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Variation in the Flash Pattern of the Firefly, *Photuris versicolor quadrifulgens*[1] (Coleoptera: Lampyridae)

T. G. Forrest[2,4] and Micky D. Eubanks[3]

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We sampled a population of signaling Photuris versicolor quadrifulgens *fireflies to quantify the variation in flash patterns emitted by males. Males produced five distinct flash patterns during their mate-searching flights. Four of the patterns consisted of two to five equal-intensity pulses and the fifth pattern type was a flicker, a group of rapid modulations in intensity. We found that the proportions of each pattern remained relatively constant from night to night throughout the season. The different flash patterns produced varied significantly with time of night; patterns having fewer pulses occurred earlier in the evening. Local density, an estimate of competition, did not significantly correlate with flash pattern type. On consecutive emissions, individuals changed their flash types with a mean probability of 0.12 (over all males), and they usually switched between patterns differing by a single pulse (from a two- to a three-pulse pattern, from a three- to a two-pulse pattern, etc.). The nightly temporal changes in flash patterns may be related to tradeoffs between female availability and energetic costs of signaling or the changes may be related to increased predation risk from visual predators.*

KEY WORDS: Lampyridae; *Photuris;* firefly; bioluminescent signal; flash pattern; signal variation.

[1] *Photuris versicolor quadrifulgens* was originally described by Barber (1951) as a subspecies of *P. versicolor*. The genus *Photuris* is currently under revision by Dr. James E. Lloyd, and this firefly will be given species status.

[2] Section of Neurobiology and Behavior, S. G. Mudd Hall, Cornell University, Ithaca, New York 14853-2702.
[3] Department of Entomology, University of Maryland, College Park, Maryland 20742.
[4] To whom correspondence should be addressed.

INTRODUCTION

Flash patterns of male fireflies are species-typical signals produced to elicit flashed answers from conspecific females. The bioluminescent signals of North American flashing fireflies, especially those of *Photinus* and *Photuris*, are diverse compared with those of European and Asian species. A typical search scenario of a North American firefly can be generalized as follows: Males fly throughout their habitat emitting a particular flashed signal and their stationary receptive females respond with flashed answers having a characteristic form and time delay relative to the male's pattern. Once a female answers a male, the pair continue a pattern–answer dialogue as the male approaches. Mating follows.

In this general and simple signaling system, males of a firefly species produce a single type of flash pattern. Thus the signals of *Photinus* and *Pyractomena* fireflies have been used extensively in detecting cryptic species and as an aid in understanding these group's systematics (McDermott, 1958; Lloyd, 1966). *Photuris* flash patterns, however, are more problematical because flashing occurs in contexts other than mating and males may produce several different patterns during their mate-seeking flights (Barber, 1951; Lloyd, 1969, 1990). Some flash patterns emitted by male *Photuris* are close matches and possibly mimics of the patterns emitted by their females' prey. Perhaps these patterns are related to the hunting of their own females and are used to locate aggressive mimics that can be inseminated (see discussion by Lloyd, 1980). Although more than half of the "species" of North American *Photuris* emit more than one distinct flash pattern, little is known about the multiple signals of male *Photuris* and how they relate to mate-finding tactics of the males that use them (Lloyd, 1990). *Photuris* males may show temporal changes in the types of flash patterns used during a night (Lloyd, 1990). These multiple signals and the temporal changes in the signals could represent different tactics in the mate-search strategy set of a male firefly.

We systematically quantified flash signaling in a population of male *Photuris versicolor quadrifulgens*, hereafter referred to as *quadrifulgens*. Our objectives were, first, to determine the seasonal and nightly variation in flash patterns of male *quadrifulgens*. By observing two consecutive flash pattern emitted by individuals, we were able to determine whether, and to what extent, individuals within the population used multiple patterns. We show that five different flash signals are emitted by *quadrifulgens* males and that individuals change between (among) patterns. Because the emission of these alternative signals might be conditional on the environmental or the social circumstances during a male's search, we also tested the dependence of the different signals on time of night and on local male density.

MATERIALS AND METHODS

Flash Patterns

We categorized the flash patterns of male *quadrifulgens* based on the following description of flash pattern types. Pulsed flashes of male *quadrifulgens* consist of two to five equal-intensity pulses with (temperature-dependent) intervals of 0.5–0.75 s. Pulsed patterns are repeated at intervals of about 5–10 s (Fig. 1). Males emit these patterns while flying low over fields and at the tops of trees. The bright, greenish pulses emitted by flying males are easily counted and the patterns are clearly distinguishable from those of other sympatric, synchronic fireflies. Another flash pattern emitted by males is a rapidly modulated flicker (ca. 9 Hz at 20°C) produced while flying over fields. Our flash pattern categories were the pulsed flashes having different pulse numbers and the flicker pattern.

Seasonal Census

During each week of the spring of 1990, a 3.5-km route near Oxford, Lafayette Co., MS (sec. 1 T8S R4W), that included residential lawns, an old field, and mixed forest was walked and the number of flashing male *quadrifulgens* counted. Because other species at this locality have flickering flash patterns, flickering fireflies were not counted for the census. When more than one census was taken during a week an average is reported.

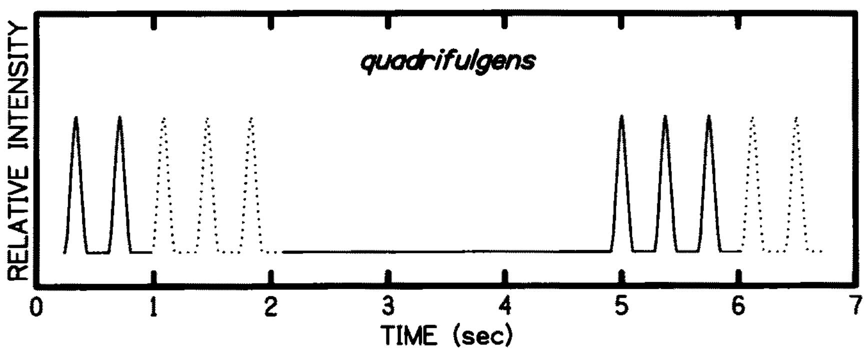

Fig. 1. Schematic representation of *Photuris versicolor quadrifulgens* pulsed flash patterns. The patterns consist of two to five pulses of approximately equal intensity (sometimes the last pulse is less bright). Depending on temperature, the pulses are given at a rate of about 2 to 3 s^{-1} and the pattern is produced every 5 to 10 s. The dotted line represents "missing" pulses and the figure shows a two-pulse pattern followed by a three-pulse pattern.

Nightly Census

To determine the extent to which male *quadrifulgens* emit different patterns, during 1991 we studied a population in the old field included in our 1990 census (above). The field was bordered by mixed pine and hardwood forest. On 10 nights we sampled the flashing males in the population to determine (1) the flash pattern variation within the population, (2) the seasonal and within-night temporal relationship among the different patterns, and (3) the extent that individuals change their flash pattern.

During each 5-min period beginning with the start of male activity, we counted males that flew into a 20 × 20-m sampling quadrat that moved along the edge of a 150 × 80-m field (Fig. 2). After a focal (flashing) male entered the quadrat, we noted his second and third flash pattern. We designated the second pattern as the male's current pattern and used his third flash pattern to ascertain whether he changed (switched) patterns. After noting two consecutive

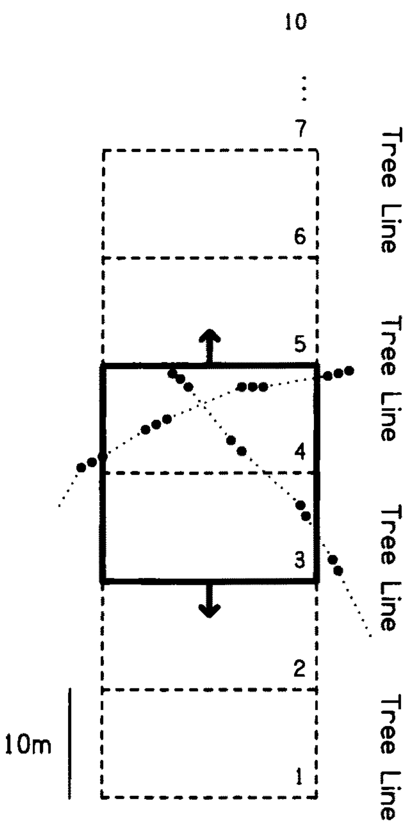

Fig. 2. Sliding sampling area. The 400-m^2 sampling quadrat represents a sampling unit. Male *Photuris versicolor quadrifulgens* that entered the quadrat during a 5-min period were counted and the second flash pattern of a male was taken as his current pattern. His third flash pattern was used to estimate the frequency of pattern switching. The figure shows representations of two flashing males entering the quadrat. The second flash pattern of the male entering from the right has two pulses followed by a three-pulse pattern (i.e., the male switched flash patterns). The male from the left has a three-pulse pattern for both his second and his third flash. After sampling for 5 min the quadrat was moved 10 m (50% overlap). A total of 10 sampling stations was located along the edge of an old field bordered by trees in north Mississippi. During a night all 10 locations were sampled at least twice.

flash patterns of an individual, a different focal individual was observed. After 5 min, the sampling quadrat was moved 10 m (50% overlap) along the 100-m transect (Fig. 2). On most nights (seven) there were two observers and two sampling quadrats. Sampling continued from onset of activity until ≤ 2 males entered a quadrat during a 5-min sampling period, usually 150–180 min. We have converted all times to minutes after sunset to normalize time for each day's data. The duration of civil twilight, a crep unit, varied by less than 2 min (27–28 min) during the 10 sampling nights.

The flash patterns of *quadrifulgens* were categorized by pulse number or as a flicker. We discarded observations where there was ambiguity about whether two consecutive patterns were emitted by the same male. We also discounted instances when our view of the male's flight path was obstructed by vegetation or when the male flew into any obstacle. Because two other fireflies with flickering flash patterns occur at this site, *Pyractomena dispersa* and *Pyractomena angulata*, we captured flickering males that entered the quadrat to insure that our census included only male *quadrifulgens*. Flickers of unconfirmed fireflies were not counted.

The flash pattern given by a male might depend on the flash patterns given by others nearby or on the intensity of competition (local male density). We estimated local density as the number of males counted in a sampling unit during each 5-min sampling period.

Analysis

We used stepwise logistic regression (SAS Institute, 1989) to analyze relationships between the categorical response variable, flash pattern, and predictor variables of date and time of night. We also tested for the effects of date, time, and flash pattern on pattern switching. Because the local male density was the same for each individual during each 5-min sampling period, we randomly chose an individual from each period and then used logistic regression to examine the effects of local density, time of night and date on flash pattern. The logistic analysis examines the hierarchical importance of the predictor variables, and uses a χ^2 statistic with df $= 1$ to test whether models including subsets of the predictor variables are superior to the full model that includes all predictor variables and their interaction terms.

RESULTS

Seasonal Census

P. v. quadrifulgens in Lafayette Co., Mississippi, is an early spring species, active from the end of April to the first or second week in June (Fig. 3). The nightly censuses in 1991 were spread throughout the season and included the peak of seasonal activity.

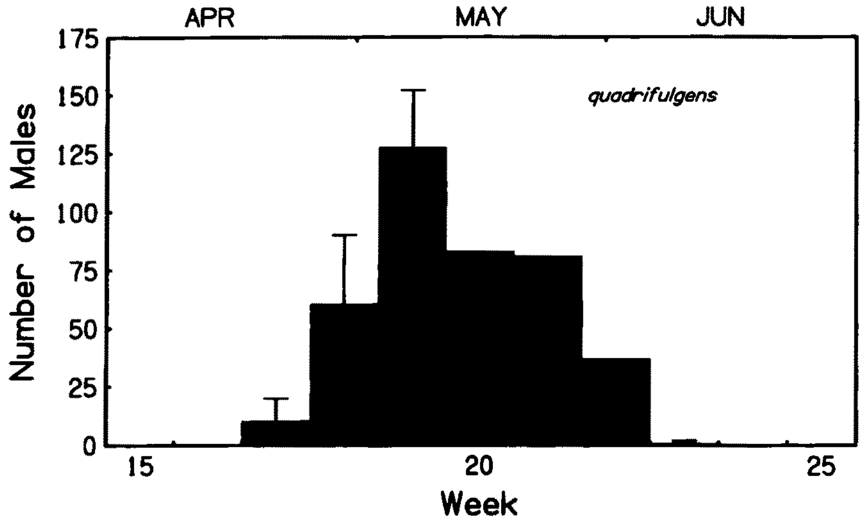

Fig. 3. Seasonal distribution of flashing male *Photuris versicolor quadrifulgens*. Bars are the number of males counted along a 3.5-km route in northern Mississippi during weeks 15 to 25 (9 April–24 June 1990). Vertical lines at the tops of the bars are the range when two censuses were taken during the same week, and the bar is the average number of males for those two censuses.

Nightly Census

We observed 1484 pairs (second and third) of flash patterns emitted by males in our sampling quadrat during our 10 1991 censuses. Summarizing only the males' current (second) flash patterns, 66% of the flash patterns had three pulses, 26% were four-pulse patterns, and 6% had two pulses (Fig. 4). We saw only 5 (<1%) five-pulse patterns and only 31 (2%) flickers during our 10 sampling nights. There were no significant seasonal changes in flash patterns, and the nightly proportions of each pattern remained relatively constant throughout the season (Fig. 4).

Because there was no statistically significant seasonal trend in flash patterns, we combined data across nights and examined the within-night variation in pulse patterns. Time of night (adjusted relative to sunset) was a significant predictor of flash pattern (Fig. 5; $\chi^2 = 164$, 1 df, $P < 0.001$). There were no significant interactions in the analysis. Males emitted patterns with fewer pulses early in the evening and the number of pulses in flashes increased during the nightly activity. Two-pulse patterns were produced almost exclusively during the first 20 min of activity (Fig. 5). After 20 min, three-pulse patterns were produced most often but their prevalence within a 5-min sample period declined from 80 to about 45% during the remainder of the evening. Four-pulse patterns were not produced until 35 min after sunset and increased in prevalence until

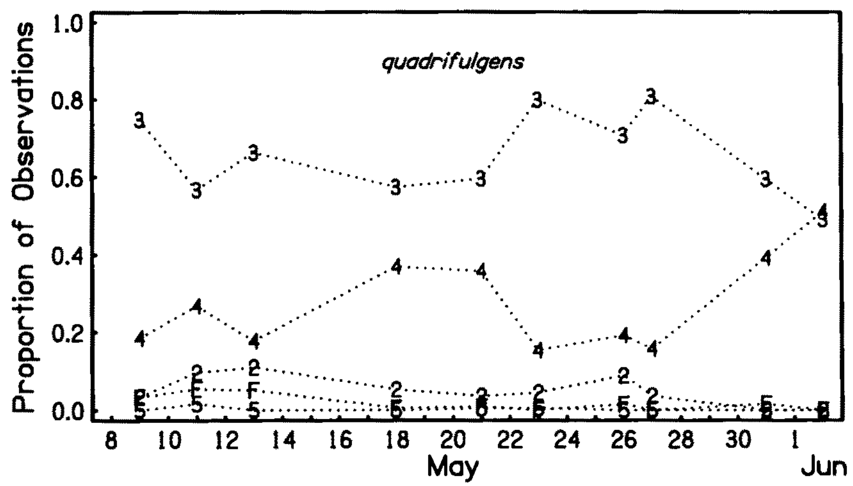

Fig. 4. Nightly proportions of different flash patterns emitted by 1484 male *Photuris versicolor quadrifulgens* during the spring 1991 in northern Mississippi. Numbers represent the flash pattern 2 = two-pulse pattern, 3 = three-pulse pattern..., F = flicker). For each of 10 nights in May and June the proportions of each pattern are plotted. Nightly proportions remained relatively constant throughout the adult season, with three-pulse and four-pulse patterns being most common (66 and 22% of nightly totals, respectively). Only 3 of the nights (26 and 31 May and 2 June) represent fewer than 100 patterns.

the end of nightly activity. All five-pulse patterns were seen during the last half of the activity period (Fig. 5). Flickers were produced late in the evening and early in the season. Of the 31 flicker patterns we observed, 27 occurred during the first 4 nights of observation. Interestingly, all ($N = 8$) of the *Pyractomena angulata* males, having the same flicker pattern, were observed only during the last half of the season.

Of the 1484 males entering the sampling quadrats, 177 (12%) changed their flash patterns between their second and their third flash (Table I). For all but 5 of the 177 observed changes in flash pattern, males switched between patterns whose pulse number differed by one (i.e., from a two-pulse to a three-pulse pattern, or vice versa). On one occasion a male switched from a two-pulse to a one-pulse pattern, the only one-pulse pattern seen. Switching between patterns was consistent with nightly temporal changes in flash patterns. For instance, switching involving two-pulse patterns (from two- to three-pulse pattern or from three- to two-pulse pattern) occurred early in the evening (compare Figs. 5 and 6). Switching between any two patterns was equally likely in both directions (Fig. 6), and three-quarters of the switching occurred between three- and four-pulse patterns. There was no significant difference in the frequency of switching from or to a particular pattern ($G = 8.2$, $P > 0.2$; Table I), but

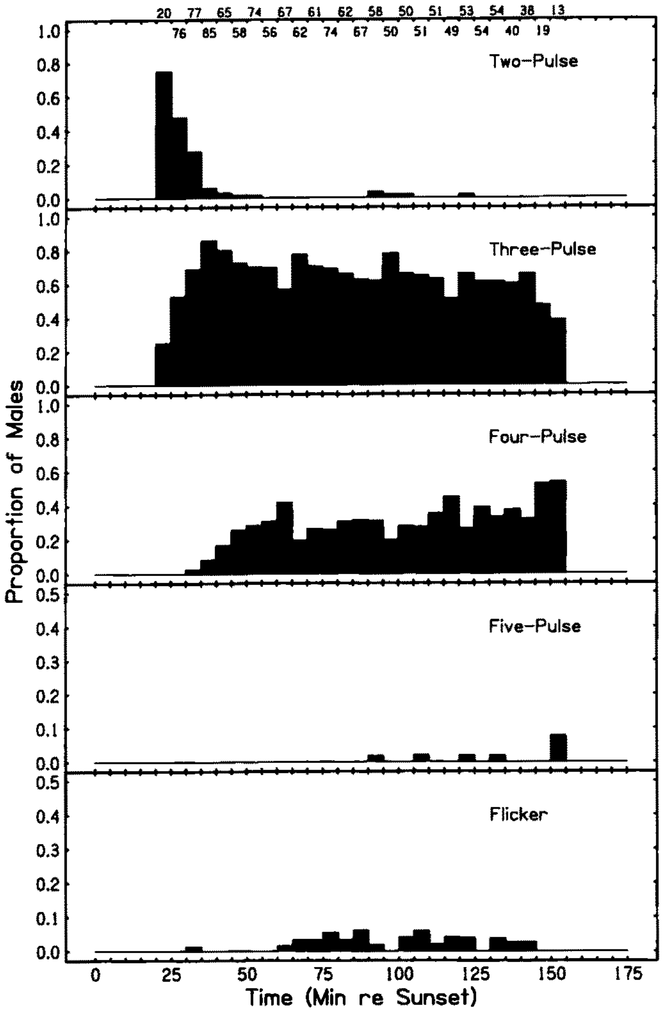

Fig. 5. Nightly change in prevalence of flash patterns of *Photuris versicolor quadrifulgens*. The bars are the proportion of each flash pattern observed during each 5-min sampling period beginning at sunset. Early in the evening males produce almost all two-pulse patterns. The number of pulses in a pattern is significantly correlated with the time of night, and patterns containing more pulses become more prevalent later in the evening (logistic regression $\chi^2 = 164$, df $= 1$, $P < 0.001$). The numbers at the top of the graph are the total number of males represented by the bars for each 5-min sampling period. Note that all samples have more than 50 males except those at the beginning (first 5 min) and the end (last 20 min) of the activity period.

Table I. Frequency of Flash Pattern Switching by *Photuris versicolor quadrifulgens* Males[a]

To	From 2	3	4	5	F	Σ to
1	1	0	0	0	0	**1**
2	—	15	0	0	0	**15**
3	20	—	64	2	0	**86**
4	0	67	—	2	0	**69**
5	0	1	3	—	0	**4**
F	0	2	0	0	—	**2**
Σ from	**21**	**85**	**67**	**4**	**0**	
Expected[b]	**10**	**118**	**46**	**1**	**4**	

[a] Numbers are the numbers of males that changed from the flash pattern indicated at the top to the pattern indicated on the left. For instance, 67 males switched from a three- to a four-pulse pattern and 64 males switched from four- to three-pulse patterns. F represents the flicker flash pattern.
[b] Expected distribution was based on total numbers observed in each flash pattern type and the overall proportion of males switching patterns (0.12). There was no difference in the frequency of switching *from* or *to* a particular pattern (log-likelihood ratio, $G = 8.2$, $P > 0.1$), but there was a significant difference between the observed distribution of switching and expected ($G = 40.8$, $P < 0.001$). Switching was less likely to involve a three-pulse pattern.

switches involving three-pulse patterns were significantly less likely than expected given the overall switching probability of 0.12 ($G = 40.8$, $P < 0.001$). Switching patterns did not correlate significantly with any main effect (time of night, season or pattern), but there was a significant interaction among time, pattern, and date in their effects on switching.

DISCUSSION

At our Mississippi site, the seasonal activity of *quadrifulgens* lasted 6 or 7 weeks and peaked during the middle of May. This seasonal activity is consistent with seasonal records of *quadrifulgens* for this latitude made by Lloyd since 1967 (J. E. Lloyd, unpublished). The males at this site emitted five different flash patterns, four pulsed patterns and a flicker. On one occasion each, Lloyd (unpublished) has seen *quadrifulgens* males emit six- and seven-pulse patterns, but these are rare compared to patterns with two to four pulses. We found that the proportions of individual *quadrifulgens* producing different patterns was relatively constant throughout the season (Fig. 4) but changed significantly within a night. Patterns with fewer pulses occurred early in the evening and pulse number increased during the activity period. The probabilistic nature by which the different flash patterns are emitted might suggest a mixed strategy (Cade, 1984; Dominey, 1984), however, the flash types had a predictable within-night change (Fig. 5). Males apparently use light intensity as a cue for changing

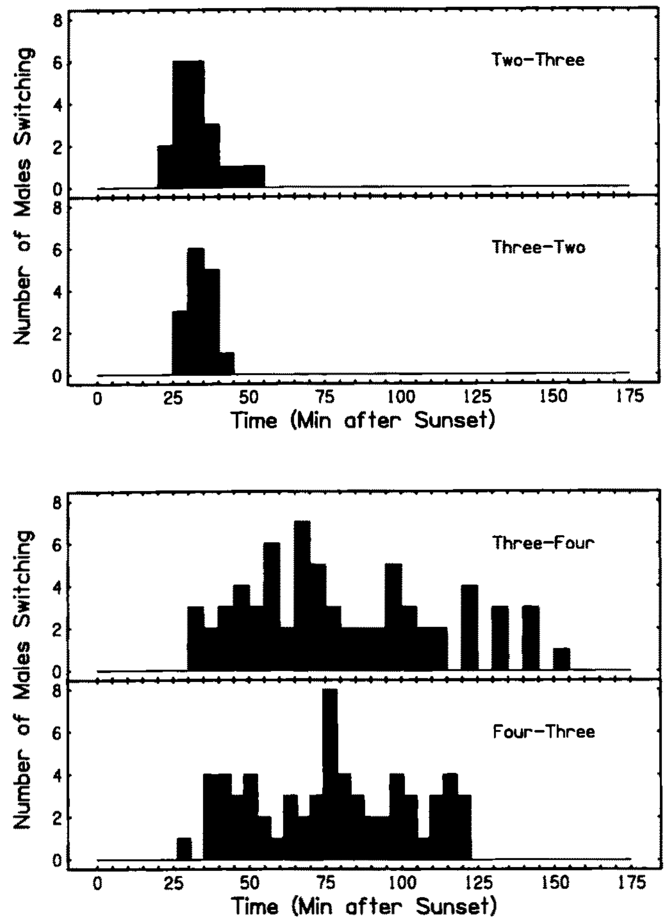

Fig. 6. Temporal switching in flash patterns of *Photuris versicolor quadrifulgens*. Bars represent the number of males changing consecutive flash patterns during each 5-min sampling period beginning at sunset. The two top panels show switching between two-pulse and three-pulse patterns, and the bottom two panels are switching involving three- and four-pulse patterns. There are approximately equal numbers switching in each direction, and the timing of switching corresponds to nightly changes in flash patterns (switching involving two-pulse patterns occurs only early in the evening; see Fig. 5).

patterns, because four of the five two-pulse patterns we observed late in the evening (>50 min after sunset; Fig. 5) occurred on a night when the sky was clear and the moon was full and high. A further test of the influence of light intensity on pulse pattern could come from a geographical study of the nightly flash patterns of *quadrifulgens*. It would be interesting to examine the nightly distribution of flash types in northern and southern *quadrifulgens* populations to see whether distributions differ with different duration of twilight at different latitudes. Interestingly, we did not find any influence of local density on *quadrifulgens* flash patterns, and this may be due to all individuals using the same strategy, an expectation of game theory (Maynard Smith, 1982).

Other fireflies change their mate-searching behavior during the night. Adams (1981) and Burk (in Grier and Burk, 1992) have shown that the search flights of male *Photinus* become more directed, with fewer turns, and that the flights are higher later in the evening. Burk (personal communication) suggested that these behavioral changes in *Photinus pyralis* represent a shift in search tactics of the males. Early in the evening males are in a "search-advertising" mode and use visual cues to concentrate their search around perches likely to harbor females. Later, when visual cues are no longer available, males switch to a "harvest-advertising" tactic using longer, straighter search paths.

Lloyd (1990) has observed temporal shifts in the use of mimetic flash patterns by *Photuris* males. A population of male *Photuris pennsylvanica* switched from 100 to 0% mimicry pattern during the first hour of their activity, the "window" when the presumptive *Photinus* prey species of their females is active. In another unnamed *Photuris* (LIV), the proportion of males producing the mimicry pattern increased sharply, to about 80% during the first hour of activity and then gradually declined to 40% over the next 2 h. We observed most of the flicker patterns emitted by *quadrifulgens* males during the last half of the activity period. The flicker pattern is a possible mimic of a species of *Pyractomena* found at our site, and the increase in flickering *quadrifulgens* late in the evening may represent a shift by males from searching for receptive females to searching for mimetic females.

We have shown that some individual *quadrifulgens* switch flash patterns during a night (Table I). Whether all male *quadrifulgens* switch patterns is not known and would require following individual males during a single evening of mate searching. There were equal numbers switching from and to a particular pattern (Table I). We used the overall switching probability (0.12) to calculate the expected switching frequencies for each flash type and found that individuals were less likely than expected to switch to (or from) three-pulse patterns (Table I). Possibly some individuals do not switch and emit only three-pulse patterns, the most common pattern observed at our Mississippi site.

One possible explanation for nightly changes in flash pattern involves a tradeoff between energy expenditure and female encounter rate. Presumably the energy required for a two-pulse flash is two-thirds that required to produce a three-pulse pattern. Thus, males are expending more energy (incurring greater costs) in locating mates as the night goes on. Early in the evening receptive females may not be as numerous as later (Wing, 1991), and the active space of a male may be smaller due to noise from ambient light (Lall *et al.*, 1980). The decreases in female availability and signal-to-noise ratio will decrease the probability of encountering a female (decrease benefits) early in the evening. If males are balancing the costs and benefits associated with increasing pulse number, males would be expected to expend more energy (higher pulse number) later in the evening when the benefits increase. Another possible explanation for males increasing pulse number during the night is a temporal change in predation risk. Early in the evening when flying males are visible, visual predators could use the flash patterns to locate males. Fewer pulses may be a countermeasure against such an aerial attack early in the evening (Lloyd and Wing, 1983). It is interesting that *Photuris* (LIV) also has a pulsing pattern and also produces patterns with fewer pulses early in the evening (J. E. Lloyd, unpublished).

The discrete signals of *quadrifulgens* are ideal for studying multiple mating tactics. If male flashing is a mixed strategy that changes dynamically with light intensity one would expect to find equal reproductive success for each of the tactics. No data on male reproductive success are available, however, *quadrifulgens* males will emit different pulsed patterns and will often switch patterns on an approach to simulated female answers (J. E. Lloyd, unpublished; T. G. Forrest, personal observations). This suggests that answering females may not discriminate against males on the basis of pulse number. As with several *Photuris*, flickering *quadrifulgens* males always switch (default) to their pulsed pattern when answered by a female or a decoy simulating a female (J. E. Lloyd, unpublished). In this study we have described the variation in *quadrifulgens* signals; whether and how females use these signals in choosing mates remain to be explored.

ACKNOWLEDGMENTS

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