Sexual selection in *Photinus* fireflies: The roles of male courtship signals and nuptial gifts

A dissertation submitted by

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ABSTRACT

Male fireflies (Lampyridae) produce bioluminescent courtship signals and provide a nuptial gift during mating in the form of a spermatophore. Females may prefer male courtship signals that indicate high nuptial gift quality, and nuptial gift quality may also affect the relative costs and benefits of mate competition and mate choice. I used Photinus ignitus fireflies as a model system to determine the potential for sexual selection on male courtship signals and the role of nuptial gifts in mate competition and choice.

I observed variation in courtship behavior, sex ratio and mating success of Photinus ignitus in the field, and I measured variation in spermatophore mass in the lab. Female responsiveness increased as the number of males dialoging with each female decreased. Males with wider lanterns mated successfully early in the season, while females with larger egg counts were more likely to mate and spermatophore mass was lower late in the season. These results are consistent with a role reversal from male competition and female choice to female competition and male choice with decreasing relative male density as well as with decreasing spermatophore investment.

Female preference for male courtship signals in both species was tested by scoring female responsiveness to simulated male flashes representing the conspecific range of male flash durations. Females of both species showed preference for longer duration flashes, but within each species there was no relationship between male flash duration and spermatophore mass. Females may not use flash duration as an honest indicator of spermatophore quality, but female courtship behavior is influenced by male spermatophore
contributions since females mate multiply, and both mated and fed females showed significantly lower responsiveness to simulated flashes than control females.

I explored the relationships between female preference, mate competition and mating success by testing female responses to simulated male flashes in the lab, and observing courtships in field enclosures. Although females preferred flashes from larger artificial lanterns, large males experienced no increased mating success. Male ability to orient towards and approach females may be just as important as male size for mating success since females preferred flashes from closer artificial lanterns.
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Chapter 1:

Introduction

Sexual selection was first identified as an evolutionary process by Charles Darwin (1859). Darwin identified sexual selection as a form of natural selection that does not result from the differential survival of individuals. Instead, sexual selection occurs through differential reproductive success among individuals of the same species as a result of differential success in mating and fertilization. Darwin viewed sexual selection as the process whereby elaborate traits that might otherwise be selected against by natural selection, can emerge in one sex because individuals possessing those traits gain an advantage in competition for access to mates. Darwin further noted that this competition usually occurs between males for access to females, and that females in turn have the opportunity to choose between potential mates. Intrasexual selection results from differential reproductive success in one sex that occurs through competition for mates, independent of any choice by the other sex. On the other hand differential reproductive success in one sex as a result of choice on the part of the other sex is referred to as intersexual selection or mate choice (Andersson, 1994). Of these two processes proposed by Darwin, mate choice was the most controversial part of Darwin’s theory from the outset since animals were not considered capable of discriminating among potential mates. Although mate choice has now been demonstrated in females of many species (reviewed by Andersson, 1994; Andersson and Iwasa, 1996; Johnstone, 1995), debate continues concerning how female choice for elaborate male traits has evolved in these species.
Darwin (1871) observed a general pattern in the preferences of females for male characteristics that has been supported by much recent research; females appear to choose to mate with males with the most vigorous or elaborate displays (Andersson and Iwasa, 1996). Fisher (1915, 1930) recognized that female choice could evolve if females were mating with males that offered some reproductive advantage to the female. He proposed that if both the elaborate trait and the preference for it are heritable, offspring inheriting the trait will have increased reproductive success because the preference for the trait will also be passed into the next generation. This hypothesized process is referred to as runaway selection. A second hypothesis to explain female choice, the good genes hypothesis, states that elaborate traits may be good indicators of male genetic quality because of the inherent cost of producing a conspicuous trait (Zahavi, 1975). Both of these hypotheses assume that the only benefit females will receive from males is the male’s genetic material. In many cases, in addition to the genetic contribution of males, females or their offspring also receive direct benefits from males such as paternal care or material benefit from males at mating (reviewed by Andersson, 1994). In this direct benefits hypothesis, if a male trait consistently indicates the quality of the male contribution, then female choice can evolve for that particular trait even if the trait is not heritable (Heywood, 1989; Hoelzer, 1989). It is also possible that female choice is independent of the quality of the male as a mate. Instead, female choice for particular male characteristics may have evolved to allow females to identify conspecific males (Andersson, 1994), or female choice may result from pre-existing sensory biases, the sensory bias hypothesis (Ryan, 1990; Andersson and Iwasa, 1996).
The occurrence of female choice in a species depends not only on its evolution through one of the aforementioned processes, but also requires conditions in which females have the opportunity to exhibit mate choice. The general pattern of male competition and female choice in many species has been explained by the fact that males generally produce large quantities of small gametes, while females produce relatively small quantities of large, energetically costly gametes (Andersson, 1994). As a result, an individual male has the potential to fertilize many more eggs than an individual female produces. Therefore males generally compete for access to a more limited number of fertilizable eggs, while females have the opportunity to choose between multiple potential fathers for their offspring (Andersson, 1994). However, research is beginning to demonstrate that the degree to which males compete and females choose can be influenced by a number of other factors (Andersson, 1994; Andersson and Iwasa, 1996; Reynolds, 1996). To represent the availability of mates for both males and females, Emlen and Oring (1977) defined the operational sex ratio (OSR) as the ratio of fertilizable females to sexually active males. The adult sex ratio and differences between the sexes in potential reproductive rate (PRR: the reproductive rate independent of mate availability, Clutton-Brock and Parker, 1992) combine to determine the OSR (Clutton-Brock and Parker, 1992; Kvarnemo and Ahnesjö, 1996; Parker and Simmons, 1996). Changes in environmental conditions that influence the operational sex ratio should lead to flexibility in courtship behavior such that under some conditions males may compete for access to females and females may be choosy, while in other conditions females may not be choosy as they compete for access to a limited number of males (Emlen and Oring, 1977).
Variation in mate choice in particular may also be influenced by other factors such as the variability of mate quality (Owens and Thompson, 1994; Johnstone et al., 1996; Jennions and Petrie, 1997; Widemo and Saether, 1999). Parental investment can potentially influence mate choice and mate competition both through its influence on male and female PRR, and through its influence on variability in mate quality (Johnstone et al., 1996). Male contributions made during mating, nuptial gifts, contain nutrients which can increase a female's reproductive output (reviewed by Boggs, 1995; Gwynne, 1997; Vahed, 1998). Therefore nuptial gifts represent a cost of mating for males, a benefit of mating for females, and a potential benefit of mate choice. Intraspecific variation in mate competition and mate choice will influence the intensity of selection acting on elaborate male traits. This study explores sexual selection acting on male courtship signals, and the potential influence of male nuptial gifts on variation in mate competition and mate choice.

Background

The influence of male nuptial gifts on mate competition and mate choice has been demonstrated for the pollen feeding katydid *Kawanaphila nartee* (Orthoptera: Tettigoniidae) in Australia (Simmons and Bailey, 1990; Gwynne and Simmons, 1990). In this species, at mating, males transfer a sperm-containing ampulla attached to a large spermatophylax that the female eats (Simmons and Bailey, 1990). Under early season conditions in the field when pollen was scarce due to reliance on kangaroo paw flowers, there was a high incidence of female competition and male choice. Later in the season however, when a richer source of pollen was available due to the flowering of the grasstree plant, mating behavior changed, with males becoming less choosy (Simmons and
Bailey, 1990). Experimental manipulation of food availability in cages also demonstrated that *K. nartee* male choice for heavier females occurred only in control cages, not in food-supplemented cages (Gwynne and Simmons, 1990). At low food abundance nutrient availability limited spermatophylax production so the OSR became female-biased, and males that could produce a spermatophylax chose among many potential mates. At high food abundance the OSR shifted as many males produced large spermatophylaxes, and they competed for access to females by producing more calls (Gwynne and Simmons, 1990; Simmons and Bailey, 1990). Furthermore, Shelly and Bailey (1992) demonstrated that male preference for larger females was lower in males that had encountered fewer females. These studies demonstrate how variation in male investment and the operational sex ratio can interact to generate variation in sexual selection through mate choice and mate competition.

The operational sex ratio can be influenced by many other factors in addition to male investment in nuptial gifts. For instance, male milkweed beetles, *Tetraopes tetraophthalmus*, like katydids, choose to mate with heavier females (Lawrence, 1986). Males only exhibit this choosy behavior when the sex ratio is female biased, and exhibit competitive behavior when the local sex ratio is male biased. In this case the differences in sex ratio are not brought about by changes in food availability but by the spatial and temporal grouping of females in the field. Although both the work on milkweed beetles and research on katydids focused on male choice, changes in the temporal and spatial distribution of males are predicted to produce changes in female mate choice as well (Emlen and Oring, 1977). Very few studies have looked at the influence of male spermatophore production and the OSR on competition and choosiness in males or
females (Shelly and Bailey, 1992) or documented variation in female choice in particular (Jennions and Petrie, 1997; Widemo and Saether, 1999).

Many recent studies indicate that male spermatophores represent direct material contributions to females and offspring, and females that mate multiply have higher fecundity, produce larger eggs and live longer than females that mate only once (reviewed by Boggs, 1995; Gwynne, 1997; Vahed, 1998). In females that do not feed as adults or females that do not feed on protein as adults, the nutrients provided by the male spermatophore may become the most important factor in determining female reproductive success. Therefore, females of some species may benefit most by actively foraging for matings (Kaitala and Wiklund, 1994), and to the extent that females choose among prospective mates, they may be choosing males with the most nutrient rich spermatophores.

Female choice may occur for traits that are correlated with spermatophore quality even if the spermatophore itself cannot be assessed by the female prior to mating. For instance in the butterfly *Pieris napi*, spermatophore mass increases with male mass (Wiklund and Kaitala, 1995). Positive relationships between measures of body size and spermatophore size have also been observed in a number of species of katydids (Gwynne, 1982; Gwynne and Bailey, 1988; Simmons, 1993; Wedell and Sandberg, 1995). In at least two katydid species female choice occurs for males with larger body size (Gwynne, 1982; Wedell and Sandberg, 1995). Therefore, it is possible that in these species, females preferentially mate with larger males in order to obtain a large spermatophore contribution.
In katydids, males advertise their readiness to mate by producing courtship calls. Females may be able to assess both male body size and spermatophore size through the calls they produce, since characteristics of these calls such as the carrier frequency can be correlated with body size (Wedell and Sandberg, 1995). Experiments using recorded courtship calls in a variety of species of Orthoptera have demonstrated that females can discriminate between potential mates as a function of the calls they produce (Hedrick, 1986; Gwynne and Bailey, 1988; Tuckerman et al., 1993). However, the relationship between the characteristics of the call produced and measures of the size and quality of the spermatophore needs to be directly assessed. In some species females preferentially respond to calls presented with a timing structure characteristic of larger males, even though there is no correlation between body size and spermatophore size (Tuckerman et al., 1993), while in other species females preferentially respond to calls at the frequency of smaller males which generally produce smaller spermatophores (Gwynne and Bailey, 1988).

In order to establish that female choice for male courtship traits occurs in a particular species, it is necessary to demonstrate that variation in a male characteristic is correlated with female behavior that leads to differential male mating success. Charles Darwin first suggested (1871) that the evolution of the peacock’s train must have occurred through female choice. However, it was not until recently that Petrie, Halliday and Sanders (1991) experimentally demonstrated that peahens assess a number of different males and choose a mate based on specific characteristics of his train. They found that male mating success was highly correlated with the number of eye spots a male could display, rather than any other aspect of male behavior, and the number of eye spots a male
possessed was only correlated with train length, rather than any other aspect of body size. Therefore, females were directly assessing the male train and choosing to mate males with the longest, most elaborate trains (Petrie, Halliday and Sanders, 1991). Female preferences for male traits will not always lead to differential mating success because female-female interactions, male-male interactions and environmental conditions may influence the potential for females to assess and discriminate among potential mates (Jennions and Petrie, 1997; Wagner, 1998; Widemo and Saether, 1999). Therefore, it is essential to combine experiments investigating female preference with experiments to determine the courtship behaviors and traits that are correlated with mating success.

Study system

Fireflies are beetles belonging to the family Lampyridae most of which possess a bioluminescent lantern as larvae and adults, and are found in a wide variety of climates and geographical locations. The genus Photinus is found in temperate and tropical America as well as the Caribbean islands (McDermott, 1964). The bioluminescent flash patterns produced by the adult lanterns in male and female Photinus fireflies have been surveyed in 25 of the 31 identified North American species (Lloyd, 1966), and further research has been conducted for several Photinus species on bioluminescent flash behavior as a courtship signal (Branham, 1995; Buck, 1937, 1990; Buck and Buck, 1972; Carlson and Copeland, 1988; Carlson et al., 1976, 1977; Cicero, 1983; Lewis and Wang, 1991; Lloyd, 1966; Venclo and Carlson, 1998; Wing 1984, 1985).

Photinus courtship occurs during one or more months of the summer depending on the climate, and throughout this period of the summer, the male flight season, males fly

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across their habitat advertising with a species-specific pattern of bioluminescent flashes for a limited period of time each night, the male flight period (Lloyd, 1966). Males produce single, double, or multiple pulse flash patterns, and females respond to male flash advertisements after a species-specific time delay with flashes of their own directed towards the male from the female’s perch in the vegetation (Lloyd, 1966). Once a male observes the female response he moves towards the female and flashes again. This pattern of male flash and female response will continue either until the male has contacted the female, until the male stops flashing, or the female stops responding. Therefore, females have the opportunity to determine which males they will mate with by preferentially responding to courtship flashes, and males may differ in their ability to locate and contact females.

As a result of their unique system of courtship, fireflies of the genus Photinus provide an excellent system for examining courtship behavior both in the field and in the lab in order to determine how male nutritional contributions can influence the potential for sexual selection on male courtship signals through mate competition and mate choice. The short nightly flight period and annual mating season makes it possible to accurately characterize variation in courtship behavior and male spermatophore contribution for the entire season. In addition, the photic nature of the courtship signals makes it possible to record and simulate them in order to test female preferences. Finally, the conspicuous nature of bioluminescent courtship interactions makes it possible to directly observe differential mating success generated by mate competition and female choice in the field.

By simulating male flashes and observing female response, male flash characteristics influencing the likelihood of eliciting a female response have been identified.
(Branham and Greenfield, 1996; Venclo and Carlson, 1998). Branham and Greenfield (1996) reported the first evidence for differential female responsiveness to naturally occurring intraspecific flash variation in *Photinus consimilis*, a species that produces flash trains of multiple pulses. Branham and Greenfield (1996) determined that *P. consimilis* females respond more frequently to simulated flash trains in which the pulse rate is higher than the mean pulse rate in *P. consimilis* males. This result demonstrates the potential for female fireflies to discriminate among the flashes of conspecific males.

Once a male has located and contacted a female, mating can occur. In stage I of mating (Lewis and Wang, 1991) the male mounts the female dorsally inserting his aedeagus into her genital opening. The mating pair remain in this position until the male turns 180° with his aedeagus still inserted within the female so the mating pair are interlocked abdomen to abdomen facing away from each other, stage II of mating (Lewis and Wang, 1991). In many *Photinus* species males and females mate multiply during the season (Lewis and Wang, 1991; van der Reijden et al., 1997; Wing, 1985, but see Wing 1984 for *P. colustrans*). However, males and females can only mate once per night because once mated, males and females remain in stage II of copulation, abdomen to abdomen, for the rest of the male flight period (Lewis and Wang, 1991; Rooney, personal communication). The extended length of stage II has been proposed to serve as mate guarding behavior (van der Reijden et al., 1997; Wing, 1985).

*Photinus ignitus* and *P. marginellus* males produce a complex spermatophore that is transferred to females during stage II copulation (Rooney and Lewis, 1999, van der Reijden et al., 1997). The spermatophore is digested within the female, and nutrients from the spermatophore are incorporated into the oocytes (Rooney and Lewis, 1999, van der
Reijden et al., 1997). Since neither males nor females feed as adults (Williams, 1917; Lloyd, 1997), this spermatophore production represents a substantial energetic cost imposed on males and a substantial benefit to females that mate multiply. Therefore, spermatophore production may influence mating competition and mate choice in two possible ways: variation in spermatophore quality among males may favor female choice for male traits indicating spermatophore quality, or females may compete for matings because spermatophores represent a limiting resource. These potential effects on female mating behavior could influence male behavior as well.

Mate choice and mate competition in both male and female Photinus fireflies may also be influenced by the operational sex ratio. Because both sexes can mate at most once on any given night, the relative abundance of available males and females each night should play a crucial role in determining the relative levels of competition and choice in males and females. If the nightly sex ratio is heavily male-biased, males should compete heavily for access to the limited number of females and females have the opportunity to choose between potential mates. On the other hand, if the sex ratio is female-biased, males have the opportunity to be choosy and females should compete for access to the limited number of males. This appears to be the case, as a seasonal change in sex ratio in Photinus aquilonius and Photinus marginellus was accompanied by changes in the mating behavior of both males and females (Lewis and Wang, 1991). P. marginellus female responsiveness to male flashes was higher later in the season when males are very scarce and lower early in the season when males are abundant.

Firefly courtship behavior makes it possible to observe two or more different males simultaneously courting the same female and to identify which male successfully mated
(Lewis and Wang, 1991; Vencl and Carlson, 1998). Therefore, it has been possible to demonstrate that differential female responsiveness to male flashes determines male mating success in *P. marginellus* (Lewis and Wang, 1991), suggesting a role for female choice. However, male competition may also be important for determining male mating success. The outcome of competitive interactions between males could be determined by male flashing activity since successfully mating *P. pyralis* males produced more flashes than their competitors (Vencl and Carlson, 1998). Alternatively, male walking speed may influence the outcome of competitive interactions since smaller *P. pyralis* males were more successful mating in groups of multiple dialoging males, and also walked more quickly than their larger counterparts (Vencl and Carlson, 1998).

**Summary of Research**

Using *Photinus* fireflies as a model system, I investigate how female preferences for male courtship signals may be influenced by variation in male spermatophore contribution, and I examine related patterns of male and female choosiness and competition. The present study was carried out on a population of the firefly *Photinus ignitus* located in the Smith-Andover field in Lincoln, Massachusetts, USA with additional research on *P. pyralis* collected in the Princeton Collection development in Plainsboro, New Jersey, USA. The research consists of three investigations designed to provide insight into the potential for sexual selection on male courtship signals in *P. ignitus* fireflies, and to examine how male spermatophore contribution may influence mating competition and mate choice. Chapter II examines a field population of *P. ignitus* to determine the relationships between patterns of variation in sex ratio, courtship behavior,
mating success, male spermatophore quality and female egg count in *P. ignitus*, and explores how these factors affect mate competition and mate choice. Chapter III reports laboratory investigations of factors influencing female preference for male courtship signals by measuring variation in male flash duration, the relationship between flash duration and spermatophore mass, and female preference for simulated flashes in *P. ignitus* and *P. pyralis*, as well as the influence of female mating and nutritional status on female responsiveness in *P. ignitus*. Chapter IV combines laboratory experiments on female preference for simulated flashes from artificial lanterns of varying sizes at varying distances with observations of courtship and mating in field enclosures to determine how mating competition and female preference may interact to generate differential mating success in *P. ignitus*. By synthesizing data from field observations with the results of controlled experiments, this study tries to provide insight into the complex processes that influence sexual selection in natural populations of *P. ignitus*. 
Chapter 2:

Variation in courtship behavior of *Photinus ignitus* fireflies corresponds with local sex ratio and male investment.

Abstract

Patterns of mating competition and mate choice vary both between and within species with changes in the relative costs and benefits of mating and mate choice to each sex. We examined variation in sex ratio, courtship behavior, mating success, spermatophore production and egg counts in *Photinus ignitus* fireflies to explore the factors influencing sexual selection in this species. We measured local sex ratio, female flash responsiveness and mating behavior of focal females in the field, as well as several morphological traits in both sexes, male spermatophore mass and female egg count. Female flash responses to male flashes increased as the number of males courting the female decreased over the course of the season. Comparisons of morphological traits between successfully mating and unsuccessful individuals revealed that successful males had larger lanterns only during early season, successful females had larger body mass, and females from aborted matings had fewer eggs. Spermatophore mass, male lantern size, and female egg count were related to body size, and spermatophore mass decreased from early season to late season and with successive matings. These results suggest that early in the season when spermatophore quality is high, several males compete for access to each female, and females choose males with wider lanterns. Later in the season when male spermatophore size declines, males may choose to mate selectively with females possessing high egg counts, while females may compete for males by increasing their flash responsiveness.
Introduction

The operational sex ratio (OSR: the ratio of fertilizable females to sexually active males, Emlen and Oring, 1977) is one of several factors which may determine levels of competition and choosiness exhibited by males and females during courtship because it influences the costs of choice due to lost mating opportunities and the benefits of competition for increased mating opportunities (Andersson, 1994; Andersson and Iwasa, 1996; Reynolds, 1996). The OSR is determined by the adult sex ratio and differences between the sexes in potential reproductive rate (PRR: the reproductive rate independent of mate availability; Clutton-Brock and Parker, 1992; Kvarnemo and Ahnesjö, 1996; Parker and Simmons, 1996). The relative costs and benefits of mate choice are also influenced by many other factors including variation in mate quality (Owens and Thompson, 1994; Johnstone et al., 1996; Jennions and Petrie, 1997; Widemo and Saether, 1999). Parental investment influences the costs and benefits of mating by influencing the PRR of each sex, and parental investment can also influence variation in mate quality for each sex (Johnstone et al., 1996).

Variation in paternal investment in the form of parental care or nuptial gifts can shift the intensity of competition and choice both between and within species (e.g. Gwynne, 1984; Gwynne and Simmons 1990; Vincent et al., 1994). Recent studies have demonstrated that male fireflies in the genus Photinus transfer a spermatophore to females at mating (van der Reijden et al., 1997), and nutrients from the spermatophore are subsequently incorporated into the female’s eggs (Rooney and Lewis, 1999). This male investment may be particularly important in Photinus fireflies because they do not feed as adults (Williams, 1917; Lloyd, 1997). Therefore, male investment through spermatophore
production in *Photinus* fireflies may influence the relative occurrence of mate choice and
competition in firefly courtship behavior.

*Photinus* females may choose mates through differential responsiveness to the
bioluminescent flashes produced by males (Lloyd, 1979; Lewis and Wang, 1991; Branham
and Greenfield, 1996; VencI and Carlson, 1998; this thesis, Chapter 4). However, in *P.*
aquilonius and *P. marginellus*, female responsiveness increased over the course of the
season as male density decreased and the local sex ratio became increasingly female-biased
(Lewis and Wang, 1991). The seasonal change in sex ratio was also accompanied by a
change in mating behavior as the duration of the first stage of copulation prior to
spermatophore transfer decreased and in some cases copulation was prematurely
terminated (Lewis and Wang, 1991). These changes in female responsiveness and mating
behavior may represent a shift from female choice and male competition to female
competition and male choice as the sex ratio varies from male-biased to female-biased.
Seasonal changes in the relative investment of males and females may also explain the
observed variation in courtship behavior.

In this study we examined seasonal changes in courtship behavior and investment
in spermatophores and eggs for *Photinus ignitus* fireflies. We measured variation in
female responsiveness in relation to season and sex ratio, as well as changes in mating
behavior over the course of the season. In addition we examined whether mating success
covaried with male or female morphology throughout the season. Finally, we explored the
relationship between morphological traits, time of season, male spermatophore mass, and
female egg count. We sought to determine how variation in sex ratio, spermatophore
mass, and egg counts might influence mate competition and mate choice in *P. ignitus.*
Methods

Study System

Adult *P. ignitus* fireflies are found primarily in open fields in eastern North America, ranging from North Carolina to Canada (Lloyd, 1966). Like most *Photinus* fireflies they spend up to two years as predaceous larvae living underground (Williams, 1917) before emerging as adults in the late spring and early summer. Adult *Photinus* live approximately two weeks (Buschman, 1977; Lewis and Monchamp, 1994; Lewis and Wang, 1991; Williams, 1917) during which time both males and females engage in courtship and mating on multiple nights (Lewis and Wang, 1991; van der Reijden et al., 1997; but see Wing, 1984 for singly-mating *P. collustrans*). Courtship and mating behavior has been previously documented for two species of polygamous *Photinus* fireflies in Massachusetts: *P. aquilonius* and *P. marginellus* (Lewis and Wang, 1991). The general pattern of behaviors involved in courtship and mating for these species is shared by most other *Photinus* species (Cratsley, unpublished data; Lewis, personal communication; Lloyd, 1966; Vencl and Carlson, 1998; but see Cicero, 1983; Wing, 1984, 1985). Males fly and produce bioluminescent flashes for a limited period of time each night, referred to as the male flight period. Females respond to male flashes with bioluminescent flashes of their own after a species-specific time delay. Males land shortly after observing a female response and begin a courtship dialog of male flashes and female responses that continues as males approach the female. Once a male contacts a female he usually stops flashing, mounts the female dorsally and inserts his aedeagus into her genital opening. This is defined as stage I of mating (Lewis and Wang, 1991). Stage II of mating occurs when the male swivels around 180 degrees, but remains positioned abdomen to
abdomen in copula with the female (Lewis and Wang, 1991). During this stage of mating the male transfers a spermatophore into the female's bursa (van der Reijden et al., 1997; Rooney and Lewis, 1999). This second stage of mating usually lasts from two to eight hours (Lewis and Wang, 1991; Rooney, personal communication), limiting both males and females to one mating per night.

**Seasonal patterns of courtship and mating behavior:**

*P. ignitus* flight season at the field site, Smith Andover field, Lincoln MA, USA, lasted 6 weeks from late June to early August. Field observations of *P. ignitus* courtship and mating behavior were conducted throughout the entire flight season from June 26 to August 1, 1995. Focal females were located by their responses to simulated male flashes produced by penlights starting at approximately 2100 h each evening. The dialog area for each female was defined as a 1 m radius circle around the female's perch site, because previous observations had found this to be the maximum distance across which bioluminescent courtship dialogs occurred. Each female was observed for several 5 min periods during which we recorded the number of males flashing within the dialog area, the number of flashes from each male, and the number of female responses to each male. We observed females repeatedly throughout the male flight period until the female mated with a male, the female was contacted by a male but failed to mate, or male flight activity had ceased for the night (approximately 2300 h). Whenever possible we carefully observed male and female behavior during mating with illumination from a blue filtered headlamp. We recorded the times of initial male contact, duration of stage I of copulation, and initiation of stage II of copulation. Regardless of whether male contact led to mating we
attempted to collect both the contacting male and focal female, as well as nearby males who had been observed in dialog with the focal female. In addition, near the end of the male flight period we sampled additional spontaneously flashing males as well as other, non-focal females that remained responsive to simulated flashes.

Each female's local sex ratio (Lewis and Wang, 1991) was calculated as the maximum number of males observed dialoging with her within a 1 m radius during a 5 min observation period. This measurement was used as an estimate of the OSR because it reflects the number of reproductively active males per each responsive and presumably fertilizable female we observed. In addition, each female's total responsiveness was calculated as the proportion of total flashes by all males within her dialog radius to which she responded. For females that eventually mated, we also calculated female responsiveness to particular males (the mating male as well as to any other dialoging males). We conducted a paired t-test to explore differences in female responsiveness to the male with which she ultimately mated compared to the most actively dialoging unsuccessful male. We used regression analysis to examine seasonal changes in maximum local sex ratio experienced by females on each night. Regression analysis was also conducted to determine the relationship between maximum local sex ratio and total female responsiveness. For successful matings we used regression analysis to determine seasonal changes in duration of stage I copulation.

In cases where a male contacted the female but did not mate, we distinguished three outcomes defined by female behavior and the initiation and length of stage I copulation: 1) female-terminated courtships, in which females walked away from the male immediately after contact, 2) aborted matings, in which females remained stationary, but
after contacting the female, the male either did not initiate stage I of copulation or terminated it within 5 min without proceeding to stage II (spermatophore transfer).

3) mating failures, in which pairs engaged in lengthy stage I copulation ($\geq 1$ h) that never progressed to stage II (spermatophore transfer).

**Variation in morphology and mating success**

Field-collected *P. ignitus* were brought into the lab for measurements of male and female morphological traits. Each firefly was anesthetized with CO$_2$, video imaged under a dissecting microscope, and measured to the nearest 0.01 mm using NIH Image version 1.59. We measured male and female elytral length (distance anterior to posterior margin of left elytron measured parallel to body axis), elytral width (distance from medial edge to lateral edge of left elytron at widest point), pronotal width (maximum distance between lateral edges of pronotum), eye span (distance between lateral margins of eyes including head width), lantern width (maximum distance across lantern measured perpendicular to body axis), lantern length (maximum distance from anterior to posterior margin of lantern measured parallel to body axis), and lantern area (surface area of lantern outlined and calculated as polygon with NIH image). The male lantern occupies both the 6$^\text{th}$ and 7$^\text{th}$ abdominal sternites, and measurements of lantern length and total area vary with the position of the male's abdomen. Therefore in males, lantern width was used as the sole estimate of lantern size. Fireflies were also anesthetized and weighed on a Mettler AT261 balance to the nearest 0.1 mg. Once measured, fireflies were maintained in the lab for the remainder of their lifespan. We tested the relationships among morphological traits separately for males and for females with Pearson correlation coefficients.
We grouped males and females based on mating outcomes in the field. Individuals were classified as unmated if they were collected actively flashing or responding to simulated flashes at the end of the male flight period. We classified males collected dialoging with a female as unmated if the female mated with another male. All males and females that mated were classified as such. Males who contacted a female but did not mate, and females from aborted matings were excluded from analysis. Morphological characteristics of mated vs. unmated males and females were compared for the season as a whole as well as for only early vs. late season observations using 2 way ANOVAs. Early season was defined as the first 10 d of field observations when the mean local sex ratio remained above 1, while late season was defined as the last 9 d of field observations when the mean local sex ratio fell below 1 (Figure 1b). In this manner we divided the season into the period of time when the operational sex ratio was male-biased, and the period when the operational sex ratio was female-biased.

Variation in male spermatophore mass, and female egg counts

During summer 1997 and 1998 males and females were collected from Smith Andover field, Lincoln, MA, USA over the course of their entire mating season from June 22 to August 5. Male and female morphological traits were measured as described above. In order to assess seasonal and intraspecific variation in spermatophore quality, each male was placed in a container with a female during the male flight period (≈2100 to 2300 h), and pairs observed every 15 min. Mated pairs (n = 34) were separated after 45 min of stage II copulation by gently pulling them apart. Females were then frozen to prevent degradation of the male spermatophore inside the female’s reproductive tract. We chose
45 min as our time point because preliminary research indicated that this was the minimum time after which we could be sure the spermatophore had been transferred.

Females were later dissected in physiological saline, and mature eggs (≥ 600 μm diameter) were counted in the female reproductive tract. The male spermatophore was removed from the female reproductive tract, and spermatophore dry weight determined by rinsing in dH₂O, placing it on a pre-weighed plastic disc in a desiccation chamber for 24 h, and weighing on a Cahn model 21 microbalance to the nearest μg. The firefly spermatophore is proteinaceous, and amino acids from spermatophores are incorporated into a female’s eggs (Rooney and Lewis, 1999). Therefore, a subset of spermatophores were macerated and solubilized for determination of protein content using a dye binding protein assay (Biorad). We used regression analysis to determine the relationship between spermatophore mass and protein content, and to determine the relationships between body mass and spermatophore mass in males as well as body mass and egg count in females. Separate analyses were conducted for early season and late season males and females. We defined early season fireflies as any collected by July 6, and late season fireflies included any collected beginning July 12 based on observations from 1995 that sex ratio became increasingly female biased after July 10 (Figure 1b).

Additional data on the change in spermatophore size across successive matings was collected in the lab using field-collected males and females in the summers of 1997-1999. Males were provided the opportunity to mate nightly with different field-collected females. Spermatophores were dissected out of mated females, and dry weights obtained using the techniques described above. Mean male spermatophore masses from 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd}, and 5\textsuperscript{th} or later lab matings were compared using ANOVA.
During 1999 we observed aborted matings both in the field and in the lab. In many cases the same female failed to mate when offered two different males. Females (n = 9) involved in aborted matings were frozen and dissected in order to determine egg counts (J. Neumann, unpublished data). Mating females (n = 14) were collected from the field during the same portion of the flight season, frozen during mating, and dissected. Female egg counts were compared between mated females and females involved in aborted matings using a Mann-Whitney U test, because data were not normally distributed. We also compared elytral lengths of females from each group using an unpaired t-test.

Results

Seasonal patterns of courtship and mating behavior:

Female flash responsiveness in the field varied during the flight season, as well as between individual females on the same night. Female responsiveness ranged from 0 to 100% and there was no clear pattern of change in female responsiveness over the course of the season (Figure 1a). The number of days from the start of the season did not explain a significant proportion of the variation in female responsiveness (linear regression, \( r^2 = 0.002, n = 45, p = 0.76 \)). The maximum local sex ratio, defined as the maximum number of males dialoging with a female during a 5 min observation, decreased significantly over the course of the season (Figure 1b: linear regression, \( r^2 = 0.21, n=71, p<0.001 \)) indicating that fewer males were competing for access to each female as the season progressed.
Figure 1

a) Percentage of *P. ignitus* male flashes to which females *(n = 45)* responded during 1995 flight season *(6/25 to 8/02)* (Linear regression equation: \( y = .116x + 42.0 \)).

b) Maximum number of *P. ignitus* males dialoging within 1 m of each female *(n = 71)* during 1995 flight season (Linear regression equation: \( y = -.05x + 2.3 \)).

Although the number of males dialoging with each female declined over the course of the season, on a given night different females experienced different numbers of males.

When female responsiveness was analyzed relative to the maximum number of dialoging males, we found that female responsiveness decreased significantly as the number of dialoging males increased (figure 2: linear regression, \( r^2 = 0.24, n=32, p= 0.003 \)). Finally, when two or more males courted a single female, females responded to a significantly greater proportion of the flashes produced by the male with whom she eventually mated (female response to successful male: mean = 65.3%, female response to unsuccessful male, mean = 33.3%, paired t-test, \( t = 2.9, df = 6, p = .027 \)).
Both female and male behavior also varied once a male had contacted a female. In cases where contact led to stage I and ultimately to stage II, stage I duration varied from 30 s to 43 min (n = 22). There was no relationship between stage I duration and day of season (linear regression, $r^2 = 0.02$, $n = 22$, $p = 0.42$). Contact led to stage II of mating in 70% of cases observed (n = 47). We observed 2 mating failures, 2 cases of female terminated courtships in which the female walked away from the male, and 8 aborted matings in which the male left the female after less than 5 min in stage I. The aborted matings all occurred between days 12 to 38 of the season (7/6-8/01), representing the latter half of the early season as well as late season.

Variation in morphology and mating success

There was considerable morphological variation within males and females, with particularly large coefficients of variation for measurements of body mass and lantern size (Table 1). *P. ignitus* male mass, elytral length, elytral width, pronotal width, and eye-span were all significantly positively correlated with one another (Pearson correlation, $r > .55$ for all, $n = 41$, $p < .005$ for all). Male lantern width was not significantly correlated with
body mass (Pearson correlation, $r = .42$, $n = 41$, $p = .137$), but was significantly correlated with all other aspects of male morphology (Pearson correlation, $r > .59$ for each, $n = 41$, $p < .001$).

Table 1
Mean and coefficient of variation (CV = standard deviation/mean x 100) for morphological measurements of *P. ignitus* males and females collected during 1995 flight season.

<table>
<thead>
<tr>
<th>Morphological Trait</th>
<th>Mean</th>
<th>Male CV</th>
<th>n</th>
<th>Mean</th>
<th>Female CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (mg)</td>
<td>15.91</td>
<td>34.21</td>
<td>76</td>
<td>18.54</td>
<td>41.98</td>
<td>67</td>
</tr>
<tr>
<td>Elytral length (mm)</td>
<td>7.26</td>
<td>6.88</td>
<td>77</td>
<td>6.28</td>
<td>9.69</td>
<td>69</td>
</tr>
<tr>
<td>Elytral width (mm)</td>
<td>1.40</td>
<td>9.55</td>
<td>77</td>
<td>1.32</td>
<td>13.76</td>
<td>69</td>
</tr>
<tr>
<td>Pronotal width (mm)</td>
<td>2.39</td>
<td>6.95</td>
<td>77</td>
<td>2.36</td>
<td>9.33</td>
<td>69</td>
</tr>
<tr>
<td>Eye span (mm)</td>
<td>1.61</td>
<td>6.16</td>
<td>77</td>
<td>1.27</td>
<td>7.70</td>
<td>69</td>
</tr>
<tr>
<td>Lantern length (mm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.66</td>
<td>18.67</td>
<td>69</td>
</tr>
<tr>
<td>Lantern width (mm)</td>
<td>2.30</td>
<td>10.55</td>
<td>77</td>
<td>1.01</td>
<td>13.26</td>
<td>69</td>
</tr>
<tr>
<td>Lantern area (mm$^2$)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.60</td>
<td>25.85</td>
<td>69</td>
</tr>
</tbody>
</table>

Comparisons among successfully mated vs. unsuccessful males as well as early vs. late season revealed a seasonal decline in body mass, but no differences in morphological traits based on mating status (Figure 3a, 2-way ANOVA: season $F_{1,30} = 11.5$, $p = .002$; mating status $F_{1,30} < .01$, $p = .95$; season x status $F_{1,30} = 0.5$, $p = .50$; all other morphological comparisons $n \geq 34$, $p > .11$). However, considering only males active during early season, successfully mated males had significantly wider lanterns than unsuccessful males (Figure 3b, t-test, $t = -2.1$, df = 36, $p = 0.04$). Much of this difference came from courtships involving multiple males dialoging with a single female. Males that
successfully mated in the presence of at least one other dialoging male had significantly wider lanterns \( \text{mean} = 2.37 \text{ mm} \pm 0.06 \text{ SE} \) than males observed dialoging with females that ultimately mated with another male \( \text{mean} = 2.14 \text{ mm} \pm 0.07 \text{ SE} \): \( t = -2.6, \ df = 10, \ p = 0.03 \).

**Figure 3**

a) **Mean (± SE) body mass of *P. ignitus* males during 1995: early season (6/25-7/10) successful males \( n = 12 \) males reaching phase II of mating in the field), early season unsuccessful males \( n = 16 \) males observed dialoging but failing to locate the female or collected flashing at the end of the male flight period), and late season (7/11-8/2) successful \( n = 3 \) and late season unsuccessful \( n = 3 \) males.

b) **Mean (± SE) lantern width of: Early season successful \( n = 19 \), early season unsuccessful \( n = 19 \), late season successful \( n = 7 \) and late season unsuccessful \( n = 9 \) *P. ignitus* males.

For *P. ignitus* females, mass, elytral length, elytral width, pronotal width, eye span, and lantern area were all variable (Table 1) and significantly positively correlated with one another (Pearson correlation, \( r > .45 \) for all, \( n = 51, p < .028 \) for all) with the largest coefficient of variation for measurement of body mass. Eye span was significantly larger in successfully mating females relative to unsuccessful females, but did not differ between early season and late season females (Figure 4a, 2-way ANOVA: mating status, \( F_{1,36} = \)
4.8, p = .04; season, F_{1,36} = 0.2, p = .69; mating status x season, F_{1,36} = 0.7, p = 0.4). Body mass was also significantly larger in successfully mating females, with no difference between early season and late season females (Figure 4b, 2-way ANOVA: mating status, F_{1,34} = 8.4, p = 0.01; season, F_{1,34} = 1.2, p = 0.28). There was a significant interaction between mating status and season with a more pronounced difference in body mass between mated and unmated females occurring in the latter half of the season (mating status x season, F_{1,34} = 6.1, p = 0.02).

Figure 4

a) Body mass of *P. ignitus* females (mean ± SE) during 1995 for: Early season (6/25-7/10) successful females (n = 17 females that reached stage II copulation in the field), early season unsuccessful females (n = 10 females dialoging but not mating at end of flight period), late season (7/11-8/02) successful females (n = 6), and late season unsuccessful females (n = 5).

b) Mean female *P. ignitus* eye span (± SE) of: Early season successful (n = 18) early season unsuccessful (n = 10), late season successful (n = 7), and late season unsuccessful (n = 5).
Variation in male spermaphore mass, and female egg counts

Male body mass was the single best predictor of spermaphore mass (linear regression, \( r^2 = 0.83, n = 11, p < .001 \)). Spermaphore protein content increased significantly with spermaphore mass (Figure 5: linear regression, \( r^2 = 0.75 \) n = 10, p = .001). Male spermaphore mass was also positively correlated with several other morphological traits; for instance male light organ width predicted a significant portion of the variation in male spermaphore mass (linear regression, \( r^2 = 0.61, n = 11, p = 0.003 \)). Both male body mass and spermaphore mass decreased significantly with day of the 1997 flight season (linear regression, body mass: \( r^2 = 0.72, p < .001 \); spermaphore mass: \( r^2 = 0.43, n = 11, p = 0.02 \)).

Figure 5
Male *P. ignitus* spermaphore protein content as a function spermaphore dry mass (n = 10; Linear regression equation: \( y = 0.11x + 4.88 \)).

The relationship between male body mass and spermaphore mass changed significantly between early season and late season (Figure 6, ANCOVA, n = 34, season * body mass: \( F = 8.1, p = 0.008 \)). Body mass predicted a greater proportion of the variation in spermaphore mass for early season males (\( r^2 = 0.69, n = 14 \)) than for late season males (\( r^2 = 0.23, n = 20 \)). Late season spermaphores (mean = 58.2 \( \mu \)g + 5.7 SE) were significantly lighter than early season spermaphores (mean = 103.0 \( \mu \)g + 16.7 SE)(t-test,
When males were given access to different females each night, spermatophore mass declined across successive matings by individual males (Figure 7, ANOVA, $F_{3,112} = 11.0$, $p < 0.001$). We observed a maximum of 8 lab matings by a single male over 12 nights (N. Levesque, unpublished data).

Figure 6
Male *P. ignitus* spermatophore dry mass as a function of wet body mass for early season (6/22-7/6; n = 14) and late season (7/12 - 8/05; n = 20) males (combined data for 1997 and 1998: Separate linear regression lines plotted for early season and late season).

Figure 7
Mean *P. ignitus* male spermatophore dry mass (+ SE) for spermatophores obtained from 1st (n = 77), 2nd (n = 30), 3rd (n = 5) and 5th or later (n = 4) matings.

Female egg number increased significantly with increasing body mass with no significant seasonal effect (Figure 8, ANCOVA, n = 56, body mass: $F = 65.4$, $p < 0.001$; season: $F = 0.3$, $p = 0.585$; all females pooled: $r^2 = 0.58$). Females collected during late season had significantly lower body mass compared to females collected during early season (t-test, $t = 2.3$, n = 56, $p = 0.030$), as well as slightly, but not significantly fewer eggs (t-test, $t = 1.9$, n = 56, $p = 0.065$).
Figure 8
Number of mature eggs in *P. ignitus* female reproductive tracts as a function of body mass for early season (6/22-7/6; n = 20) and late season (7/12-8/05; n = 34) females (combined data for 1997 and 1998: separate regression lines plotted for early season and late season).

Females involved in aborted matings had significantly fewer eggs than females that were involved in successful copulations (Figure 9, J. Neumann, unpublished data: Mann-Whitney U test, U = 108.5, n = 9 and 14, p = 0.004). This difference in female egg count was not associated with differences in female elytral length, as there was no significant difference in elytral length between females involved in aborted matings and successfully mated females (t-test, t = -0.394, n = 9 and 14, p = 0.177).

Figure 9
Mean number of mature eggs in *P. ignitus* female reproductive tracts (± SE) for aborted females (left by males after less than 5 min in stage I of mating, n = 9), and successful females (reached stage II of mating with males, n = 14).
Discussion

We have examined patterns of variation in local sex ratio, courtship behavior, differential mating success and parental investment in *Photinus ignitus* fireflies. Our findings show that early in the season when the sex ratio is male biased, females show reduced responsiveness, males with large lanterns experience increased mating success, and the strength of the relationship between spermatophore mass and body size is strongest. These early season conditions may generate differential male mating success based on intense male competition for access to a more limited number of females, and possible female choice for male characteristics such as large lantern size that may be correlated with large spermatophore contribution. As the season progresses aborted matings increase in frequency, and as the sex ratio approaches unity and then becomes female-biased, female responsiveness increases. Throughout the season, females with larger body mass and wider eye spans experience increased mating success, but female body mass and egg number declines later in the season. The increased frequency of aborted matings later in the season may represent male choice for females with large egg numbers as male spermatophores become more limiting. On the other hand, the increase in female responsiveness with decreasing male density suggests increased competition between females for access to a limited number of males as the operational sex ratio becomes female-biased.

In katydids the shift in OSR has been attributed to changes in food plant availability for male spermatophore production (Gwynne and Simmons, 1990; Simmons and Bailey, 1990). We found that male spermatophore mass in *P. ignitus* decreased with day of season, with decreased male body mass, and with successive male matings. The
observed decline in spermatophore mass from early season to late season may result from successive male matings as the season progresses. Although the seasonal change in spermatophore size may not have had a direct effect on OSR since all males collected were actively flashing and therefore sexually active, it may have influenced relative male and female PRR. Male spermatophore production is limited because *Photinus* do not eat as adults, so nutritional reserves available for spermatophores are likely to decrease over the course of the season and with successive matings. Therefore, early in the season when nutritional reserves for spermatophore production are high, males may benefit by mating as frequently as possible, while later in the season males could benefit by choosing to which females they will donate a spermatophore. Seasonal variation in male spermatophore production may in turn influence female mate choice because variability of male spermatophore mass is much higher early in the season and male traits such as lantern width may provide an honest indicator of spermatophore mass.

Lewis and Wang (1991) found seasonal decreases in male density and the number of males dialoging with each female in *P. aquilonius* and *P. marginellus* as well as a negative relationship between female response rate and number of dialoging males in *P. marginellus*. We found the same pattern of seasonal change in local sex ratio and the same relationship between local sex ratio and female responsiveness in *P. ignitus*. Female responsiveness to male bioluminescent flashes has been proposed as a possible mechanism for female choice (Lloyd, 1979; Lewis and Wang, 1991; Branham and Greenfield, 1996; Venci and Carlson, 1997; this thesis, Chapter 3). Preferential female responsiveness directed towards the male with which the female ultimately mates both in our study as well as in other *Photinus* species (Lewis and Wang, 1991) support this hypothesis. Therefore,
low levels of female responsiveness under male-biased sex ratios may represent high levels of female choosiness as a result of reduced costs of female choice through lost mating opportunities in addition to the high benefits of mate choice in the form of larger spermatophores early in the season.

On the other hand, increased female responsiveness when the sex ratio reaches unity or becomes female biased may represent decreased female choosiness and may also represent female competition for access to male spermatophore contributions. Lewis and Wang (1991) found that two or more *P. marginellus* females often responded simultaneously to flashing males during late season, and that the most responsive females ultimately mated with the males. Shifts between choosy and competitive courtship behavior as a function of the operational sex ratio have been observed in other insects (Lawrence, 1986; Gwynne and Simmons, 1990; Simmons and Bailey, 1990), and females compete for access to male spermatophore contributions in some katydids (Gwynne, 1981; Gwynne, 1985; Gwynne and Simmons, 1990; Simmons and Bailey, 1990).

We demonstrated the potential for male and female choice or competition to generate differential mating success in *Photinus ignitus* by comparing the morphology of fireflies that mated or failed to mate on a given night in the field. We found evidence for increased mating success by males with large lantern widths only during the early season. Differential mating success based on lantern width may be the product of male visual signal competition as males fly, advertising to females with bioluminescent flashes, or after males have landed and multiple males are dialoging with a single female. Alternatively female choice for flash characteristics of males with wider lanterns could occur through preferential female responsiveness to these males. Since both male competition and female
choice are more likely to occur early in the season when the sex ratio is male biased, this
may explain why males with larger lanterns were more likely to mate only during the early
season. Male competition or female choice likely occurs in groups of multiple males
dialoging with a single female since we found males that mated in those conditions had
wider lanterns than males that failed to mate in those conditions.

Vencl and Carlson (1998) found evidence for differential mating success as a
function of lantern size and body size in *P. pyralis*. They compared male body mass and
lantern area for successfully mating males dialoging with a female alone or in the presence
of competitors to the overall population means. Males who successfully mated in the
absence of competitors had significantly larger lanterns and body sizes than the population
mean, while males who successfully mated in the presence of three or more competitors
had significantly smaller lanterns and body sizes (Vencl and Carlson, 1998). Vencl and
Carlson (1998) reported the occurrence of direct competition in which “love knots”
(Mauer, 1968) of multiple suitors interact by crawling over the female as well as each
other, and the first male to locate the female does not necessarily succeed in mating with
her. In *P. ignitus* (personal observation) as well as in *P. marginellus* and *P. aquilonius*
(Lewis and Wang, 1991) males do not generally engage in direct competitive interactions.
Although extraneous males do locate mating pairs, climb on them, and in rare instances
knock them off their perch in the vegetation, extraneous males do not generally succeed in
mating with the female (Lewis and Wang, 1991; personal observation). This difference in
mating behavior among *Photinus* species may explain why our evidence for differential
mating success is similar to that described for *P. pyralis* when only single males dialog
with a female.
We observed increased mating success of *P. ignitus* females with particular morphological traits as well, but differential mating success in females did not vary seasonally. Larger eyespans and body masses were observed in mated females across the entire season. In spite of the small sample size of late season matings, mated females also had larger body masses than unmated females during late season. Since body mass is correlated with egg mass, males may benefit from choosing to mate with heavier females since they have the potential to fertilize more eggs. This may be particularly true late in the season when the operational sex ratio is female biased so there is relatively little cost in lost mating opportunities if a male rejects a female. In other insects male choice for large female body size has been demonstrated to vary with changes in the operational sex ratio in this manner (Lawrence, 1985; Gwynne and Simmons, 1990; Simmons and Bailey, 1990).

No previous evidence of male choice for female characteristics has been reported in *Photinus* fireflies. However, *P. marginellus* males terminated an unusually high proportion of late season matings before reaching stage II (Lewis and Wang, 1991). We saw a similar increase in the occurrence of aborted matings over the course of the season in *P. ignitus*. We observed three distinct behaviors (female terminated, aborted matings, and mating failure) following male and female contact but not leading to mating. While female terminated and mating failure may represent female choice or mating incompatibility possibly due to interspecies interactions, the relative inactivity of the female and short duration of stage I (< 5 min) during aborted matings may indicate that males are assessing females and rejecting them as potential mates. We found that aborted females had fewer mature eggs in their reproductive tracts than mated females, suggesting
that males may use female traits such as body mass or size to assess female egg count and mate preferentially with the most fecund females. Males mount females dorsally during the abbreviated stage I in aborted matings, so it is possible that males can assess females size at this point. If that is the case, the increase in this behavior as the season progresses could represent increased male choosiness as the sex ratio becomes female biased, and as male PRR decreases because of increasing constraints on spermatophore production.

Emlen and Oring (1977) proposed that variation in the operational sex ratio could generate variable courtship and mating behaviors within a species. Variation in parental investment can also influence mating choosiness both through its effect on the relative costs and benefits of mating as well as its effect on the benefits of mate choice (Johnstone et al., 1996). Our research suggests that seasonal changes in both male and female courtship behavior may be influenced by seasonal changes in the local sex ratio as well as seasonal changes in male spermatophore quality. We observed increases in female responsiveness with decreasing local sex ratio consistent with a change from female choice to female competition as potential mates became limited. In addition we observed preferential mating success for males with wider lanterns exclusively during the early season when both the quality and variability of male spermatophore contributions were high. Finally, we observed potential male choice for female egg number through aborted matings which may have increased later in the season as constraints on male spermatophore production increased. These results support the potential for variation in both the costs and benefits of mate choice to generate variable mating behavior within a species.
Chapter 3:

Factors influencing female preference for male flash duration in two fireflies:

*Photinus ignitus* and *P. pyralis* (Coleoptera: Lampyridae)

Abstract

In *Photinus* fireflies males produce spontaneous bioluminescent courtship flashes. Females preferentially respond to particular male flashes with flashes of their own. This study explored variation in female flash responsiveness as a function of male flash duration, and the relationship between spermatophore mass and flash duration in two closely related species, *Photinus ignitus* and *P. pyralis*. Variation in flash duration and spermatophore mass was measured in males of both species. Female preference was determined by scoring female flash response to simulated flashes of varying duration. In addition, variation in overall female flash responsiveness was determined for lab-mated, lab-fed, and control *P. ignitus* females. Females of both species exhibited greater preference for simulated flashes representing the upper range of conspecific male flash duration. However, there was no relationship between male flash duration and spermatophore mass in either species. Both lab-mated and lab-fed *P. ignitus* females showed lower overall responsiveness across all flash durations relative to control females that did not mate or feed in the lab. These results demonstrate the potential for female preference to produce directional selection for longer duration male flashes, but find no evidence for a direct benefit to female preference. Intraspecific variation in female choosiness as a function of female mating and nutritional status may preserve variation in male flash duration of *Photinus* fireflies in spite of the potential for directional selection.
Introduction

Female choice for particular male courtship traits has been demonstrated in many species (Andersson, 1994; Johnstone, 1995) and proposed to play a role in the evolution of elaborate male traits (Darwin, 1871) and speciation (Endler, 1989; Lande, 1981; Ryan, 1990). Mate choice is the pattern of mating that arises at least in part through the mating preferences of one sex (Heisler et al., 1987). Until recently variation in female preference and the factors underlying this variation have received little attention (Jennions and Petrie, 1997; Widemo and Saether, 1999). Variation in mating preferences can result from either differences in preference functions, the ranking of potential mates as a function of their phenotype, or differences in choosiness, the amount of effort that will be put into obtaining a preferred mate (Jennions and Petrie, 1997). Variation in female preference functions between species can potentially produce differential selection for male traits, while variation in female choosiness within a species will influence the intensity of selection acting on those male traits.

Courtship flash behavior has been described for several Photinus firefly species (Branham, 1995; Buck, 1937, 1990; Buck and Buck, 1972; Carlson and Copeland, 1988; Carlson et al., 1976, 1977; Cicero, 1983; Lewis and Wang, 1991; Lloyd, 1966; Vencl and Carlson, 1998; Wing, 1984, 1985). Male Photinus fly while spontaneously emitting a species-specific flash pattern, which may consist of single, double, or multiple flash pulses (flash trains) that are repeated after a characteristic time interval. Females perched in the vegetation may or may not respond to the flashes of particular males with bioluminescent flashes of their own given after a species-specific time delay.
Once a female has responded to a male flash, the male and female engage in a
dialog of repeated male flashes and female responses as the male lands nearby and
approaches the female. Males require female responses to their flashes to locate and
eventually mate with females. Therefore, males that elicit a higher female response rate
are more likely than competing males to successfully mate with the responding female
(Lewis and Wang, 1991; Vencl and Carlson, 1998; this thesis, Chapter 2). Females
discriminate between conspecific and heterospecific males on the basis of the duration of
single flash pulses, the number of flash pulses in each flash pattern, and the time interval
between flash patterns (Lloyd, 1966). Although Lloyd’s work demonstrates the potential
for female preference for male flash pattern, little is known about female preferences for
variation in male flash pattern within a species. Female preference for male flash pattern
has been examined in *P. consimilis* (Branham and Greenfield, 1996). Male *P. consimilis*
fireflies produce flash trains of multiple pulses repeated at 20 s intervals. Females respond
preferentially to simulated male flash trains with higher pulse rates (Branham and
Greenfield, 1996).

In most *Photinus* species male flash patterns consist of single pulses repeated at
intervals (Lloyd, 1966). Flash duration varies across species; *Photinus ignitus* males
produce relatively short 0.2 s duration flashes, while others like *Photinus pyralis* produce
flashes approximately 0.5 s in duration (Lloyd, 1966). Female choice for male flash
duration may help explain this diversity, and variation between species in female
preference for flash duration can provide clues about the strength and direction of
selection.
Female choice for a male trait such as flash duration, can evolve either because the trait provides an honest indicator of some aspect of male quality (Zahavi, 1975), through runaway selection (Fisher, 1915 1930) or as a result of female sensory biases for long duration flashes (Ryan, 1990). When male characteristics are honest indicators of male quality, the covariance of the preferred traits and male quality can influence variation in female preference both within and between species (Jennions and Petrie, 1997).

Female choice can evolve for male traits which act as honest indicator of either male genetic quality (reviewed by Kirkpatrick and Ryan, 1991; Andersson, 1994), or some direct benefit of mating with a particular male (Heywood, 1989; Hoelzer, 1989; Wolf et al., 1997). However, direct mating benefits also may represent a cost to female choice since rejecting a potential mate can mean missing out on the direct mating benefit (Johnstone et al., 1996). Therefore, nutritional contributions provided by males during courtship or mating can influence variation in female choosiness within a species, because under certain conditions females may benefit from choosing carefully among prospective mates, while under other conditions females may benefit more by obtaining a male’s contribution regardless of the male phenotype.

In many species females can increase their reproductive output by obtaining spermatophores through additional matings (reviewed by Boggs, 1995; Gwynne, 1997; Vahed, 1998). Males of several Photinus species transfer a protein-rich spermatophore to females during mating (van der Reijden et al., 1997). Amino acids obtained from male spermatophores are distributed to the female’s eggs within two days after mating (Rooney and Lewis, 1999). Since adult Photinus fireflies apparently do not feed in the field (Williams, 1917; Lloyd, 1997), male spermatophores may represent the only nutritional
supplementation that females receive as adults. Female preference for male flash behavior may occur for flash characteristics that are correlated with male spermatophore quality. Furthermore, females who have not mated recently may be less choosy than recently mated females because the costs of rejecting any male and his spermatophore contribution may outweigh the benefits of choosing a particular mate.

In this study we investigated female preference for male flash duration in two species of Photinus fireflies. P. pyralis and P. ignitus females were presented with simulated male flashes to determine whether females exhibited preference for different male flash durations, and to examine how those preferences differed between species. We also examined the relationship between male flash duration and male spermatophore contribution in both species. Finally, in P. ignitus, we experimentally examined the prediction that supplemental protein obtained through either mating or artificial feeding would decrease overall female responsiveness.

Methods
Study organisms

P. pyralis and P. ignitus share life history characteristics with most Photinus fireflies, spending up to two years as predaceous larvae living underground (Williams, 1917). Adults emerge in the late spring and early summer. Adult Photinus live approximately two weeks (Buschman, 1977; Lewis and Monchamp, 1994; Lewis and Wang, 1991, Williams, 1917), and in most species both males and females can mate multiply (Lewis and Wang, 1991; van der Reijden et al., 1997; but see Wing, 1984 for P. colustrans). Prolonged copulation durations effectively limit both sexes to a single
mating per night (personal observations; Lewis and Wang, 1991). *P. pyralis* and *P. ignitus* occupy overlapping geographic ranges and habitat distributions (Lloyd, 1966). *P. pyralis* occurs in open fields and lawns across the central and eastern United States from Texas to New York. *P. ignitus* has a more northerly distribution, ranging from North Carolina to Canada, and utilizes a narrower range of habitats, primarily open fields (Lloyd, 1966).

*P. pyralis* and *P. ignitus* were studied at two separate field sites, and did not co-occur at either site. *P. pyralis* were collected during June and August 1998 and August 1999 from a field in the Princeton Collection development in Plainsboro, NJ, USA. *P. pyralis* at this location are active from the middle of June through the middle of August, and males fly and produce spontaneous flashes each night from approximately 2000 to 2100 h EDT. Flash duration and interflash interval varies inversely with temperature in *Photinus* fireflies (Lloyd, 1966). Therefore, all measurements of flash timing include the corresponding temperature measured as °F for greater precision. Male *P. pyralis* produce single flashes every 5.9 s at 73°F. These flashes range from approximately 0.4 to 0.6 s in duration at temperatures of 67°-72°F (Lloyd, 1966). Females respond to male flashes from perches on grass or other vegetation with a 0.5 s flash given at an average delay of 2.8 s at 67°F (Lloyd, 1966). Females and males engage in a courtship dialog that consists of a series of male flashes, with female responses to a subset of these flashes. Females respond an average of seven times to a male’s flashes before contact and mating (Vencel and Carlson, 1998).

*P. ignitus* fireflies were collected throughout their mating season from late June to early August from 1997 to 1999 at the Smith Andover field in Lincoln, MA, USA. Male
*P. ignitus* fly and produce spontaneous flashes from approximately 2100 to 2200 h each night at this location. Males produce single flashes every 5 s approximately 0.16 s in duration at temperatures of 72°-74° F (Lloyd, 1966). Females respond to male flashes from perches in the vegetation with 0.24 s flashes given at an average delay of 3.1 s at 77°F (Lloyd, 1966). Females respond to a subset of the many flashes by a given male before contact and mating (personal observation).

**Male Flash Duration**

Male *P. ignitus* and *P. pyralis* were housed individually in clear 250ml plastic containers with moistened filter paper on a natural light cycle. Spontaneously flashing males were observed in these containers (*P. pyralis*) or in larger mesh containers (*P. ignitus*). Male flashes were recorded both in the field and in the lab using a photomultiplier tube (built by G. Johnson) held 10 cm from the firefly, connected to an Intelligent Instrumentation (Tucson AZ) DASport data acquisition system. Flash data were acquired at 1,000 Hz and streamed to disc on an IBM Thinkpad 750. Efforts were made to record male flashes during each night that a male was in captivity. Male flashes were analyzed with Visual Designer software (Intelligent Instrumentation, Tucson, AZ). The duration of each flash was measured as the time interval from ½ maximal intensity at the onset of each flash to ½ maximal intensity as the flash decayed. This technique removes variation brought on by small measurement error in the tails of the flash waveform.

Flash durations were measured for 31 *P. ignitus* males and 39 *P. pyralis* males, calculated as the mean of between 2 to 73 flashes from each male. Male flash duration
was recorded at temperatures ranging from $51^\circ$ F to $83^\circ$ F in *P. ignitus* and from $70^\circ$ F to $82^\circ$ F in *P. pyralis*. Flash duration increased significantly with decreasing temperature in both species (regression, *P. ignitus*: $r^2=.84$, n=31, p<.001, *P. pyralis*: $r^2=.20$, n=39, p=.003). Therefore, male flash durations were adjusted to the temperature used for most flash recordings in each species. *P. ignitus* durations were adjusted to $75^\circ$F and *P. pyralis* durations were adjusted to $76^\circ$F using a least squares linear regression of flash duration on temperature determined for each species (*P. ignitus*: duration = $-4.8$(temp. °F) + 448.1, *P. pyralis*: duration = $-7.3$(temp. °F) + 739.9).

In order to determine whether males showed consistent differences in flash duration, variation within and between males was partitioned using a one-way random effects ANOVA. To eliminate any possible variation due to temperature correction, repeatability (Falconer, 1989) of flash duration in each species was determined only for males recorded at identical temperatures. This analysis involved 8 *P. ignitus* males recorded flashing at $75^\circ$F (2 to 9 flashes each, 33 flashes total), and 6 *P. pyralis* males recorded at $72^\circ$F (2 to 13 flashes each, 36 flashes total).

**The relationship between spermatophore mass and flash duration**

For measurements of spermatophore mass and flash duration, male *P. ignitus* were collected throughout the 1998 male flight season (June to August), while male *P. pyralis* were collected at both the beginning and end of their flight season (early June and middle of August). In order to assess spermatophore quality each male was placed in a container with a field-collected female during the male flight period (app. 2100 h to 2300 h), and the pair was observed every 15 min for stage II of mating. When we observed mated pairs in
stage II, they were allowed to mate for 45 min after which point they were separated by physically pulling them apart. The female was then frozen, insuring that the male spermatophore would not degrade in the female's reproductive tract. We chose 45 min as our time point because previous research indicated that this was the minimum time after which we could be sure the spermatophore had been transferred (personal observation).

Frozen females were dissected in physiological saline to remove the spermatophore from within the bursa copulatrix. The spermatophore was rinsed in dH₂O, placed in a desiccation chamber for 24 h, and weighed to the nearest μm on a Cahn model 21 microbalance to determine spermatophore dry weight. We used regression analysis to determine the relationship between spermatophore mass and flash duration for 14 P. ignitus and 10 P. pyralis males.

Female preference for male flash duration

Female P. ignitus were collected during summer 1998, while P. pyralis females were collected in both 1998 and 1999. Females were maintained in the laboratory in clear 250 ml plastic containers with moistened filter paper on a natural light cycle. All testing was conducted between 2100 and 2300 h at approximately 75°F for P. ignitus and 76°F for P. pyralis, because the range of male flash durations were estimated at these temperatures, and the range of flash durations to which females respond varies with temperature in the same manner as male flash duration (personal observation). Simulated male flashes were created using Visual Designer 3.0 software (Intelligent Instrumentation, Tucson, AZ) on an IBM Thinkpad 750; flashes alternated between two light emitting
diodes (Radio Shack T-1\(\frac{3}{4}\) size yellow LEDs) driven by the output channel of an Intelligent Instrumentation DASport data acquisition system.

Females were presented with simulated male flashes at five flash durations chosen to represent the range of male flash durations measured for each species: 55, 63, 71, 79, and 87 ms for *P. ignitus*; 108, 140, 180, 212 and 244 ms for *P. pyralis*. Flashes at each of the five durations were presented in groups of four flashes for a total of twenty flashes. Each flash was presented 8 s after the previous flash for *P. ignitus* and 6 s after the previous flash for *P. pyralis* to approximate conspecific male interflash interval at the testing temperature. Twenty-five females of each species were tested with flashes in one of five different presentation orders in a Latin-squares randomized block design. Female flash response to each male flash was scored as yes or no, depending on whether the female produced a flash before presentation of the next simulated male flash. Females that did not respond to any flashes were not included in the analysis because they offered no information on relative preference.

In a second *P. ignitus* experiment, 33 females were tested to determine if female preference extended beyond the range of duration observed in conspecific males. The same testing regime described above was used with three longer flash durations of 80, 108, and 132 ms. These longer durations represent respectively the average *P. ignitus* male flash duration, an extreme (approximately 2 SD above the mean) *P. ignitus* flash duration, and a flash duration beyond the range observed in *P. ignitus*.

Statistical analysis was conducted using the Generalize Estimating Equations (GEE) procedure of SAS version 6.12 (SAS Institute, 1994). For this experimental design the binary response variable precluded using a 2 way ANOVA, and logistic
regression could not have specifically accounted for both the repeated measurements made at each treatment level within subjects and the repeated measurements made on the same subjects across treatments. GEE analysis treats each female response or lack of response as a correlated binary event, and allowed testing for the effect of flash duration with the presentation order effect removed. Duration was treated as a continuous variable for the experiments in both species, with five duration treatment levels. In the second *P. ignitus* experiment, female responsiveness to the two shorter flash durations, 80 msec. and 108 msec., was compared to the longest duration to examine female responsiveness beyond the observed range of male flash duration.

**Effects of mating and feeding on female responsiveness**

To test for changes in female responsiveness as a result of female mating and nutritional status, the female testing protocol described above was used on field-collected *P. ignitus* females assigned to 3 experimental treatments: unmanipulated, lab-mated and lab-fed. For females in all treatments, mating history prior to collection was unknown, but *Photinus* fireflies do not feed as adults in the field (Williams, 1917; Lloyd, 1997).

A total of 28 unmanipulated *P. ignitus* females (including the 25 females analyzed for preference across 5 flash durations) were collected and tested without any lab mating or food supplement. Ten additional females were collected from the field and allowed to mate once in the lab to field-collected males 1-3 nights before testing. A third group of 10 females was provided with 0.75ml of 1% casein, 5% glucose solution in water, which females drank from a sponge. Females in each treatment were individually presented with
a series of 20 flashes, four flashes at each of 55, 63, 71, 79, and 87 msec duration as described above.

Female responsiveness was measured as total percentage of simulated flashes across all durations to which a female responded. A nonparametric Kruskal-Wallis test was used to compare female responsiveness across the treatments because data were not normally distributed.

Results

Male flash duration

Flash durations measured for individual *P. ignitus* males (corrected to 75°F) ranged from 56 to 123 ms with a mean duration of 77.3 ms (± 12.8 SD, Figure 1a). Flash durations measured for individual *P. pyralis* males (corrected to 76°F) ranged from 117 to 283 ms with a mean of 180.6 ms (± 41.7 SD, Figure 1b). Ninety percent of males produced flashes with a duration in the range of 56 to 89 ms in *P. ignitus* or 108 to 240 ms in *P. pyralis*, corresponding to the range of flashes used in the female preference experiments. Flash durations of both *P. ignitus* and *P. pyralis* males were highly repeatable (one-way ANOVA, *P. ignitus*: repeatability = .92, *P. pyralis*: repeatability = .79).
Figure 1

a) Frequency histogram of male flash duration for *Photinus ignitus* (n = 31 males, durations averaged across 2 to 73 flashes per male). Flash durations adjusted to common temperature of 75°F using the equation: corrected duration = measured duration (msec) - 4.8 (75°F - measurement temperature°F).

b) Frequency histogram of male flash duration for *Photinus pyralis* (n = 39 males, durations averaged across 2 to 29 flashes per male). Flash durations adjusted to common temperature of 76°F using the equation: corrected duration = measured duration (msec) - 7.3 (76°F - measurement temperature°F).

The relationship between spermatophore mass and flash duration

We found no relationship between flash duration and spermatophore mass for either *P. ignitus* or *P. pyralis* (Figure 2). Flash duration predicted less than 2% of variation in *P. ignitus* spermatophore mass (Figure 2a, linear regression, \( r^2 = 0.017, n = 14, p = 0.656 \)). Flash duration predicted 20% of variation in *P. pyralis* spermatophore mass (Figure 2b, linear regression, \( r^2 = 0.203, n = 10, p = 0.192 \)).
Figure 2
The relationship between body mass and spermatophore mass with the regression line plotted for a) 14 *Pholcus ignitus* males, and b) 10 *Pholcus pyralis* males.

**Female preference for male flash duration**

Responsiveness for both *P. ignitus* and *P. pyralis* females tested in the lab ranged from 15 to 100% responsiveness when presented with 20 simulated male flashes varying in duration (Figure 3a, 3c). Female response increased as flash duration increased, reaching a maximum at 87 ms in *P. ignitus* (Figure 3a) and 212 ms in *P. pyralis* (Figure 3c). After accounting for variation in female response produced by order of flash presentation, female responsiveness increased significantly with increasing flash duration in both species (GEE, *P. ignitus*: \( Z = 2.46, p = .014 \), *P. pyralis*: \( Z = 3.48, p = .0005 \)).

In the second experiment testing *P. ignitus* females with longer flash durations, females maintained high responsiveness to simulated male flashes up to 108 ms in duration (Figure 3b), representing the upper extreme of flash durations observed in *P. ignitus* males.
(Figure 1a). However, female response declined significantly when females were presented with 132 ms simulated flashes, which are longer than any flashes observed in *P. ignitus* males (GEE, 132 msec. vs. 80 msec.: $Z = 5.29, p<.00005$, 132 msec. vs. 108 msec.: $Z= 6.40, p<.00005$).

Figure 3

a) Mean percentage of simulated flashes (+ SE) at each flash duration to which female *P. ignitus* responded. Female response based on 25 females each presented with four flashes at five flash durations within the observed range of male flashes.

b) Mean percentage of simulated male flashes (+ SE) at each flash duration to which 33 *P. ignitus* females responded. Durations represent average *P. ignitus* male flash duration, long flash duration, and flash duration beyond the range of male flashes recorded.

c) Mean percentage of simulated male flashes (+ SE) at each flash duration to which female *P. pyralis* responded. Female response based on 25 females presented with four flashes at five flash durations representing the observed range of male flashes.
Effects of mating and feeding on female responsiveness

For individual, field-collected, unmanipulated *P. ignitus* females, responsiveness to 20 simulated male flashes (four flashes at each of five durations: 55, 63, 71, 79, and 87 ms) ranged from 0 - 100%, with a mean responsiveness of nearly 50% (Figure 4). Responsiveness to the same series of simulated flashes was considerably lower for both lab-mated females and fed females, and differed significantly among the three treatments (Kruskal Wallis test, H = 6.42, p = .04).

**Figure 4**
Percentage (mean + SE) of 20 flashes to which *P. ignitus* females responded. Treatments are: field-collected females tested without further manipulation (*n* = 28), females mated once in lab to field-collected males between 1 and 3 days before testing (*n* = 10), and fed females (*n* = 10) provided with 1% casein, 5% sucrose solution for 1-5 days prior to testing.

Discussion

In this study female responsiveness to male flashes varied as a function of male flash duration. Females of both *P. pyralis* and *P. ignitus* preferentially responded to longer duration simulated flashes within the range of flash duration observed in conspecific males. Our results suggest that female preference for longer duration male flashes could be a widespread phenomenon within single-flashing *Photinus* fireflies. This finding is consistent with previous evidence documenting female preference for increased pulse rate in the flash pattern of *P. consimilis* (Branham and Greenfield, 1996). Both of these
studies suggest potential directional selection for flash duration through female preference. In 16 of the 22 Photinus species surveyed by Lloyd (1966), male flash patterns consist of single pulses repeated at intervals, as in P. ignitus and P. pyralis. Therefore, female preference for male flash durations representing the upper end of the species range may be common within the genus Photinus.

Female preferences for male traits do not always generate preferential mating success for males possessing those traits (Jennions and Petrie, 1997; Wagner, 1998; Widemo and Saether, 1999). For instance, female preference for long duration flashes in P. ignitus or P. pyralis may not be as important as female preferences for other male traits or male competitive interactions in determining male mating success. P. pyralis females prefer both more intense flashes as well as the first flash presented when simulated flashes are generated in pairs separated by 500 msec (Vencl and Carlson, 1998). P. ignitus females prefer simulated flashes from artificial lanterns of larger size or closer proximity (this thesis, Chapter 4). In both species, female preferences for flash characteristics and competitive male interactions such as the relative timing of male flashes or male ability to rapidly approach the female may all mediate male mating success.

Larger lantern and body sizes relative to the population mean in P. pyralis males that successfully mate in the absence of competitors (Vencl and Carlson, 1998), may indicate female preference for large male body size or lantern size. Wider lanterns in successfully mating P. ignitus males relative to unsuccessful males (this thesis, Chapter 2) also suggest female preference for a trait other than flash duration. However, flash duration, body size and lantern size could all represent intercorrelated traits which females use as indicators of male quality. The strong relationship between P. ignitus male body
mass and spermatophore mass early in the season (this thesis, Chapter 2) indicates that females could potentially benefit by mating with males with large body sizes early in the season in order to obtain a larger spermatophore contribution.

The potential benefits of female preference have been widely debated (Andersson, 1994). On the one hand, female preferences have been theorized to result either from direct benefits (Heywood, 1989; Hoelzer, 1989; Wolf et al., 1997) or indirect genetic benefits (reviewed by Kirkpatrick and Ryan, 1991; Andersson, 1994). In contrast, female preference has also been suggested to occur in the absence of any benefit to females, as a byproduct of existing biases in the female sensory system (Kirkpatrick and Ryan, 1991; Ryan, 1990; Ryan and Rand, 1993). We found no evidence that female preference for longer male flash duration directly benefits females by allowing them to identify males having large spermatophores. However, we sampled males throughout the season, and since male spermatophore mass decreases with successive matings, it is possible that a strong relationship between flash duration, spermatophore mass and body mass only occurs early in the season when most males are virgins. Female preference for male flash duration could also benefit females either directly or indirectly through some aspect of spermatophore quality other than spermatophore mass that covaries with flash duration, or through genetic benefits associated with long male flash duration. Finally, the similar trend in two different species may suggest that underlying sensory biases in Photinus fireflies produce female preference for longer duration flashes.

Although female responsiveness in both P. ignitus and P. pyralis increased with longer male flash durations within the observed range in each species, female P. ignitus response declined when flashes were longer in duration than those produced by
conspecific males. This decrease is consistent with previous evidence (Lloyd, 1966) that male flash duration is one aspect of flash timing along with the interval between paired pulses and the number of pulses within a flash pattern that provide mechanisms for species recognition. Geographic ranges of *P. ignitus* and *P. pyralis* overlap (Lloyd, 1966), although the two species did not co-occur at either study site. *P. ignitus* female responsiveness declined significantly when presented with long duration flashes corresponding to the lower range of *P. pyralis* male flash duration, and *P. pyralis* female responsiveness was lowest for short duration flashes corresponding to the upper range of *P. ignitus* flash durations. This suggests that in areas of sympatry, female preferences may allow them to avoid heterospecific courtship interactions. Both species also co-occur with predatory *Photuris* fireflies (personal observation), which prey on male *Photinus* by mimicking female *Photinus* flash responses (Carlson and Copeland, 1978; Lloyd, 1965, 1975; Nelson et al., 1975; Vencel et al., 1994; Zorn and Carlson, 1978). In addition *Photuris* females have been shown to use spontaneous flashes as cues to locate and attack *Photinus* fireflies (Lloyd and Wing, 1983). Therefore, inappropriate responses by females to heterospecific flashes could expose them to an increased risk of predation.

In order to understand the potential for sexual selection through female choice, it is necessary to understand variation in female choosiness within a species (Jennions and Petrie, 1997; Widemo and Saether, 1999). In this study *P. ignitus* female responsiveness declined for mated females as well as for females fed an artificial diet, indicating that female nutritional status can influence female courtship behavior. Previous work on *P. ignitus* has demonstrated that a majority of radiolabeled amino acids derived from male spermatophores are incorporated into a female’s developing oocytes within two days after
mating (Rooney and Lewis, 1999). Our results support the hypothesis that changes in female nutritional status produce variation in female choosiness, since similar reductions in female responsiveness were observed with lab-mated and lab-fed females. Female nutritional status has been shown to influence mate choice for other species in which males transfer spermatophores. For instance, hungry female water mites (*Neumania papillator*, Proctor, 1991) initiate contact with a greater number of males, and hungry katydids mate more frequently (*Kawanaphila nartee*, Simmons and Bailey, 1990).

Female mate choice within populations can influence the evolution of male courtship signals (Andersson, 1994; Bakker and Pomiankowski, 1995; Kirkpatrick and Ryan, 1991; Ryan and Keddy-Hector, 1992). This process represents one possible mechanism through which speciation may occur (Endler, 1989; Lande, 1981). However, variation in female preference can influence the strength and direction of sexual selection via mate choice (Jennions and Petrie, 1997). Female preference in *P. ignitus* and *P. pyralis* fireflies occurs for longer duration male flashes. No relationship was found between male flash duration and spermatophore mass, but female condition as a result of spermatophore contributions from prior matings can produce variation in female choosiness. Therefore, directional selection for longer duration male flashes may vary as a function of female mating and nutritional status.
Chapter 4:

Sexual selection in *Photinus ignitus* fireflies: Exploring the roles of female preference and male competition

Abstract

Female preference and male competition can each generate sexual selection for male traits. Although it is possible to experimentally isolate male characteristics that females prefer, the extent to which such female preferences generate differential reproductive success may depend on how male competitive interactions limit a female's ability to discriminate among potential mates. We combined observations of *Photinus ignitus* courtship and mating in field enclosures with tests of female preference for male flash characteristics to determine the roles of female preference and male competition. We paired large vs. small and lab-mated vs. control males in field enclosures with single females. There was no effect of either treatment on courtship or mating. Successfully mating males produced more flashes, and elicited female responses to a greater percentage of their flashes than their unsuccessful counterparts in the enclosures. Successfully mating males also moved closer to the female during the first 5 min of courtship than did unsuccessful males. We tested female preference for simulated flashes produced by artificial lanterns of varying size and distance from the female. *P. ignitus* females preferred both larger and closer lanterns. These data suggest that male flash activity, male movement, and female responsiveness may all combine to determine male mating success, because female preference may be influenced as much by male proximity as by male flash characteristics.
Introduction

Female preferences across a range of natural variation in male courtship signals are often used as evidence for the potential for mate choice in a species (Jennions and Petrie, 1997; Wagner, 1998). Auditory, olfactory, visual and tactile signals have been experimentally manipulated to isolate specific elements of the signal eliciting female response (Andersson, 1994). This approach is invaluable for identifying traits which may be subject to sexual selection through mate choice. However, female preferences for particular male traits may not always lead to differential mating success for males with those traits (Jennions and Petrie, 1997; Wagner, 1998; Widemo and Saether, 1999). The potential for females to assess and discriminate among potential mates under natural conditions may be influenced by many factors including female condition, female-female interactions, male-male interactions, and environmental conditions (Jennions and Petrie, 1997; Widemo and Saether, 1999). Patterns of differential mating success may result from female preferences for particular male phenotypes, but they may also be influenced by male competition or constraints on a female’s ability to assess male phenotype.

Photinus fireflies provide an excellent system for examining female preferences for male signal traits because male fireflies produce bioluminescent courtship flashes to which females respond with courtship flashes of their own, and males require female responses in order to locate, contact and mate with females. By simulating male flashes and observing female response, male flash characteristics influencing the likelihood of eliciting a female response have been identified (Branham and Greenfield, 1996; Vencl and Carlson, 1998; this thesis, Chapter 3). However, by focusing on female preferences for male courtship
signals we run the risk of ignoring male competitive behaviors that can generate differential mating success.

In both *P. ignitus* and *P. pyralis*, male body size has been found to predict mating success under certain conditions. *P. pyralis* males that successfully mated in the absence of competitors had significantly greater lantern areas and elytral lengths than the population mean (Vencel and Carlson, 1998). We have found that mated *P. ignitus* males have significantly larger lanterns than unmated males, particularly early in the season when male densities are high (this thesis, Chapter 2). Female preferences for temporal characteristics of male courtship flashes have been demonstrated (Branham and Greenfield, 1996; this thesis, Chapter 3), as well as female preference for greater flash intensity (Vencel and Carlson, 1998). Previous studies also suggest two possible sources of male scramble competition, total male flash production and male walking speed (Vencel and Carlson, 1998). Males that produce more flashes can get more information about female location from female responses to those flashes, and faster males can reach the female first. We hypothesized that differential mating success as a function of male body size in *Photinus* fireflies may be based on male flashing frequency and male searching speed rather than female preference for male flash characteristics.

In order to determine the importance of male competitive activity and female preference in producing differential mating success, we experimentally paired large vs. small males as well as lab-mated vs. lab-isolated, control males in mating enclosures with single females. We observed courtship and mating to determine the relative importance of male size, prior mating history, male flash activity, male searching speed and female preference on the mating outcome. To further explore how male competition might
generate differential mating success we used simulated male flashes to test how male size and proximity to the female could influence female preference. We manipulated flash intensity by altering both artificial lantern size and distance from female to test the hypothesis that female preference for simulated male flashes in *P. ignitus* would increase with both decreasing distance and increasing lantern size. By combining experiments that examine female preference for male courtship signals with observations of mating behavior and differential mating success in field enclosures, we examine the potential for female preference and male competition to generate differential mating success in *Photinus ignitus* fireflies.

**Methods**

**Male mating success in enclosures**

Paired male and single female *P. ignitus* were observed in mating enclosures from early season (June 21) to mid season (July 18) 1999 in Smith Andover field, Lincoln, MA USA. Males were collected each night and held in clear 250 ml plastic containers with moistened filter paper. Male elytral length and elytral width were measured to the nearest 0.1 mm with calipers the following day and males were weighed to the nearest 0.1 mg. Females were captured each night and maintained overnight in the field. Each night paired males and single females collected the prior night were observed in one of four 32 x 32 x 78 cm screen enclosures. Males were distinguished by blue or pink paint dots on their left elytron.

Enclosure observations were initiated between 2100 and 2330 h. Before each observation the female was placed on or adjacent to a synthetic plant at the bottom of the
enclosure and allowed to acclimate for 5 min. Two containers, each containing one male, were then suspended on opposite sides of the enclosure with their lids 10 cm from the top, and the container lids were removed. Observations of flash behavior included the time of each male’s first flash, as well as the time the female first responded to each male. Total number of flashes from each male and female responses to each male were also recorded until male and female flashing ceased. During periods of flash quiescence male location and identity were verified by illuminating the males with blue-filtered light. If flash cessation indicated that a male had contacted the female, the times of initial male contact, and the timing of stages I and II of mating were also verified under blue light and recorded. If neither male had contacted the female or if the male did not achieve stage II of mating we resumed observations of flash behavior. Observations were terminated when one of the males successfully mated with the female, when 30 min had passed with no male or female flashing, or if no mating occurred by 2400 h.

Two separate experiments were conducted in the field enclosures, pairing males by either body size or mating history. The first of these experiments was conducted during the early portion of the flight season from June 21 to July 6. In the first experiment males differing in body size were paired based on differences in body mass, and elytral length. These measurements were compared using paired t-tests to confirm that there were significant size differences. Large males had significantly greater wet weights than small males (paired t-test, t = 9.9, df = 19, p < .001; mean ± SE = 20.9 ± 0.8 mg vs. 13.6 ± 0.5 mg) and significantly longer elytral lengths (t test, t = 13.4, df = 17, p < .001 mean ± SE = 7.7 ± 0.1 mm vs. 6.8 ± 0.1 mm).
The second experiment was conducted during the middle of the season from July 6 to July 18, and compared males differing in mating history. Since male *P. ignitus* produce proteinaceous spermatophores (Rooney and Lewis, 1991; van der Reijden et al., 1997), do not feed as adults (Lloyd, 1997; Williams, 1917), and produce smaller mass spermatophores with successive matings (this thesis, Chapter 2), we manipulated male condition by mating males in the lab. Field-collected males were given the opportunity to mate in the lab the night after they were collected. Males that did mate were then paired with control males (males who did not mate the previous night) of similar mass and elytral length. Every other night, paired males collected two nights before and single females collected the prior night were observed in the enclosures. For these observations the enclosures were divided into thirds vertically (top, middle, and bottom, each 26 cm high), and the vertical position in the enclosure of each male was noted when spontaneous male flashing began and again 5 min later. Male movement was scored on a scale of 0 to 4: 0 indicated males that did not leave their containers, 1 indicated males remaining in the top third of the enclosure (farthest from the female), scores of 2 or 3 represented movement to the middle or bottom third of the enclosure, and 4 indicated males who contacted the female within the 5 min period.

Enclosure observations were selected for statistical analysis if they met a series of minimum criteria chosen to insure that both males were actively attempting to court and mate with the female. Observations were excluded in which neither male mated with the female, one male contacted the female but did not attempt to mate with her, the female failed to respond to either male, or one of the males never flashed. For enclosure observations pairing males by body size, 20 out of 31 met the criteria, while for males
paired by mating history, 11 out of 20 observations met the criteria. Paired t-tests were used to compare the number of flashes produced by the two males, as well as to compare the percentage of each male's flashes receiving female responses. We used binomial tests to determine whether large vs. small and lab-mated vs. control males were equally likely to successfully mate.

Because we found no effect of either experimental treatment on mating success, we pooled the data from the two enclosure experiments in order to test what variables predicted mating success overall. For each enclosure experiment, the male that mated with the female was considered the successfully mated male, while the other male in the enclosure was categorized as the unsuccessful male. Paired t-tests were used to compare the number of flashes produced as well as the percentage of flashes to which the female responded for pairs of successfully mating males and their unsuccessful counterparts.

Movement of successfully mating and unsuccessful males was compared using a Wilcoxon signed ranks test for ordinal data. Male flash activity and female responsiveness during these 5 min observations were compared using paired t-tests.

**Female responsiveness as a function of male lantern size and distance**

Female *P. ignitus* collected during July and August 1999 were maintained in the laboratory in clear 250 ml plastic containers with moistened filter paper on a natural light cycle. All testing was conducted between 2100 and 2300 h at approximately 75°F. Simulated male flashes were created using Visual Designer 3.0 software (Intelligent Instrumentation, Tucson, AZ) on an IBM Thinkpad 750; flashes were presented using one of three light emitting diodes (Radioshack T-1 3/4 size yellow LED) driven by the output
channel of an Intelligent Instrumentation DASport data acquisition system. To approximate the range of lantern sizes observed in male *P. ignitus* (mean ± SD lantern area = 3.4 ± 0.7 mm², n = 77), each LED was enclosed in a 0.5 ml microcentrifuge tube covered with black electrical tape, except for openings of 2, 4, and 6 mm² created by varying the width of the openings while holding height constant. These artificial firefly lanterns were framed by 12 mm long cardboard firefly silhouette, and mounted 10 cm apart on a black background.

Females were presented with four flashes from each of these three differently-sized artificial lanterns for a total of 12 flashes at a particular distance. Each group of four flashes was presented with an 8 s delay between successive flashes, and a 30 s time delay between groups of four flashes. Each female was presented with the sequence of 12 flashes at distances of approximately 12, 24, and 48 cm to represent distances within the 1m radius within which courtship dialogs occur (Lewis and Wang, 1991; this thesis, Chapter 2). Females were allowed to acclimate for at least 5 min after being moved to a new distance. Twenty-seven females were tested with one of 9 combinations of distance and lantern size orders in a Latin-squares randomized block design. Female flash response to each male flash was scored as yes or no, depending on whether the female produced a flash before presentation of the next simulated male flash. Females that did not respond to any flashes were not included in the analysis because they offered no information on relative preference.

Statistical analysis was conducted using the Generalize Estimating Equations (GEE) procedure of SAS version 6.12 (SAS Institute, 1994) which treats each female response or lack of response as a correlated binary event. We tested for the effect of both
lantern size and distance while accounting for the presentation order effect. Distance and lantern size were treated as discrete variables for the experiment, with the largest lantern size and the furthest distance used as reference categories.

Results

Male mating success in enclosures

There were no significant differences between large and small males in either the number of flashes they produced during courtship or the percentage of those flashes to which females responded (Figure 1a,b: paired t-tests; number of flashes $t = -0.891$, df $= 19$, $p = 0.384$, $1-\beta = 0.14$; % response $t = 0.017$, df $= 19$, $p = 0.987$, $1-\beta = 0.06$). The larger male mated in 45% of the observations, which shows no significant deviation from 50% (binomial test, $n = 20$, $p = 0.8238$, $1-\beta = 0.07$).

Figure 1
Comparison of flash behavior for large ($n = 20$) vs. small ($n = 20$) *P. ignitus* males in enclosures: (a) Mean (+ SE) number of flashes produced by large and small males (b) percentage of flashes eliciting a female response for large and small males.
Similarly, there were no significant differences between lab-mated and control males in either the number of courtship flashes they produced or the percentage of those flashes to which females responded (Figure 2a,b: paired t-tests; number of flashes \( t = -0.258, df = 10, p = .802, 1-\beta = .06 \); % response \( t = 0.302, df = 10, p = .769, 1 - \beta = .06 \)). Control males successfully mated with the female in 73% of observations, which shows no significant deviation from 50% (binomial test, \( n = 11, p = .2266, 1 - \beta = .35 \)).

![Figure 2](image)

Comparison of flash behavior for lab-mated (\( n = 11 \) mated the night prior to enclosure observation) vs. control (\( n = 11 \) that did not mate in the lab) \( P. \) ignitus males in enclosures: (a) Mean (+ SE) number of flashes produced by lab-mated and control males (b) percentage of flashes eliciting a female response for lab-mated and control males.

When results from both sets of enclosure experiments were pooled, males who had successfully mated were found to have produced significantly more flashes and elicited female responses to a significantly greater percentage of those flashes than their unmated counterparts (Figure 3a,b: Paired t-tests; number of male flashes, \( t = 2.757, df = 30, p = .010 \); % female response, \( t = 2.743, df = 30, p = .010 \)).
Figure 3
Comparison of flash behavior for successfully mated (n = 31) vs. unsuccessful mating (n = 31) P. ignitus males in enclosures: (a) Mean (+ SE) number of flashes produced by successful and unsuccessful males (b) percentage of flashes eliciting a female response for successful and unsuccessful males.

When lab-mated and control male pairs were observed for 5 minutes from the start of flash activity, there were no significant differences detected in number of male flashes or percentage of female responses between successfully mating males and their unsuccessful counterparts in the same enclosure (paired t-tests: number of male flashes, t = .644, df = 10, p = .534, 1-β = .09; % female response, t = 0.067, df = 6, p = .949, 1-β = .05). There was, however, a significant difference in how far males moved toward the female during their first five minutes of flash activity. We observed males approaching females by walking and flying from the top of the enclosure down towards the female’s perch. Males that eventually mated had moved closer to the female during the first 5 min of their flash activity than their unsuccessful counterparts (Figure 4: Wilcoxon signed ranks test, Z = -2.058, n = 11 pairs, p = .04).
Figure 4
Number of *P. ignitus* males in each movement category for males who successfully mated (n = 11) and males who were unsuccessful in mating (n = 11) during courtships in second enclosure experiment. Male movement was scored on a scale of 0 to 4: 0 indicated males that did not leave their containers, 1 indicated males remaining in the top third of the enclosure, scores of 2 or 3 represented movement to the middle or bottom third of the enclosure, and 4 indicated males who contacted the female within the 5 min period.

Female responsiveness as a function of male lantern size and distance

Female responsiveness increased with increasing artificial lantern area across all distances tested (Figure 5). Simulated flashes from the largest lantern elicited significantly greater female response than flashes from the smallest lantern (GEE, n = 27, Z = -6.46, p < .0001), but there was no significant difference in female response between largest and intermediate lanterns (GEE, n = 27, Z = -1.598, p = .1101). Female responsiveness decreased with increasing distance from the artificial lantern (Figure 5). The longest distance (48cm) elicited significantly lower female response than either the shortest distance or the intermediate distance (GEE, n = 27, for 48cm vs. 24cm lantern distance Z = 4.2696, p < .0001, and for 48cm vs. 12cm lantern distance Z = 6.6973, p < .0001).
Figure 5
Mean (+ SE) percentage response by 27 P. ignitus females presented with simulated flashes from 3 different sizes of artificial lanterns (2, 4, and 6 mm²) placed at 3 different distances from females (12, 24, and 48 cm).

Discussion

This study failed to find evidence for differential mating success based on male body size or prior mating history, as neither number of flashes, % female response, or mating success differed between large vs. small males or lab-mated vs. control males. This lack of influence of male body size on mating success is in stark contrast to one other field study of Photinus mating success. In P. pyralis, large males experienced increased mating success in courtships without competitors, while smaller males were more likely to mate in the presence of three or more competitors (Vencel and Carlson, 1998). In contrast, field studies of P. ignitus showed that large males experienced increased mating success only early in the season when the sex ratio was male-biased so males had many competitors (this thesis, Chapter 1). Our enclosure experiments did not take into account male searching behavior and female responsiveness during long distance male flights, nor did our enclosure experiments simulate conditions of extremely high male density. Therefore, differential mating success in the field may be the product of interactions which were not incorporated into our field enclosure experiments.
This study provides evidence that male movement rate, male flash behavior, and female responsiveness each contribute to determining male mating success in field enclosures. In both enclosure experiments, males that eventually mated successfully had produced more courtship flashes than their unsuccessful counterparts, and in the second enclosure experiment successful males had moved closer to the female during the first five minutes of courtship. Both of these findings are consistent with observations in *P. pyralis* that mated males had produced more flashes than unmated males and that there were intraspecific differences in vertical climbing speed (Vencl and Carlson, 1998). Vertical climbing speed in *P. pyralis* was measured by timing males as they climbed out of a vertical tube at dusk (Vencl and Carlson, 1998). Our results more directly assess a male’s search ability by measuring his rate of approach towards a responsive female. Since males both flew and walked in the enclosures, the ability to correctly orient towards the female rather than climbing speed likely explains differences in male movement towards the female. Males that ultimately successfully mated also elicited a higher % female response than unsuccessful males, consistent with previous field findings in *P. ignitus, P. aquilonius* and *P. marginellus* (this thesis, Chapter 2; Lewis and Wang, 1991).

Since male searching behavior, male flashing behavior and female responsiveness are all associated with mating success, it is very difficult to infer causation. Increased female responsiveness towards a particular male could stimulate that male to flash more frequently, and could also provide the male with more information about the female’s location, allowing him to move towards her more quickly. In contrast, as males approach females, their proximity may stimulate both increased male flashing activity and increased female responsiveness.
Two lines of evidence from our research support the possibility that male proximity influences female responsiveness. In the second enclosure experiment, males that ultimately mated had moved significantly closer to the female than their competitors, in spite of there being no significant difference in the number of flashes they produced or female responsiveness to those flashes during the initial 5 min observation of courtship. Since successful males ultimately did produce more flashes and receive responses to a greater proportion of their flashes over the course of an entire courtship, this increased flashing and responsiveness may be the result of closer male proximity after this first 5 min of observation. Our findings in the lab that female responsiveness to simulated male flashes increased with decreasing distance between the female and the artificial lantern also support the possibility that male proximity may influence female responsiveness. We also found that *P. ignitus* female responsiveness increased with higher intensity generated by larger LED surface area. Female responsiveness to all three lantern area treatments decreased with increasing distance so that females responded to a greater proportion of small lantern flashes from 12cm than large lantern flashes from 48cm. This result suggests that male distance may be just as important as male lantern size in determining female responsiveness.

Female *P. ignitus* preference for closer flashes may be due to a general female preference for greater flash intensity. Vencel and Carlson (1998) demonstrated female preference in *P. pyralis* for higher intensity flashes produced by bare LEDs. Closer flashes may be perceived as being more intense because light intensity varies as the inverse of distance squared. Larger artificial lanterns may be perceived as more intense as well since less of the underlying LED is obscured. Our current knowledge of insect visual acuity
suggests that fireflies may be unable to distinguish between lanterns that differ in size by only a few millimeters at our minimum testing distance of 12 cm. Since the angle between detectors (1.8° in larger, predatory Photuris versicolor fireflies, Land, 1997) would subtend a width of approximately 4mm at this distance, size differences smaller than 4mm could not be distinguished by the firefly visual system. The angle between detectors in the firefly eye also combines with a small eye-span to limit the potential for distance estimation through binocular vision in Photinus fireflies, with 12 mm representing a distance that theoretically could not be distinguished from any further distances. Simple binocular vision does not represent the only method of distance estimation in insects (e.g. Sobel, 1990), and our understanding of the limitations of the firefly visual system is still incomplete. Female preferences for greater flash intensity in P. ignitus could explain increased female responsiveness to both closer and larger artificial male lanterns.

By combining careful observation of P. ignitus mating behavior in enclosures in the field with lab tests of female preference for male flash characteristics, we have demonstrated that mating outcomes in P. ignitus represent a complex interaction of male advertising behavior, male searching ability, and female responsiveness. While female mate choice and male competition each have the potential to generate sexual selection, male competitive behaviors and female preferences can also directly influence one another during courtship. Therefore, complete understanding of the potential for sexual selection in a species requires a careful combination of tests for female preference, observation of courtship behavior under controlled conditions, and data on the correlates of mating success in the field.
Chapter 5:

Discussion

This research used *Photinus ignitus* fireflies as a model system to explore sexual selection on male courtship signals through female preference, and to determine how male spermatophore contributions influence mate choice through their effects on mate preference and mate competition. There is increasing evidence that female *Photinus* fireflies show preference for certain male flash characteristics (Branham and Greenfield, 1996; Vencel and Carlson, 1998). This study provides additional evidence of female preference for male flash characteristics in both *P. ignitus* and *P. pyralis*. However, this study also demonstrates variation in female preferences, male competition, and male mate choice, all of which may influence differential mating success and mitigate the role of female preference in generating sexual selection for male flash characteristics.

Mating competition and mate choice within a species have been shown to vary with the relative investment made by males in spermatophores (Gwynne, 1985; Gwynne and Simmons, 1990; Simmons and Bailey, 1990). Patterns of variation in *P. ignitus* spermatophore mass, courtship behavior, and the traits correlated with mating success were consistent with predictions that male competition and female choice would be prevalent when male spermatophore masses were relatively high and variable, while male choice and female competition would dominate when the potential for male spermatophore production was limited. Male spermatophore mass declined from early season to late season, perhaps in part due to decreasing spermatophore masses across successive matings. Early in the season successfully mated males had larger lanterns, and
received responses to a significantly greater proportion of their courtship flashes than unsuccessful males, suggesting that male flash competition or female choice may have been occurring. Later in the season no differences were observed between successful and unsuccessful males, but the occurrence of aborted matings increased as the season progressed, and females from aborted matings had fewer mature eggs than successfully mating females. Therefore, male choosiness, perhaps for larger females containing more eggs, appeared to increase as declining male nutrient reserves limited spermatophore production late in the season.

The OSR is influenced by the adult sex ratio as well as by relative parental investment (Clutton-Brock and Parker, 1992; Kvarnemo and Ahnesjö, 1996; Parker and Simmons, 1996). Male protandry has been suggested to occur in some Photinus species (Cicero, 1983; Lewis and Wang, 1991) and there is likely higher male mortality due to predation by both spiders (Lloyd, 1973) and Photuris fireflies (Lloyd, 1965). Regardless of its cause, the shift in local sex ratio from male biased to female biased was accompanied by the corresponding shift in courtship behavior predicted from OSR theory. Female responsiveness increased over the course of the season with decreasing male densities, supporting the hypothesis that low female responsiveness under male biased sex ratios represent female choosiness, while high female responsiveness under female biased sex ratios reflects increased female competition (Lewis and Wang, 1991).

This change in female responsiveness may at least in part be attributable to female mating history. In the lab both lab-mated and lab-fed P. ignitus females showed lower overall responsiveness to all male flash durations relative to control females. This result suggests that female condition as influenced by female mating and nutritional status can
influence female responsiveness. Seasonal variation in female responsiveness may result from variation in female mating status with early season females more likely to have recently mated and less likely to be actively seeking the nutritional benefits of a spermatophore, while under low male densities late in the season females are less likely to have recently mated resulting in higher responsiveness.

Female responsiveness could also potentially be influenced by male spermatophores if females responded preferentially to males possessing flash characteristics that were honest indicators of spermatophore quality. Female preferences measured as differential female responsiveness have been demonstrated across the natural range of variation in male flash characteristics for one other Photinus species (Photinus consimilis, Branham and Greenfield, 1996). However, nothing is known about the relationship between flash characteristics and spermatophore quality in P. consimilis. Female P. ignitus and P. pyralis both exhibited greater preference for simulated flashes representing the upper range of conspecific male flash duration. However, male flash duration varied independently of male spermatophore mass in both species. These results demonstrate the potential for female preference to produce directional selection for longer duration flashes, but provide no evidence for male flash duration as an honest indicator of spermatophore mass.

Although spermatophore mass could not be predicted by flash duration, the strong relationship between spermatophore mass and both body mass and body size suggests that females could potentially use some male characteristic to assess spermatophore mass prior to mating. In addition, the strength of the relationship between body mass and spermatophore mass decreases as the season progresses and with successive male matings.
Therefore, strong relationships between male characteristics such as flash duration and spermatophore mass may only be observed early in the season, and the relationship may have been obscured in this study by sampling males over the entire season. Other male flash characteristics such as flash intensity and lantern size could predict spermatophore mass since this study demonstrated that male lantern size is proportional to body size in *P. ignitus*, and these two male traits are also highly correlated in *P. pyralis* (Vencl and Carlson, 1998). Although neither the relationship between lantern size and flash intensity nor the ability of the female sensory system to distinguish small variations in lantern size are known for any *Photinus* species, experiments in both *P. ignitus* and *P. pyralis* suggest female preference for either larger or brighter flashes. Female preference for more intense male flashes in *P. pyralis* was proposed to select for larger male lantern size and overall body size in the field (Vencl and Carlson, 1998). Furthermore, this study demonstrated that *P. ignitus* female responsiveness increased with increasing lantern size, and that in the field successfully mated males had wider lanterns than unmated males.

Female preferences for male traits observed in the lab do not always generate sexual selection through mate choice in the field (Jennions and Petrie, 1997; Wagner, 1998; Widemo and Saether, 1999). Although *P. ignitus* female responsiveness increased with increasing lantern size in the lab, paired large vs. small and lab-mated vs. control males showed no differences in courtship behavior, female responsiveness or mating success in *P. ignitus*, providing no evidence that female preference for flashes from large male lanterns or males with larger spermatophores could generate differential mating success. On the other hand, flashing frequency, searching speed and female responsiveness were greater for males that successfully mated than for the other male in
the same enclosure, suggesting that male courtship behavior and female responsiveness interact to produce differential mating success. In the lab, female responsiveness increased with decreasing distance from the artificial lanterns. Therefore, the ability of a male to quickly identify a female flash response, and then land or crawl as close as possible to the female may directly influence male mating success as male flashes that are closer to the female receive increased female responsiveness.

By combining lab and field observations this study shows that male spermatophore investment and seasonal variation in sex ratio may influence the potential for sexual selection through both mate choice and mate competition. There does not appear to be any evidence for sexual selection on spermatophore quality through precopulatory mate choice or male competition, but the potential for selection on male body size and spermatophore mass warrants further study. Female preference for long male flash duration, large lantern size and high flash intensity in male *P. ignitus* and *P. pyralis* could generate sexual selection on these male courtship signals under some conditions. However, male competition mediated by male ability to orient towards and approach a female may play an equally important role in generating differential mating success in *P. ignitus*.

**Future Studies**

The role of sexual selection in firefly evolution is particularly interesting because there are many species within the genus *Photinus* which share almost identical morphology, but differ from one another only in the structure of their external genitalia and the characteristics of their bioluminescent courtship flashes (Lloyd, 1966). As we
learn more about the potential for sexual selection on male courtship flashes (Branham and Greenfield, 1996; Vencl and Carlson, 1998; this thesis, Chapter 3) and the phylogeny (van der Reijden, 1996) of *Photinus* fireflies we may be able to begin to generate and test predictions about the evolution of species-specific flash patterns. This will necessitate a broad survey of female preferences and courtship behavior, and could provide important insights into the role of sexual selection in speciation.

More in depth research will also be needed on the relative costs and benefits of mating in the genus *Photinus*. Intraspecific variation in OSR have been proposed to influence courtship behavior in three species of Massachusetts fireflies (Lewis and Wang, 1991; this thesis, Chapter 2). Interspecific differences in the relative costs and benefits of mating could help to explain differences in courtship behaviors such as the “love knots” of multiple males competing for one female observed in some species (Mauer, 1968; Vencl and Carlson, 1998). This type of research will require a more careful quantification of male and female parental investment as well as a quantification of the energetic costs of male and female courtship behavior.

The operational sex ratio is just one of many ecological factors that have the potential to influence mating behavior. In many species variation in predation risk may play an important role in generating variation in male and female courtship activity. *Photinus* fireflies are preyed on by *Photuris* fireflies as well as by arachnids. The role of *Photuris* fireflies as aggressive mimics of *Photinus* fireflies has been well documented and likely has important implications for *Photinus* mating behavior (Lloyd, 1965, 1984). However, it has also been suggested that arachnid manipulation of captured *Photinus* fireflies can serve to attract additional fireflies which the spider also captures (Lloyd,
1973; Lewis and Wang, 1991). A complete understanding of the predation pressures on males and females from different *Photinus* species will provide important insight into how predation may influence the costs and benefits of courtship and mating behaviors, as well how predation influences seasonal changes in the operational sex ratio.

The approach used in this study of combining field observations of courtship behavior with lab measurements of mating preference and mating costs is essential to understanding the evolution of traits through sexual selection. This approach can be expanded upon by more rigorously examining the costs and benefits of mating or mate choice, and by experimentally manipulating conditions in field enclosures. Finally, by studying patterns of variation across closely related species we may begin to be able to understand the evolution of courtship signals through sexual selection, as well as how both parental and mating investment influence the strength and direction of sexual selection.
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