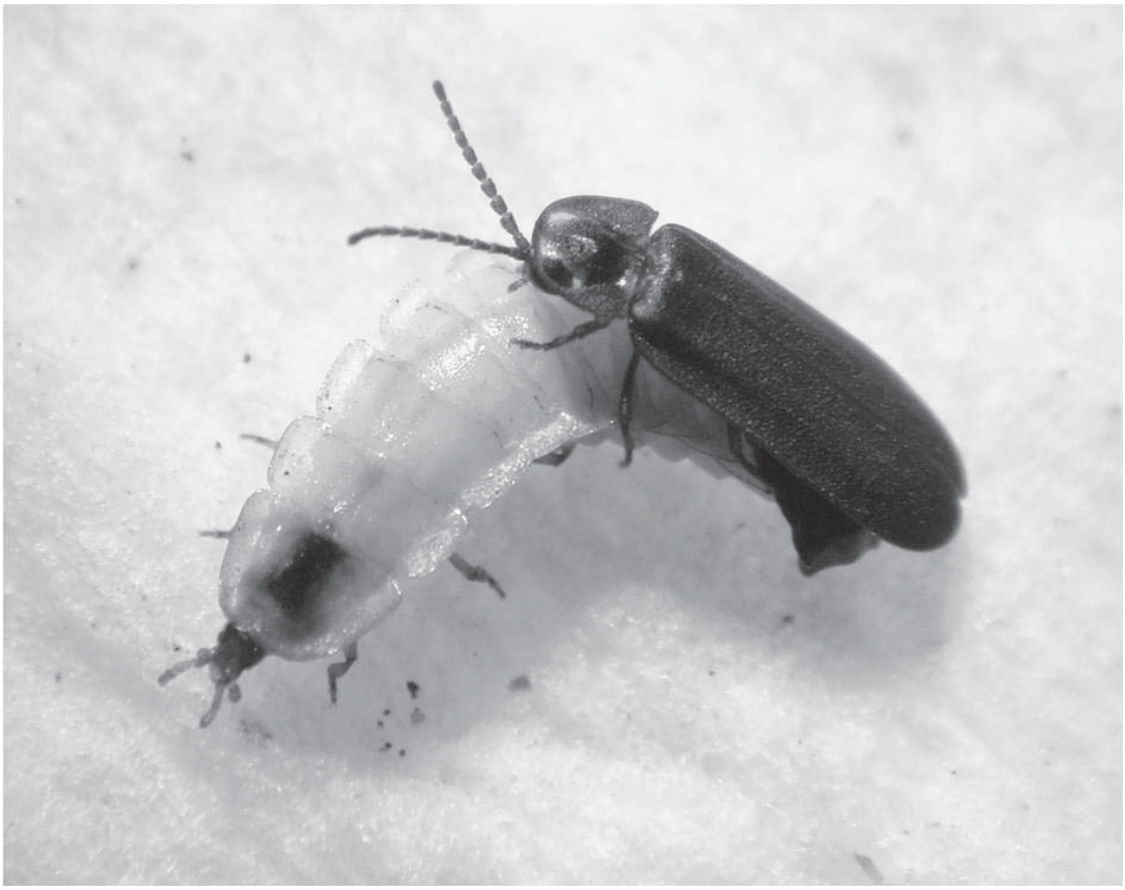


Fig. 8. Pair of *Phausis reticulata* fireflies in the initial dorsal mount phase of copulation (male positioned above female). (Photo: R. De Cock). A colored version of this figure can be seen online in Florida Entomologist 97(4) (December 2014) at http://purl.fcla.edu/fcla/entomologist/browse .

3 matings under fully natural conditions of which 2 were successful. Yet, they also report 2 successful matings in 3 trials when males were placed near dark females during manipulated mating experiments in the field.

Mated *P. reticulata* females laid their eggs singly and in groups (Fig. 9a) over a period of several hours, with a mean clutch size of 31 eggs (n = 3; range 27 to 37 eggs). Freshly laid eggs were oval, yellow-tan in color, and about 0.7 mm long. After ovipositing, females (n = 4) curled their bodies tightly around their eggs (Fig. 9b), using one or more legs to grasp them. Once they had oviposited, females rejected any additional advances from males and continued their egg-guarding behavior. When we gently disturbed these females, they briefly abandoned their clutch to move a short distance away; they then returned and resumed a position tightly clutching their eggs. After being threatened once daily, one 2013 female returned to grasp her clutch 6 days in a row, until the day of her death. When eggs were misted every few days and kept at natural temperature and photoperiod, 30% of one clutch that contained 27 eggs took 34-35 days to hatch. Newly hatched first instar larvae were unpigmented and ~1.5 mm in length (Fig. 9c).

Discussion

Courtship Signals and Behaviors: Glows and Pheromones

Despite its common name of blue ghost firefly, our spectrophotometric measurements demonstrate that bioluminescence of both sexes is yellow-green, peaking at around 552 nm. This glow color closely resembles that reported for other glow-worm fireflies: 550 –547 nm in *L. noctiluca* (Sala-Newby et al. 1996; De Cock 2004), 550 nm in *Lamprohiza splendidula* (Schwalb 1961) and Asian species *Pyrocoelia miyako* (refs. see: Day 2009). Glow-worm fireflies, which are characterized by flightless, neotenic females that emit con-

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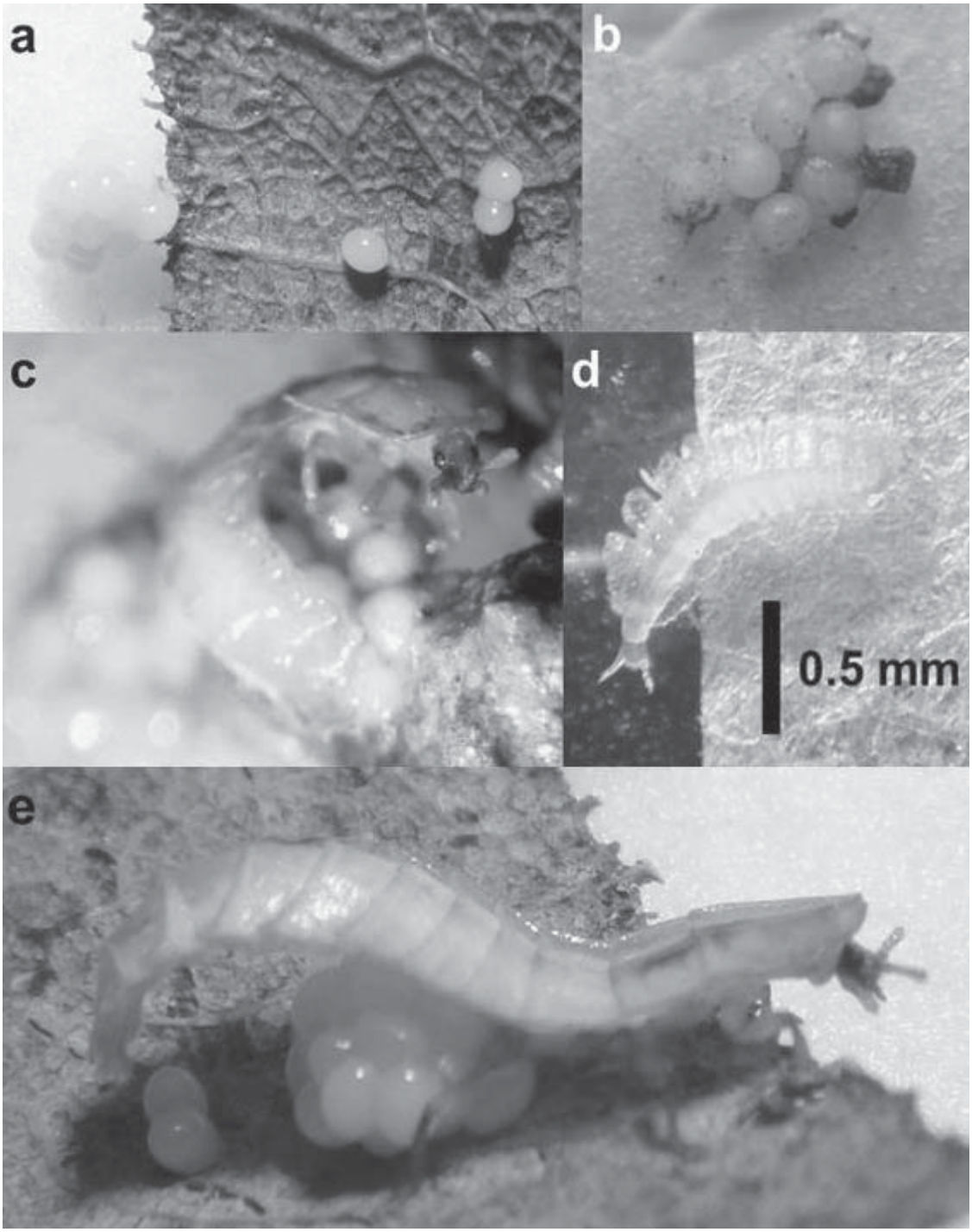


Fig. 9. *Phausis reticulata* oviposition, egg-guarding behavior, and newly hatched larva: a) Freshly laid softer, slightly oval eggs; b) round, more rigid eggs ready to eclose; c) and e) females guarding eggs 2012, 2011; d) Newly eclosed larva. (Photos a, d, e: R. De Cock; photos b, c: L. Faust). A colored version of this figure can be seen online in Florida Entomologist 97(4) (December 2014) at http://purl.fcla.edu/fcla/entomologist/browse .

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tinuous light to attract flying males, are unusual in North America (Lloyd 1997b; Branham & Wenzel 2003). Surprisingly, the yellow to amber bioluminescence colors typically seen in many flashing species (*Pyractomena* spp., *Photinus* spp., *Photuris* spp. (Day 2009; Lall et al. 2009) and Luciolinae (Day 2009) have not been reported from any glow-worm firefly species. Potential advantages of this wavelength may be that lime-green glows of the sedentary females is best enhanced by reflection off the (equally green) surrounding vegetation, and that glow-worm fireflies start activity well after sunset and thus avoid signal-to-noise issues from vegetated surroundings (Lall et al. 2009).

Yiu (2013) distinguished 2 signaling systems used by glow-worm fireflies: either both sexes are luminescent or males are entirely non-luminescent or are only faintly luminous (usually from larval lanterns and only glowing on disturbance). In *P. reticulata* both sexes are luminous with the males having specialized adult light organs. This characteristic is shared with *Lamprohiza splendidula* (Linné, 1767) from Central Europe (Schwalb 1961; De Cock 2009) and several Asian *Diaphanes* and *Pyrocoelia* species (Chen 2003; Ohba 2004; Yiu 2012, 2013; Wong 2013), South American *Cratomorphus* spp. and *Magnoculus* spp. from Brazil (Viviani 2012). *P. reticulata* closely resembles European *L. splendidula* sharing similar habitats, behaviors, habitats and ecology. Notably, females of both species show an unusual arrangement of light organs for lampyrids (Lloyd 1997b; Branham & Wenzel 2003): small glow spots spread laterally over the abdomen and visible from both ventral and dorsal sides. This glow spot arrangement resembles that of phengodid glow-worms, Rhagophtalmidae or larval luminescence in click beetles (Sivinski 1981). Similar to *Lamprohiza* species (De Cock 2009), it is possible that the glow spots of *P. reticulata* females are inherited from the larval stages. A lack of typical adult light organs with reflectors may explain why *Phausis* females are noticeably dim compared to their own males and also to females of other similar glow-worm firefly species.

The function of male glows remains unknown. It has been suggested (Lloyd 1965, 1997, Yiu 2013) that male light production might induce females to glow. Although Lloyd (1971) reported observing *P. reticulata* females beginning to glow in response to males' glows overhead, such female glow responses to males were never observed in our study or by Frick-Ruppert & Rosen (2008). Instead, in our experiments females began glowing independently within min of one another on multiple nights. Based on our observations we propose 3 alternative hypotheses for male glows in *P. reticulata*. First, male glows might trigger females to produce a second courtship signal, such as a sex pheromone (see below) that allows males to identify them as appropriate mates (also suggested for other glow-worm fireflies by Yiu 2013). Another hypothesis, not mutually exclusive, is that such glows allow the males to illuminate (Lloyd 1968), and thus avoid, potential hazards. Our observations and glow lure experiments indicate that male *P. reticulata* are attracted to other glows, including those produced by dying fireflies captured by predators (e.g. spiders, harvestmen, or *Photuris* females; Lewis et al. 2012), fireflies trapped in puddles, or the glows of *Orphelia fultoni* fungus gnats. By illuminating the vicinity with their glows before landing, males may be able to distinguish the pale-bodied outlines surrounding the glows of conspecific females from glows associated with other hazards. Thirdly, these flying males might glow to signal that they are aposematically defended to nocturnal flying predators, such as bats or caprimulgids (Moosman et al. 2009), or even to *Photuris* fireflies that are specialized firefly predators themselves but seem to reject *P. reticulata* males (Lewis et al. 2012).

As noted in previous studies (Lloyd 1964, Frick-Ruppert & Rosen 2008), *P. reticulata* often occurs in forested habitats with dense understory vegetation. In such obstacle-filled habitats, signaling systems may evolve that use a combination of pheromones for long-range attraction and bioluminescence for better short-range localization (Lewis & Cratsley 2008). Among fireflies, direct evidence for volatile pheromones in sexual communication has thus far been restricted to diurnally active species (De Cock & Matthysen 2005; Lloyd 1972, 1997b; Ohba 2004). To date no lampyrid pheromones have been isolated or characterized (Lewis 2009) except for some species of the Asian firefly genus *Pyrocoelia* (Shibue et al. 2000). It had previously been hypothesized that *P. reticulata* might use pheromones (Lloyd 1965; but see Lloyd 1997b). Both our study and Frick-Ruppert & Rosen (2008) observed males flying directly upwind toward females.

Our study represents the first attempt to experimentally unravel the relative importance of female pheromones and glow signals in the courtship system of *P. reticulata* fireflies. The observations and results from our courtship signal experiment provide suggestive, though inconclusive, evidence that female-produced pheromones may play a role in *P. reticulata* courtship. With and without putative pheromone release, glowing females did not show statistically significant differences in their attractiveness measured both as the number of airspace males and the proportion of these males that landed. It is possible that the cardboard cover used to block the female visual signal in our experimental design may have somehow interfered with the production (e.g. if pheromone release is triggered by females seeing male glows) or dissemination of any female-produced pheromones. We also cannot rule out that our experiment might have been confounded

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by the presence of wild females nearby, with the possibility that high female densities might have overloaded the air with female pheromones.

On the other hand, preliminary evidence comes from observations that males often approached females assigned to the pheromone treatment (no glow visible) from downwind and exhibited flight patterns typical of males tracking a pheromone plume. In addition, we observed 3 males that landed directly on the containers of females in this treatment, while another 3 males landed within a 1m radius of the female. It seems very unlikely that these landings and approach behaviors happened by chance.

Our field observations suggest that the *P. reticulata* signaling system may be more complex than previously thought. At the outset of the flight period, males may rely primarily on light signals to locate females, perhaps later switching to pheromones when the pheromone plumes of relatively fewer unmated females would be more distinct. Alternatively, unmated females might also release pheromone only later at night. Such possibilities are supported by our experimental observations during the late flight period in which Pheromone + Glow females often rapidly attracted several males, whereas the Glow-only females seemed less successful after male peak activity. Thus, it is possible that *P. reticulata* courtship signaling strategies may vary both in terms of the relative importance of different female signals (glows versus pheromones) as well as in male search patterns (focus on visual versus chemical signals), depending on temporal, nightly or seasonal changes in relative densities and sex ratios.

Female Light-Producing Spots as an Indicator of Body Size

There was extensive intraspecific variation in female body size, which was positively correlated with differences in how many light spots females had. Further, our glow lure experiments provide suggestive evidence that males preferentially approach female-like lures with a greater number of light spots. Further studies should reveal if it is the overall brightness or glow spot pattern that plays a role. Across many insects, female body size is correlated with fecundity (Honěk 1993), and this has also been shown for a firefly (Wing 1989). Thus, our results provide novel evidence that *P. reticulata* males, could use female courtship glows as an honest signal of female reproductive potential.

Fluctuating asymmetry has been shown to be an indicator of fitness (Tomkins & Kotiaho 2001; Reusch & Muhlhauser 1998; but: Lens et al. 2002). We observed considerable asymmetry in female glow spot patterns (also seen in European *L. splendidula*; pers. obs. R. De Cock) which may result from fluctuating asymmetry and thus another honest advertisement of female fitness possibly with implications for male mate choice. Wing (1988) also noted that the asymmetrical arrangement of light organs on *P. reticulata* females may even be sufficient to identify individuals.

Interestingly, such variation in female light spot number was absent in a lowland population of *P. reticulata* females (Knoxville area), where all females show 4 light spots irrespective of body size (pers. obs. Lynn Faust). It is possible that what is currently described as *P. reticulata* actually represents a complex of several species or subspecies, though this remains to be investigated.

Phausis reticulata Mating Systems

Our results indicate that *P. reticulata* females are polyandrous: not only did mated females continue to emit courtship glow signals on successive nights, but the females we observed mating in the field readily remated in captivity. Wing (1988) also reported a *P. reticulata* female that mated twice in captivity. This is in contrast to the monogamy displayed by the flightless, neotenic females of another North American firefly, *Photinus collustrans* (Wing 1984, 1988). However, females in the Asian glow-worm firefly *Pyrocoelia pectoralis* were also found to be highly polyandrous, mating with up to 7 different males (Fu et al. 2012). It remains to be seen whether multiple matings by *P. reticulata* females results in increased fecundity or egg hatching success, as documented by Fu et al. (2012) for *Pyrocoelia pectoralis.*

In other fireflies, prolonged copulation durations are associated with males transferring a complex spermatophore during mating (Wing 1988; Lewis et al. 2004). The relatively short copulation times we observed in *P. reticulata* suggests these males do not produce spermatophores (generally < 10 min; see also Wing 1989). Previous work by South et al. (2011) reached a similar conclusion based on male reproductive anatomy.

Expanding on Wing's (1988) photograph by Sivinski of a female grasping her egg clutch, our study provides evidence for female egg-guarding behavior in *P. reticulata.* This behavior not only includes females curling their bodies around their egg clutch initially, but continuing to guard their egg mass until their death (maximally observed up to 9 days after oviposition). Though abandoning the clutch if threatened, females would repeatedly return to this guarding position when conditions were safe. Females, but not males, glowed to substrate vibrations indicative of a relatively distant threat. It seems likely that this behavior evolved as a way to reduce egg predation most likely acting as a defensive startling or aposematic signal. Interestingly, in the Rhagophthalmidae, which is thought to be a sister clade to the Lampyridae (Branham & Wenzel 2003), flightless *Rhagoph-*

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thalmus ohbai females similarly curl around their eggs. They also glow from several lateral photic organs, apparently producing an aposematic warning display (Ohba et al. 1996).

In conclusion, this study has provided many new insights into the courtship signals and mating behavior of *P. reticulata,* a little-studied North American firefly. In addition, we hope this work inspires further research on what appears to be a complex mix of courtship signals in this and other glow-worm fireflies. Particularly useful will be future studies to characterize female pheromones, and to examine how these signals might change with female ages, mating status, and environmental conditions.

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