

Male reproductive allocation in fireflies (*Photinus* spp.)

Kristian C. Demary and Sara M. Lewis^a

Department of Biology, Tufts University, Medford, Massachusetts 02155, USA

Abstract. Resource allocation between somatic and reproductive structures has important fitness consequences for individuals, and optimal trade-offs are expected to depend not only on mating system differences among species but also on levels of resource stress within species. We tested the prediction that polyandry (associated with increased sperm competition) will increase male reproductive allocation in bioluminescent fireflies in *Photinus* spp. by comparing the relative mass of testes, seminal vesicles, and reproductive accessory glands among a monandrous and several polyandrous species. In addition, we examined a single population of a polyandrous species, *Photinus greeni*, to see how reproductive allocation might shift between years in response to different levels of larval resource stress. As predicted, males of *P. collustrans*, a monandrous species, showed a fivefold lower allocation to sperm production and a 100-fold lower allocation to reproductive accessory glands compared with males from polyandrous species. We also found evidence within *P. greeni* of a trade-off between allocation either to reproduction or to somatic tissue; following larval resource stress, males eclosed at significantly shorter body lengths, yet showed a 35% increase in their reproductive allocation. These results demonstrate that mating systems strongly influence male allocation to reproductive accessory glands as well as to sperm production. Furthermore, these results suggest that under larval resource stress males of *Photinus* spp. increase their allocation to reproduction at the expense of somatic tissue, thus maximizing their ability to produce nuptial gifts required for reproductive success.

Additional key words: Lampyridae, life history, sexual selection

The partitioning of resources between somatic and reproductive structures is an important component of organismal life histories, and such allocation decisions can have major fitness consequences (Sibley & Calow 1989; Stearns 1992; Roff 2002). Mating systems are one important factor influencing such trade-offs, with increased polyandry predicted to select for higher male reproductive allocation (Simmons 2001). Males should respond to increased risk and intensity of sperm competition under polyandry by increasing their ejaculate expenditure (Parker 1998). Larger relative testis size for species with polyandrous mating systems has been found in many taxa (Birkhead & Møller 1998). However, much less is known about how mating systems alter allocation to male reproductive accessory glands. These glands produce a variety of ejaculate components that are transferred during mating (Leopold 1976; Chen 1984), and may also play an important role in

determining the paternity success of mating males (Wolfner 2002; Gillott 2003).

Within species, levels of resource stress may also influence trade-offs between somatic and reproductive allocation. In holometabolous insects, allocation to adult structures such as flight muscles, wings, and reproductive tissue is determined during metamorphosis (Chapman 1998). Unfavorable conditions during larval development may reduce larval energy reserves before metamorphosis, thus imposing physiological constraints on adult size and reproductive investment. Leimar et al. (1994) proposed that for insects whose mating systems involve paternal investment in nuptial gifts, males faced with unpredictable or limited food should allocate resources to maximize their ability to produce such gifts. In such cases, males are expected to maintain nuptial gift production in order to maximize their reproductive success.

We chose to examine male reproductive allocation using fireflies of *Photinus* spp. (Coleoptera, Lampyridae), based on their life-history attributes and our previous work on mating systems and nuptial gifts in

^a Author for correspondence.
E-mail: sara.lewis@tufts.edu

Photinus (reviewed in Lewis et al. 2004a). These beetles overwinter for 1–2 years as subterranean larvae, which prey upon earthworms and soft-bodied insects and pupate in early summer (Williams 1917; Hess 1920). Larval feeding is the sole source of resources required for reproduction, as adults of *Photinus* do not feed during their ~2-week lifespan (Williams 1917; Lloyd 1997). Courtship takes place each evening, with males flying and emitting bioluminescent advertisement signals in search of stationary females. The majority of *Photinus* species studied to date have polyandrous mating systems in which females mate with multiple males over several nights (Wing 1985; Lewis & Wang 1991; Rooney & Lewis 2002; reviewed by Lewis et al. 2004b). The mating system in *Photinus carolinus* is unknown, although intense pre-mating competition in the form of “love knots” with many males surrounding a mating pair (Copeland & Moiseff 1995; Faust 2004) suggests that these females also mate with multiple males. Thus far only a single *Photinus* species, *P. collustrans*, is known to be monandrous (Wing 1984).

In several *Photinus* species, males transfer sperm packaged within a coiled spermatophore that is manufactured mainly by paired spiral accessory glands, along with contributions from three additional paired glands (van der Reijden et al. 1997; Cratsley et al. 2003; Lewis et al. 2004a). Males with larger accessory glands are likely to produce larger spermatophores, which in *Photinus* have been shown to lead to increased male paternity success (Rooney 2000). Previous work on *P. collustrans* indicates that in this monandrous species, males lack spiral glands and do not produce a spermatophore (Wing 1985, 1988).

In the first part of this study, we used interspecific comparisons to examine how different mating systems may affect male reproductive allocation. We predicted that males in polyandrous *Photinus* species, which encounter a greater risk of sperm competition, would show increased allocation to sperm production and accessory glands compared with a monandrous species, *P. collustrans*. In the second part of this study, we focused on a single population of *P. greeni*, a polyandrous species, to examine how male reproductive allocation might change in response to different levels of resource stress between years.

Methods

Species comparisons

To examine differences in male reproductive allocation associated with species' mating systems, we collected males of four *Photinus* species as follows:

P. greeni LLOYD 1969 ($n = 49$ collected in Lincoln, MA), *P. carolinus* GREEN 1956 ($n = 9$ collected in Elkmont, TN), *P. ignitus* FALL 1927 ($n = 10$ collected in Lincoln, MA), and *P. collustrans* LECONTE 1878 ($n = 16$ collected in Gainesville, FL).

For each male, elytral length was measured from digital photographs using ImageJ (National Institutes of Health), and male wet weight was measured to the nearest 0.1 mg. All males were preserved in 95% ethanol and stored at -20°C until dissection. Male reproductive allocation was determined for each male by removing both testes; seminal vesicles; and spiral, long, medium, and short accessory glands from the male reproductive tract. Each tissue was placed on a pre-weighed plastic platter, kept in a desiccator at room temperature for 24 h, and weighed to the nearest $1\ \mu\text{g}$ on a Mettler MT5 microbalance (Columbus, OH, USA). The remainder of each male's body was placed on a pre-weighed aluminum foil platter, dried at 45°C for 48 h, and weighed. We calculated the relative dry weights for male testes and seminal vesicles as well as for male accessory glands (all glands combined) by dividing the dry weight of these tissues by the total dry weight (including reproductive tissue) of each male. We used Kruskal–Wallis tests to compare male allocation with sperm production (testes and seminal vesicles) and with accessory glands across the four *Photinus* species, as well as to compare the three polyandrous species (*P. greeni*, *P. ignitus*, *P. carolinus*) with the monandrous *P. collustrans*.

Intraspecific variation

A single population of *P. greeni* located in Lincoln, MA, USA ($42^{\circ}26'\text{N}$, $71^{\circ}18'\text{W}$) was studied during 2 years that differed markedly in climatological conditions during spring months before pupation in late May–early June. Because larval growth rates typically increase during later instars (Chapman 1998), spring months are likely to be a crucial time for *Photinus* larval development. The contrasting environmental conditions encountered during 2001 and 2003 are evident in a comparison of spring precipitation patterns (Fig. 1). In 2001, April and May were both extremely dry, although March was extremely wet; overall, monthly precipitation (including snowfall) during 2001 was highly variable. In contrast, during the same period in 2003, monthly precipitation was consistently above the long-term average. The average temperature for March through May was similar in 2001 (8.28°C) and 2003 (6.89°C) (Blue Hills Observatory, MA; <http://climvis.ncdc.noaa.gov>). Drought conditions during late spring 2001 may thus have resulted in resource stress due

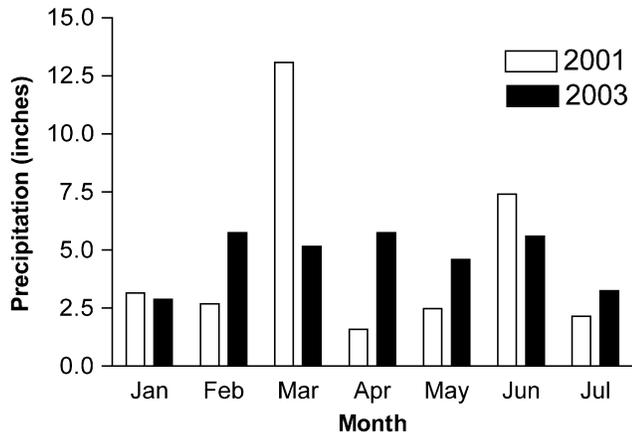


Fig. 1. Monthly precipitation for January–July 2001 and 2003 (records from the Blue Hills Meteorological Observatory, Canton, MA).

to water and possibly food limitation; *P. greeni* adult population sizes were considerably reduced during summer 2001 (unpubl. data).

We collected males of *P. greeni* from this population to compare male reproductive allocation between years (in 2001, $n = 24$; in 2003, $n = 29$). Male elytral length and wet mass were measured as described above, and a condition index was calculated as wet mass (in mg) divided by body length (measured as elytral length, in mm). Male reproductive allocation was measured as described above, except that for this study male short accessory glands and seminal vesicles were not included because their small size made accurate measurement difficult. Long and medium gland weights were combined and referred to as tubular glands for the purpose of analysis.

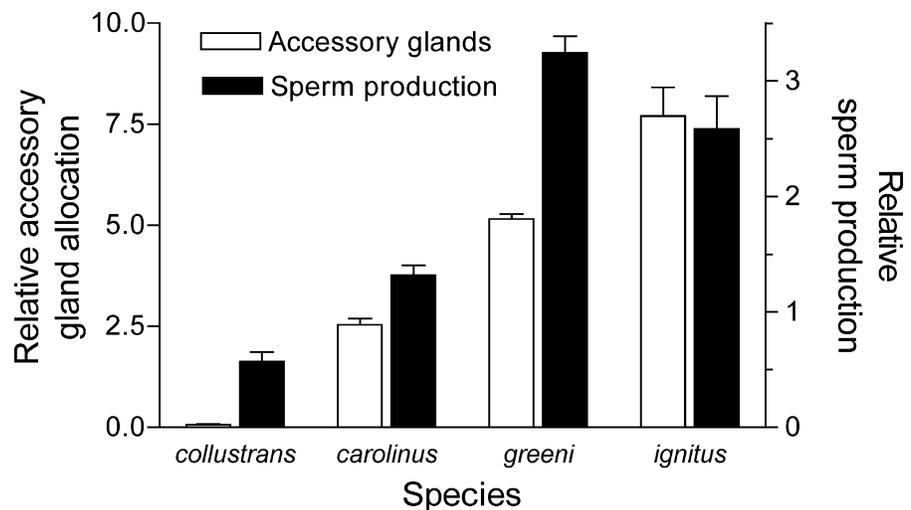
We compared males of *P. greeni* from 2001 and 2003 using separate variance t -tests to examine differences in mean body mass, elytral length, condition index, and relative reproductive allocation (combined dry weights of testes and accessory glands relative to total male dry weight). Before analysis, we verified that data conformed to assumptions of normality. We used a Levene's test (based on absolute deviations from the mean) to compare among-male variation in total reproductive allocation between the two years. All statistical analyses were conducted using Systat 10.2, and data are reported as means and standard errors.

Results

Species comparisons

Male reproductive allocation differed markedly among the four *Photinus* species (Fig. 2). Species differences in male relative allocation to reproductive accessory glands ranged over two orders of magnitude, from 0.07% in *Photinus collustrans* to 7.7% in *P. ignitus*; these differences were highly significant (Kruskal–Wallis, $H = 48.966$, $df = 3$, $p < 0.0005$). In *P. collustrans*, the monandrous species, males possessed only short accessory glands, while males in the remaining species also had spiral, medium, and long accessory glands. There were also differences based on mating system; males from the three polyandrous species showed significantly greater allocation to accessory glands compared with the monandrous species (Kruskal–Wallis, $\chi^2 = 18.927$, $df = 1$, $p < 0.0005$). Among the three polyandrous species, there were also differences in male reproductive allo-

Fig. 2. Relative male reproductive allocation (% of male dry weight) to reproductive accessory glands and to sperm production (testes and seminal vesicles combined) in four firefly species of *Photinus*. Means ± 1 SE based on sample sizes: *P. collustrans* (7, 7), *P. carolinus* (8, 7), *P. greeni* (54, 49), and *P. ignitus* (11, 10).



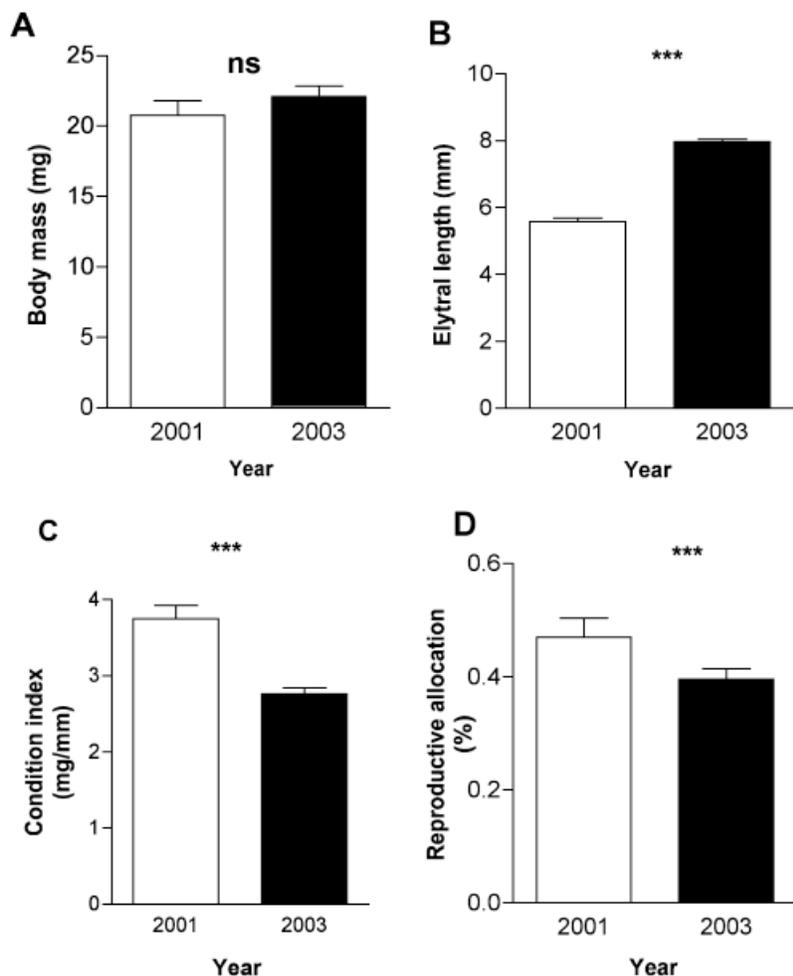


Fig. 3. Comparisons of males of *Photinus greeni* collected during 2001 (open bars, $n = 24$) and 2003 (shaded bars, $n = 29$), with means ± 1 SE shown for (A) wet mass, (B) elytral length, (C) condition index (wet mass divided by elytral length), and (D) relative reproductive allocation (combined dry weight of testes, spiral, and tubular accessory glands as a percentage of total male dry weight). $^{ns}p > 0.05$, $^{***}p < 0.0001$.

cation, with males of *P. carolinus* showing the lowest allocation to accessory glands as well as to sperm production (Fig. 2).

In addition, male allocation to sperm production (relative dry mass of testes and seminal vesicles combined) showed fivefold differences among species, ranging from 0.6% in *P. collustrans* to 3.2% in *P. greeni* (Kruskal–Wallis, $H = 36.562$, $df = 3$, $p < 0.0005$). Again, tests indicated significantly greater allocation to sperm production by males from the three polyandrous species compared with the monandrous species (Kruskal–Wallis, $\chi^2 = 18.730$, $df = 1$, $p < 0.0005$).

Intraspecific variation

Associated with differences between 2001 and 2003 in climatological conditions during late larval development, adult males of *P. greeni* showed differences in body length, body condition, and reproductive allocation (Fig 3). Although mean male mass did not differ between the two years (Fig. 3A; $t = 1.04$,

$df = 43.7$, $p = 0.305$), males in 2001 had significantly shorter elytrae compared with males from 2003 (Fig. 3B; $t = 18.2$, $df = 41.9$, $p < 0.0001$). Such differences in body length contributed to the counterintuitive result that male body condition index (body mass divided by elytral length) was higher in 2001 than in 2003 (Fig. 3C; $t = 5.03$, $df = 31.7$, $p < 0.0001$). Interestingly, following putative larval resource stress in 2001, males on average had significantly greater relative reproductive allocation ($8.4 \pm 0.4\%$) than in 2003 ($6.2 \pm 0.2\%$) (Fig. 3D; $t = 4.98$, $df = 30.4$, $p < 0.0001$). Males of *P. greeni* also showed greater variation in their total reproductive allocation (individuals ranging 5.2–14.5% of male dry weight) in 2001 than in 2003 (ranging 3.8–7.9% dry weight; Levene's test, $F_{1,51} = 6.6$, $p = 0.013$).

Several additional differences in males of *P. greeni* between years were apparent when total allocation was partitioned into component reproductive tissues (Fig. 4). Following putative larval resource stress, males exhibited significantly greater relative allocation to testes in 2001 than in 2003 ($t = 2.38$, $df = 32.2$, $p = 0.024$).

Furthermore, the relative weight of male spiral glands, the major gland responsible for producing spermato-phores, was significantly higher in 2001 than in 2003 ($t = 3.95$, $df = 36.4$, $p < 0.0001$). There was, however, no difference in tubular gland allocation (combined long and medium accessory glands) in males of *P. greeni* between years ($t = 0.82$, $df = 33.9$, $p = 0.417$).

Discussion

Under polyandrous mating systems in which females mate with multiple males, a high risk of post-mating sperm competition (or post-mating female choice) is expected to select for increased male reproductive allocation (Eberhard 1996; Simmons 2001). When sperm competition is based on a male's numerical sperm representation, males should increase their sperm production and storage capacity (Parker 1998). Our findings support this prediction, with males in several polyandrous *Photinus* species showing up to fivefold increased investment in sperm production (relative mass of testes and seminal vesicles) when compared with a monandrous species. Low reproductive allocation in males of *Photinus collustrans* is associated with low sperm competition risk; semelparous females in this species mate only once before they lay their eggs and die (Wing 1984, 1985). Previous work in other taxa supports this prediction (Birkhead & Møller 1998); for example, males in polyandrous butterfly species have larger relative testis sizes compared with monandrous species (Gage 1994). Hosken & Ward (2001) experimentally

demonstrated that polyandry selects for increased testes investment; testes weight in yellow dung flies, *Scatophaga stercoraria* LINNAEUS, increased over ten generations for males in a polyandrous treatment compared with males kept under enforced monandry.

Although male accessory gland products are also likely to play a role in mediating male post-mating paternity success (Eberhard 1996; Gillott 2003), very few studies have examined how mating systems affect male accessory gland allocation. The present study revealed highly significant, 100-fold male variation in relative accessory gland allocation associated with mating system differences, ranging from 0.07% in the monogamous *P. collustrans* to 7.7% in the polygamous *P. ignitus*. Spiral accessory glands, which are responsible for producing a major portion of the male spermatochore in *Photinus* (van der Reijden et al. 1997), are entirely absent in *P. collustrans*, and other glands are greatly reduced in size. A recent comparative study in rodents (Ramm et al. 2005) found that relative testes size (thought to reflect sperm competition level) was positively correlated with the size of two male accessory glands responsible for producing a copulatory plug; such plugs may prevent female remating.

In our study, differing degrees of polyandry could explain observed differences in reproductive allocation among the three polyandrous *Photinus* species. In both *P. ignitus* and *P. greeni*, females are known to mate with multiple males over several nights (Rooney & Lewis 2002; Demary 2005), and both species showed average allocations of > 5% dry mass to re-

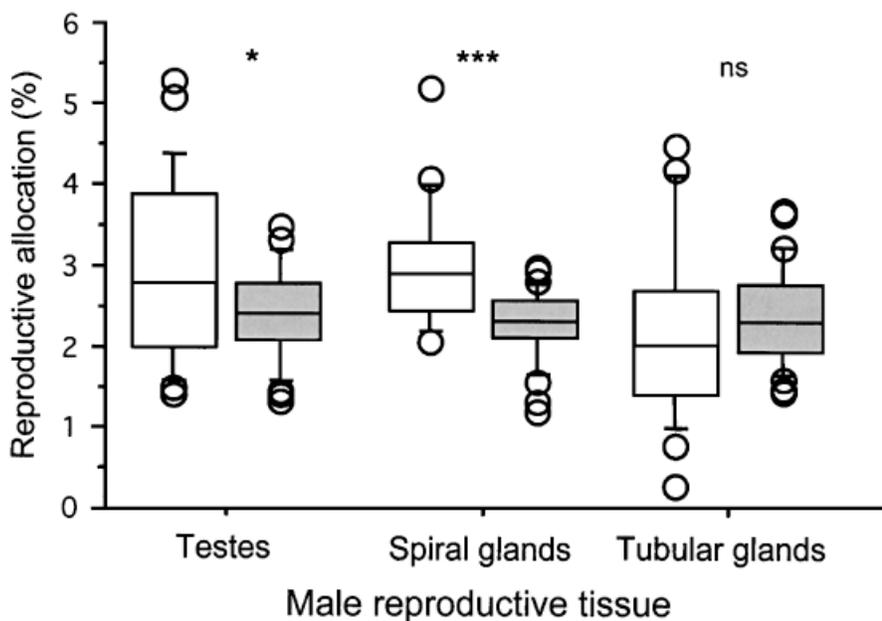


Fig. 4. Box plots comparing reproductive allocation (dry tissue mass) to specific tissues for males of *Photinus greeni* from 2001 (open boxes, $n = 24$) and 2003 (shaded boxes, $n = 29$). Within each box plot, the box represents the middle 50% of data points, the horizontal line indicates the median, whiskers are drawn to farthest data values within inner fences, and circles represent outlying data points. $^{ns}p > 0.05$, $^*p < 0.05$, $^{**}p < 0.0001$.

productive accessory glands and >2.5% allocation to sperm production. In contrast, males of *P. carolinus* showed lower reproductive allocation: accessory gland allocation averaged only 2.5% and allocation to sperm production averaged 1.3%. In spite of intense pre-mating male competition for access to females (Faust 2004), mating patterns of *P. carolinus* are unknown, and further study of this species' mating system would be worthwhile.

This study also supports the prediction that males faced with unpredictable and/or limited resources will shift their allocation to maximize their nuptial gift production (Leimar et al. 1994). Within a single population of *P. greeni*, putative larval resource stress in 2001 led to a 35% increase in reproductive allocation by adult males. This increase in reproductive allocation was accompanied by a significant reduction in male body length in 2001; male mass remained unchanged between years, perhaps due to a mass threshold for initiating metamorphosis in this species.

These results indicate a trade-off between somatic versus reproductive allocation, such that males of *P. greeni*, faced with low resource availability during larval development, maximize their allocation to reproductive tissues at the expense of somatic allocation. Higher accessory gland allocation may increase a male's post-mating reproductive success, as larger spermatophore size has been shown to increase male paternity success in *P. ignitus* (Rooney 2000). Some previous work also supports the idea that, under resource limitation, males might trade off reproductive investment at the expense of somatic structures. Adult males of *Drosophila grimshawi* OLDENBERG that were fed a low-quality diet showed higher testes allocation compared with well-fed males (Droney 1998). Additionally, Gage (1995) found that male moths of *Plodia interpunctella*, HÜBNER that were reared at high larval density (thus greater larval resource limitation and predicted risk of sperm competition as adults) showed increased relative testes weight and sperm production at the expense of lower head and thorax investment. In contrast, Leimar et al. (1994) found that relative male reproductive allocation remained unchanged when caterpillars of *Pieris napi* LINNAEUS were fed a low-quality diet. Further investigation of male reproductive allocation under differing environmental and demographic conditions will provide additional insight into the fitness consequences of such male trade-offs.

Acknowledgments. We thank L. Faust and J.E. Lloyd for providing specimens, and M.P. Scott for comments on the manuscript. Funding was provided by NSF (IBN-

9816432 and DBI-0243668) and the Tufts University Faculty Research Fund.

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