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# Chemical egg defense in *Photuris* firefly "femmes fatales"*

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**Summary.** Female *Photuris* fireflies contain defensive chemicals of two types. They sequester steroidal pyrones (lucibufagins) from male fireflies of the genus *Photinus* that they eat, and themselves produce the defensive betaine *N*-methylquinolinium 2-carboxylate. Chemical analyses of *Photuris* eggs showed that females that fed on *Photinus* males endow their eggs with both lucibufagin and the betaine, while those that did not feed on *Photinus* lay eggs that contain betaine, but virtually no lucibufagin. *Photuris* females collected in the field during the *Photinus* flight season laid eggs that invariably contained betaine, but lucibufagin only at times. Predation experiments showed that *Photuris* eggs are essentially unacceptable to larvae of a coccinellid beetle (*Harmonia axyridis*) and an ant (*Leptothorax longispinosus*), but moderately acceptable to an earwig (*Forficula auricularia*). When applied experimentally to palatable insect eggs, lucibufagin proved deterrent to these three predators, while the betaine proved deterrent to the ant and coccinellid larva only. Both types of defensive compound decreased egg predation in the field. By endowing their eggs with both exogenous and endogenous chemicals, *Photuris* females are essentially "maximizing their options" – when feeding on *Photinus*, their eggs are doubly protected, but they are not entirely defenseless when the females are unable to procure lucibufagin.

**Key words.** *Photuris* – *Photinus ignitus* – Coleoptera – Lampyridae – lucibufagin – steroidal pyrone – betaine – egg predation

## Introduction

Fireflies (Coleoptera: Lampyridae) are known to be distasteful to vertebrate and invertebrate predators (Lloyd 1973; Blum & Sannasi 1974; Sydow & Lloyd 1975; Eisner *et al.* 1978, 1997). They are even lethal to certain lizards and frogs (Knight *et al.* 1999).

Fireflies of the genus *Photinus* (*P. ignitus*, *P. pyralis*, *P. marginellus*) have been shown to contain mixtures of steroidal pyrones (lucibufagins†), which are, at least in part, responsible for their distastefulness and toxicity (Eisner *et al.* 1978, 1997; Meinwald *et al.* 1979; Goetz *et al.* 1979, 1981).

Female fireflies of the genus *Photuris*, appropriately known as "femmes fatales", also contain lucibufagin (Fig. 1A), but their content is minimal unless they fed on *Photinus* (Eisner *et al.* 1997; González *et al.* 1999a). Specifically, female *Photuris* respond to the flash signals of *Photinus* males by emitting light pulses imitative of those of *Photinus* females, thereby luring the *Photinus* males to within graspable range. They then pounce upon the males and devour them, leaving only legs and bits of wings uneaten (Lloyd 1965). The *Photuris* females sequester the defensive lucibufagin from the *Photinus* prey, thereby obtaining protection against enemies such as spiders (Eisner *et al.* 1997).

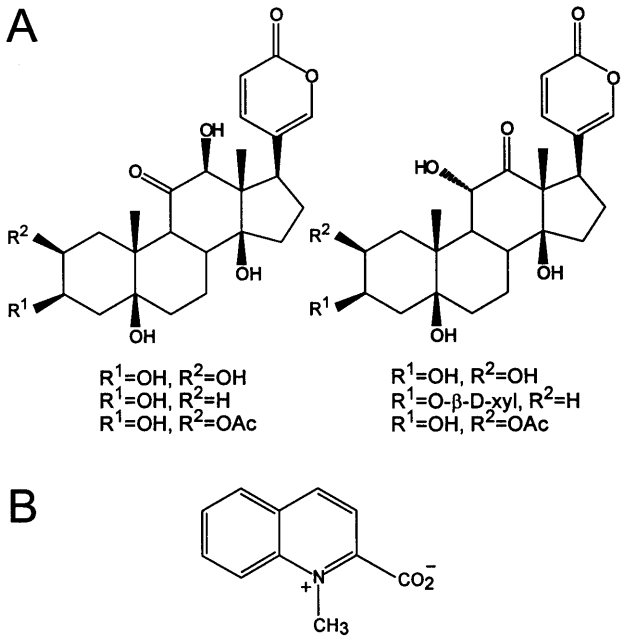


**Fig. 1** Structures of the main components of the lucibufagin mixture in *Photuris* fireflies (**A**), and of the betaine N-methylquinolinium 2-carboxylate (**B**)

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† We refer to the compounds herein in the collective, as lucibufagin.

In addition to the lucibufagin acquired by the adult females, *Photuris* larvae and adults of both sexes contain the betaine N-methylquinolinium 2-carboxylate (herein referred to as the betaine; Fig. 1B) (González *et al*. 1999b), which appears to be biosynthesized endogenously (González *et al*. unpubl.).

In this study, we investigate the defensive chemical endowment of *Photuris* eggs, and how such endowment affects the eggs' vulnerability to certain insect predators. Specifically, we show that (*i*) *Photuris* females that fed upon *Photinus* males endow their eggs with part of the lucibufagin they acquire; (*ii*) *Photuris* females also endow their eggs with the betaine, and they do so whether they fed on *Photinus* or not; (*iii*) field-collected *Photuris* females and their eggs invariably contain the betaine, but they may or may not contain lucibufagin; (*iv*) *Photuris* eggs are distasteful to some predators, irrespective of their lucibufagin content; and (*v*) both types of defensive chemical, the lucibufagin and the betaine, show anti-insectan potency when applied experimentally to palatable insect eggs, and contribute to the chemical protection of *Photuris* eggs in the field.

## Materials and methods

All fireflies were collected in the vicinity of Ithaca, Tompkins Co., New York, USA. All *Photinus* were identified as *P. ignitus*. They were collected in the field at night, and kept live individually in plastic vials, with access to water only.

The *Photuris* were identified as *P. versicolor*, but will be referred to as *Photuris* throughout this paper, since there is some question as to whether *P. versicolor* is in fact a complex of sibling species (Eisner *et al*. 1997).

The *Photuris* were either field-collected or laboratory-reared. The field-collected individuals (females only) were captured during the *P. ignitus* flight season, at the same sites as *P. ignitus*. These *Photuris* females were assumed to have mated and were kept unfed in Petri dishes (4.8 cm diameter), where they eventually laid eggs. Upon their natural death, the females were weighed and stored frozen for chemical analysis.

The laboratory-reared *Photuris* (males and females) were raised from field-collected larvae following procedures described elsewhere (Eisner *et al*. 1997; González *et al*. 1999a). The larvae were collected in their late instars, on soil, at night in early fall, and kept in the dark at 10°C until May. They were then transferred to soil-filled chambers to induce pupation (24°C, 16L:8D photoperiod), thus ensuring that adult emergence (June–mid July) coincided with the time of *P. ignitus* availability. Some *Photuris* larvae were frozen upon collection and stored for chemical analysis.

Upon emergence, laboratory-raised *Photuris* adults were kept unfed (with access to water only) in individual Petri dishes (4.8 cm diameter) under natural lighting conditions. The females were mated by enclosing them individually with single laboratory-reared *Photuris* males, shortly before dusk, in humidified plastic cages ($18 \times 13$ cm base, 10 cm height). The pairs were checked every 5 min for 3 h. Mating typically took place within the first hour of enclosure, and lasted 7–15 min. After the partners spontaneously uncoupled, they were returned to their individual dishes. Females that failed to mate were re-paired on subsequent evenings with a different male each evening. Of the 164 females tested, 102 (62%) were observed to mate, and were used thereafter in the experimental feedings.

### *(A) Experimental feedings*

About half of the mated, laboratory-reared females ($N = 53$) were offered freshly collected *P. ignitus* males. The *P. ignitus* were introduced singly into the Petri dishes that housed the *Photuris* females. After a female consumed an entire male, usually overnight, a second *P. ignitus* male was introduced. After consuming the second prey, the *Photuris* female was transferred to a new Petri dish where she laid eggs. Control *Photuris* females ($N = 49$) were kept unfed, without access to *P. ignitus* at any time.

### *(B) Egg collection*

Eggs were collected from *Photinus*-fed and *Photinus*-unfed laboratory-raised females [herein called (+) and (−) *Photuris* eggs, respectively], and from field-collected females. Females usually laid eggs in clusters, shortly before dying. Upon collection, the eggs were counted, weighed, and either stored frozen for chemical analysis, or kept on moistened filter paper for egg predation experiments. A few females laid a second egg cluster after the first had been collected. These second clusters were analyzed separately, and the values were averaged with those of the first cluster to obtain a single data point per female (first and second clusters of individual females showed similar values).

### *(C) Chemistry*

Egg clusters were homogenized in water/acetonitrile (19:1; 100 µl), extracted while sonicated for 30 min, and centrifuged. The supernatant was transferred to a 200 µl vial insert, and subjected to quantitative HPLC analysis.

*Photuris* larvae, females and males were ground in water/acetonitrile (14:1; 700 µl), extracted overnight, centrifuged, and re-extracted with 300 µl water (3 h). The two extracts were combined, filtered through a 45 µm membrane filter, and analyzed by HPLC.

Quantitative HPLC analyses of lucibufagins and the betaine were performed with a Hewlett Packard 1090 Series II HPLC system, using a BDS Hypersil C-18 column (25 cm × 4.6 mm, 5 µm; Keystone Scientific Inc.). The column was eluted with 5% acetonitrile in water for an initial 10 min, increasing the amount of acetonitrile to 30% over the next 30 min. Injection volume was 25 µl, the flow was 1 ml/min, and the analyses were monitored at $\lambda = 300$ and 325 nm for quantification of the individual lucibufagins and the betaine, respectively. A pure sample of the lucibufagin 3-*O*-acetyl-5$\beta$,11$\alpha$-dihydroxy-12-oxobufalin, isolated from *P. ignitus* (González *et al*. 1999a), and a synthetic sample of the betaine (prepared according to Quast & Schmitt 1970), were used to construct the calibration curves for the HPLC analysis, using coumarin as internal standard. The curves correlated the net amounts of both the lucibufagin and betaine standards with the ratios of the chromatographic peak areas of each compound and that of the internal standard. The regression coefficients for the calibration curves used in the analysis of *Photuris* eggs were 0.992 and 0.995 for the lucibufagin and the betaine standards, respectively. The corresponding coefficients used in the analysis of *Photuris* adults and larvae were 0.992 and 0.999. Since the lucibufagin in *Photuris* is present as a mixture of related compounds (González *et al*. 1999a), the total quantity of lucibufagin was calculated as the sum of individual lucibufagin components.

### *(D) Egg predation experiments*

We conducted field and laboratory egg predation experiments. In the laboratory we used three insect predators: larvae of the coccinellid beetle *Harmonia axyridis* (Coleoptera: Coccinellidae), colonies of the ant *Leptothorax longispinosus* (Hymenoptera: Formicidae), and *Forficula auricularia* earwigs (Dermaptera: Forficulidae).

*H. axyridis*. This coccinellid is an exotic species that has become established in much of the eastern United States (Hoebeke & Wheeler 1996). Both larva and adult are known to be voracious predators of small invertebrate prey (Hodek & Honek 1996). We used 3$^{rd}$ and 4$^{th}$ instar larvae from a laboratory colony established from adults collected in the vicinity of Ithaca, NY. The colony was kept in a greenhouse on alfalfa plants infested with the aphid *Acyrthosiphon pisum*, which constituted the only food source for the beetle. Further details on the maintainance of the colony are given elsewhere (Rossini *et al*., 1999). Prior to the predation experiments the larvae were starved for 48 h, during which they had access to water only.

*Leptothorax longispinosus*. This is a native ant species known to consume insect prey in the laboratory (Hare & Eisner 1993). It occurs throughout eastern Canada and the northeastern United States (Creighton 1950). Although it is a polydomous ant (Alloway *et al.* 1982), a group of workers and their queen(s) collected from a single hickory nut were considered to be a colony. The ants were collected at two different locations: Ithaca, NY, and Mississauga (Ontario, Canada). Unless otherwise indicated, we used colonies collected near Ithaca. The colonies were kept in plastic culture dishes (15 cm diameter, 4 cm height) and fed weekly on mealworms, 10% honey-water, and a diet developed by Bhatkar and Whitcomb (1970). One week before conducting the tests, the ants were deprived of food, and given water only. Colonies collected in Mississauga were maintained in identical fashion, except that fruit flies were given instead of mealworms.

*F. auricularia*. This earwig is also an introduced species, and it is now widely distributed throughout southern Canada and the northern United States. It feeds on small prey, decaying animal matter, and plants (Crumb *et al.* 1941). The earwigs were collected near Ithaca, NY and in Sharon, VT. Some specimens were tested shortly after capture. These were kept unfed until testing, on sand in individual plastic cages (11 × 11 cm base, 4 cm height). Earwigs used in later tests were kept in the laboratory in communal plastic cages (18 × 13 cm base, 10 cm height) and maintained on freshly cut-up mealworms (larvae of *Tenebrio molitor*). They were deprived of food for two weeks prior to testing.

1. Predation on (+) and (−) *Photuris* eggs

*a. Laboratory experiments*. *H. axyridis* larvae were placed individually in Petri dishes (4.8 cm diameter) lined with moistened filter paper. Each larva was offered a choice between (+) and (−) *Photuris* eggs (5 eggs/category), and the number of uneaten eggs was scored at 2, 4, 6 and 24 h.

*L. longispinosus* colonies were offered single clusters of 9–12 *Photuris* eggs [either (+) or (−)]. Eggs were scored as consumed when they were either taken to the nest or eaten *in situ* by workers. Records of egg consumption were made at 10 min intervals during the first hour, and at 2, 24, 48, and 72 h. This experiment was conducted at Brandon University, Manitoba (Canada), using ants collected in Mississauga and *Photuris* eggs from Ithaca.

Freshly collected *F. auricularia* earwigs were each given a single offering of 5 *Photuris* eggs [(+) or (−)] placed on wax paper squares. The experiment was conducted in Petri dishes (9.5 cm diameter) lined with moistened filter paper, and checked at 1, 2, 4, and 24 h for missing eggs.

*b. Field experiment*. This experiment was conducted in late July on the outskirts of Ithaca, using (+) and (−) Photuris eggs freshly obtained in the laboratory. Egg clusters (10–24 eggs/cluster) from individual females were placed at the center of soil-filled Petri dishes (4.8 cm diameter), and taken to the edge of a small pond, where *Photuris* fireflies occur naturally. The Petri dishes were placed in scooped-out depressions in the soil, so that their soil contents were flush with the ground. A plastic overhead shield protected the dishes from rain. The eggs were tested in pairs of (+) and (−) *Photuris* egg clusters. The two dishes containing the clusters of a pair were spaced 1 m apart, and their relative positions were switched every two days. The distance between neighboring pairs was at least 7 m. The number of missing eggs was counted every 2 days until day 10, at which time the *Photuris* larvae began hatching.

2. Predation on lucibufagin- and betaine-treated food items (*Utetheisa* eggs)

We used eggs from a laboratory culture of an arctiid moth, *Utetheisa ornatrix*, as palatable food items. In nature, *U. ornatrix* eggs are distasteful to predators by virtue of pyrrolizidine alkaloids of dietary origin they contain (Eisner & Meinwald 1995, and references therein). We maintain a colony of *U. ornatrix* on a pinto bean diet devoid of pyrrolizidine alkaloids (Miller *et al.* 1976), which provides us with a source of alkaloid-free eggs that have been shown to be acceptable to insect predators (Dussourd *et al.* 1988; Hare & Eisner 1993). To obtain such eggs, mated females were confined to containers lined with wax paper, upon which they readily laid their egg clusters.

The egg clusters (9–12 eggs/cluster) were treated by topical addition of a pure lucibufagin (2$\beta$-acetoxy-5$\beta$,11$\alpha$-dihydroxy 12-oxobufalin) that had been isolated from *Photuris* fireflies (González *et al.* 1999a), or by addition of the betaine (synthesized in accord with Quast & Schmitt 1970). The chemicals were administered in methanol solution (2.0 μg/μl) with a micropipette, at a dosage of 0.5 μl/egg. Control eggs were treated by addition of methanol only (0.5 μl/egg). While applying the compounds, it was noted that some solution inevitably spilled from the eggs onto the underlying wax paper. Therefore, to estimate the actual amount of lucibufagin and betaine applied to the eggs, we treated additional egg clusters and analyzed them by HPLC. These analyses showed that the quantity of lucibufagin and betaine actually added to the eggs was in the range of 0.2–0.6 μg/egg.

*a. Laboratory experiments*. These predation experiments were conducted as were those with (+) and (−) *Photuris* eggs. *H. axyridis* larvae were offered a choice of two *U. ornatrix* egg clusters, one treated with either lucibufagin or betaine, the other with solvent. The experiments were checked every 20 min for 3 h. Larvae that did not feed after 3 h were not tallied. *L. longispinosus* colonies were offered single egg clusters treated with lucibufagin, the betaine, or solvent, and the number of eggs consumed or taken to the nest was scored at 10 min intervals for the first hour, and then at 2 and 24 h. *F. auricularia* earwigs were individually offered a choice of two egg clusters (one control, one treated with either lucibufagin or betaine), and egg predation was scored hourly for 5 h.

*b. Field experiment*. This test was conducted in August, immediately following the field experiment with (+) and (−) *Photuris* eggs, at the same field site. The eggs were of three kinds, betaine-treated, lucibufagin-treated, and controls. They were tested in groups of three egg clusters (one cluster per treatment in every triad, 11–20 eggs/cluster). The three clusters forming a triad were placed, equidistant to one another, in a single sand-filled Petri dish (9.5 cm diameter). Neighboring dishes were placed at distances of at least 7 m from one another. The number of missing eggs was scored at 2-day intervals for 10 days. Midway through the experiment all uneaten eggs were replaced by freshly collected, equally-treated ones, since *U. ornatrix* eggs hatch at age of 5 days.

*(E) Statistics*

All values are given as mean ± SE. Data from all laboratory predation experiments were expressed as proportion of eggs consumed, and were subjected to the arc sine transformation for proportions (Snedecor & Cochran 1989). The transformed data were analyzed by repeated-measures analyses of variance, with time taken as the repeated-measures variable. Unless otherwise indicated, the Mann-Withney test was used in all two-sample comparisons. Other statistical procedures are specified where used.

## Results and conclusions

### *(A) Experimental feedings and (B) egg collection*

The laboratory-raised *Photuris* females eagerly assaulted and devoured the two *P. ignitus* males they were offered (only one of 53 females took a single *P. ignitus*). Fed and control females did not differ as to lifespan (fed: 16 ± 1; control: 17 ± 1 days, $P = 0.37$) or body mass at death (fed: 65 ± 3; control: 63 ± 3 mg, $P = 0.33$).

Not all *Photuris* females laid eggs: only 25 (of 49) control and 34 (of 53) *P. ignitus*-fed laboratory-raised females, and 13 (of 29) field-collected females, laid eggs in quantities sufficient for chemical analysis or predation experiments. A comparison between fed and control females showed no differences in the probability that a female would lay eggs in the laboratory (G-test,

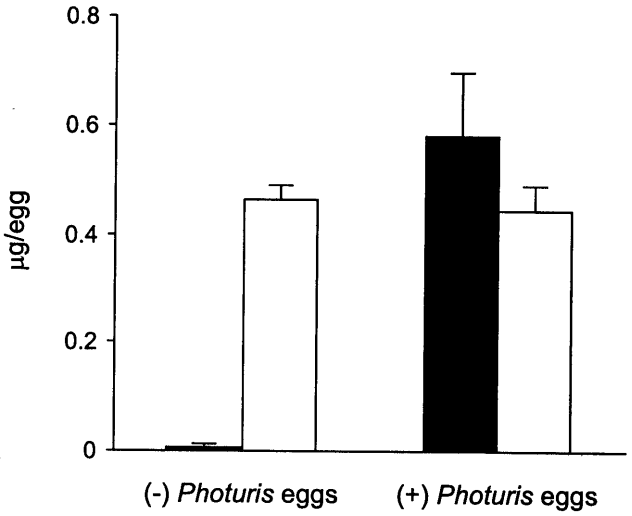


**Fig. 2** Lucibufagin (solid bars) and betaine (open bars) in (−) and (+) *Photuris* eggs [(−) eggs: N = 12 clusters; (+) eggs: N = 14 clusters]. (+) Eggs contained more lucibufagin than (−) eggs ($P < 0.001$), but the betaine endowment was equal ($P = 0.94$). Error bars represent standard errors

$P = 0.12$), in the number of eggs laid (fed: $26 \pm 6$; control: $33 \pm 7$ eggs/female, $P = 0.11$), or in the mean egg mass (fed: $0.16 \pm 0.01$; control: $0.19 \pm 0.02$ mg, $P = 0.14$).

*(C) Chemistry*

1. (+) and (−) *Photuris* eggs

HPLC analyses of (+) *Photuris* eggs from the experimental feedings showed that fed females endow their eggs with part of the total lucibufagin they sequester from *P. ignitus* (Fig. 2). Most (−) egg clusters were free of lucibufagin, although some contained trace amounts of the chemicals (range 0.006–0.04 µg/egg). This result was not unexpected, inasmuch as *Photuris* females had been shown to contain some lucibufagin even if they had no access to *P. ignitus* (González *et al.* 1999a). Furthermore, analyses of (+) and (−) *Photuris* eggs showed that the females allocate the betaine to their eggs, in amounts that do not depend on whether they fed on *P. ignitus* (Fig. 2).

2. Field-collected females and their eggs

These females were collected as adults, and therefore had the opportunity to feed on *P. ignitus* under natural conditions. Analysis by HPLC showed that both the eggs laid by these females and the bodies themselves of the females contained lucibufagin (N = 13 females, 1 egg cluster/female), although the amounts were highly variable. Three females did not contain detectable levels of lucibufagin, and the lucibufagin net amounts in the remaining females ranged from 2 to 63 µg. Likewise, there was variability in the lucibufagin concentration in the eggs of these females (the concentration in the eggs correlated positively with that in the body of the mother) (Fig. 3A and 3B). Five egg clusters contained lucibufagin at levels below 0.04 µg/egg, which could be

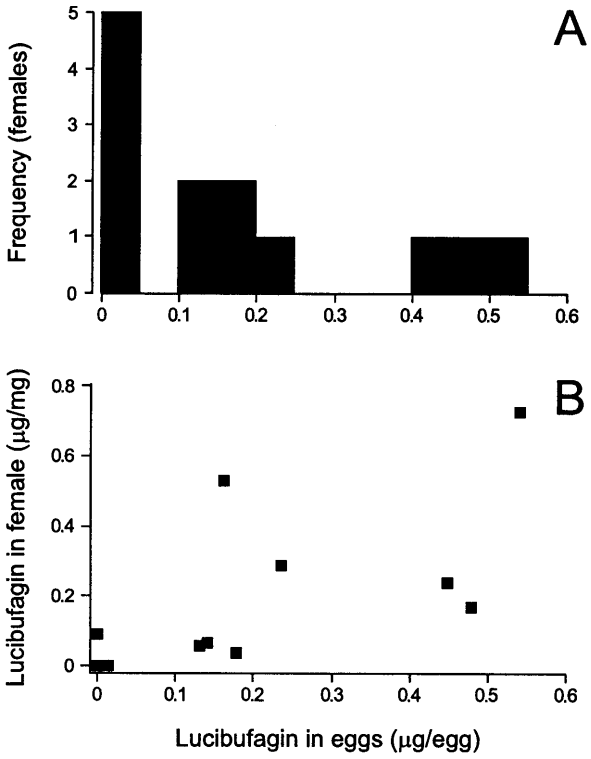


**Fig. 3** **A** – Frequency distribution of lucibufagin content in eggs of field-collected *Photuris* females. **B** – Correlation between the lucibufagin content in field-collected *Photuris* females and their eggs (Spearman's rank correlation test, $\rho = 0.738$, $P < 0.005$; N = 13)

expected for eggs from females that had no access to *P. ignitus* [(−) eggs, previous section]. Another three clusters contained 0.40–0.60 µg lucibufagin/egg, an amount comparable to that in eggs of females that ate two *P. ignitus* males [(+) eggs, previous section]. The remaining five egg clusters contained intermediate lucibufagin levels (range 0.13–0.23 µg/egg), such as one might expect from eggs laid by females that fed on a single *P. ignitus*.

Field-collected females and eggs also contained the betaine. HPLC analyses showed that all females and egg clusters contained detectable levels of the compound. The females contained a betaine net amount of $55 \pm 11$ µg, and the eggs contained a betaine level of $0.45 \pm 0.05$ µg/egg, which is equal to the betaine content of eggs from laboratory-reared females (previous section) ($P = 0.23$).

Interestingly, while there was a 5-fold enrichment in the concentration of lucibufagin in the eggs when compared to the concentration in the females, no comparable enrichment was noted for the betaine. The lucibufagin concentration in the eggs of the field-collected females that contained the chemicals was $1.0 \pm 0.3$ µg/mg, while the concentration in the females themselves was $0.2 \pm 0.1$ µg/mg (Wilcoxon signed-rank test, $P < 0.02$, N = 10). The betaine concentration in the eggs was $1.8 \pm 0.3$ µg/mg, while that in females was $1.3 \pm 0.3$ µg/mg (Wilcoxon signed-rank test, $P > 0.4$, N = 13).

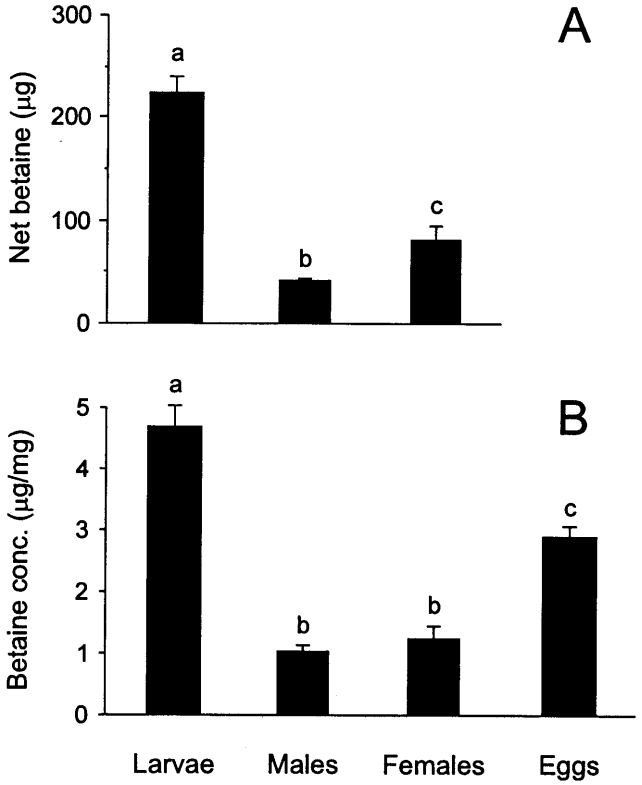


**Fig. 4** Betaine net amounts (**A**) and concentration (**B**) in *Photuris* larvae, males and females. Betaine concentration in *Photuris* eggs (**B**) was included for comparison. Different letters above bars indicate significant differences in post-ANOVA Fisher's pairwise comparisons (individual error rate 0.05). Error bars give standard errors (larvae, males and females: N = 10; eggs: N = 26 clusters)

3. Betaine in *Photuris* larvae, and adult males and females

In addition to our analyses of the betaine contents of *Photuris* eggs, we determined by HPLC the betaine contents of *Photuris* larvae, and of adult males and females. The larvae were field-collected, and the females and males were laboratory-raised from field-collected larvae.

*Photuris* larvae contained about four times the amount of betaine of adults (Fig. 4). Although relatively little is known about the predation ecology of *Photuris* larvae (Lloyd 1973; Sivinski 1981), these results suggest that the betaine may also serve as a chemical defense for the larvae. The betaine net amount in females was higher than that in males, although there was no gender difference in the concentration of the compound (Fig. 4A and 4B). The concentration in laboratory-raised females was equal to that in field-collected females (previous section) (P = 0.72).

*(D) Egg predation experiments*

1. Predation on (+) and (−) *Photuris* eggs

*a. Laboratory experiments.* Both (+) and (−) *Photuris* eggs were decidedly unpalatable to *H. axyridis* larvae. No eggs from either category were eaten within 24 h (N = 5 larvae). The eggs were then removed, and five

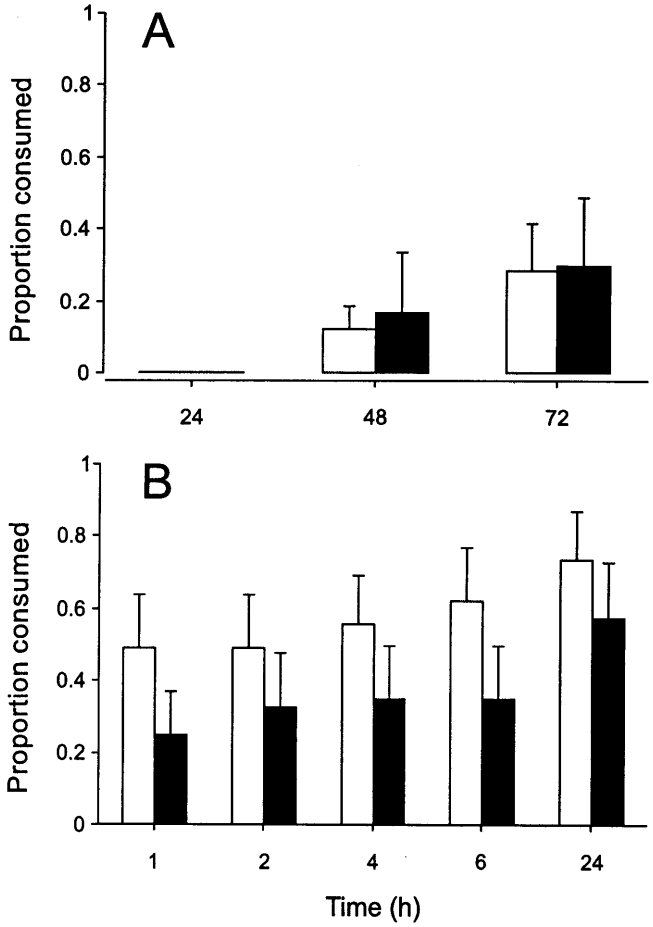


**Fig. 5** Predation rates on (−) (open bars) and (+) (solid bars) *Photuris* egg clusters in laboratory experiments with (**A**) *L. longispinosus* ants [(−) eggs: N = 7 clusters; (+) eggs: N = 6 clusters], and (**B**) *F. auricularia* earwigs [(−) eggs: N = 9 clusters; (+) eggs: N = 8 clusters]. Error bars represent standard errors

alkaloid-free *U. ornatrix* eggs were offered in their place, to ensure that it was not for lack of hunger that the *H. axyridis* rejected the *Photuris* eggs. The *U. ornatrix* eggs were all consumed within 2–24 h.

Likewise, (+) and (−) *Photuris* eggs were equally distasteful to *L. longispinosus* ants (P = 0.92) (Fig. 5A). No eggs were consumed or taken to the nests within 24 h, and only few eggs from each category were consumed within 48 and 72 h. Previous studies had shown that under comparable experimental conditions *L. longispinosus* consume palatable eggs within the first few hours after presentation (Hare & Eisner, 1993).

Our third predator, *F. auricularia*, consumed some eggs from both the (+) and (−) categories (Fig. 5B). Although (+) eggs were subjected to slightly lower predation rates, the difference was not significant (P = 0.27). On several occasions the earwigs were observed seizing an egg in their mandibles and dropping it promptly, then engaging in mouth-dragging behavior against the paper substrate. This behavior was observed at least once in 9 of the 17 earwigs tested, and its incidence was equal in tests with (+) (4 out of 8 trials) and (−) eggs (5 out of 9 trials). Interestingly, some

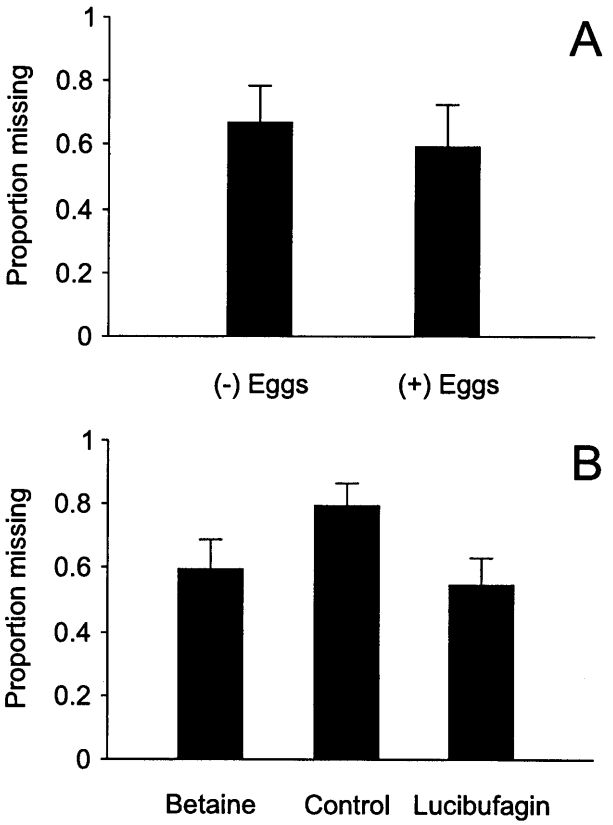


**Fig. 6** Proportion of eggs missing after 10 days in the field. **A** – Experiment with (+) and (−) *Photuris* egg clusters (N = 10 pairs). **B** – Experiment with *U. ornatrix* egg clusters topically treated with lucibufagin, betaine and control (N = 21 triads). Error bars represent standard errors

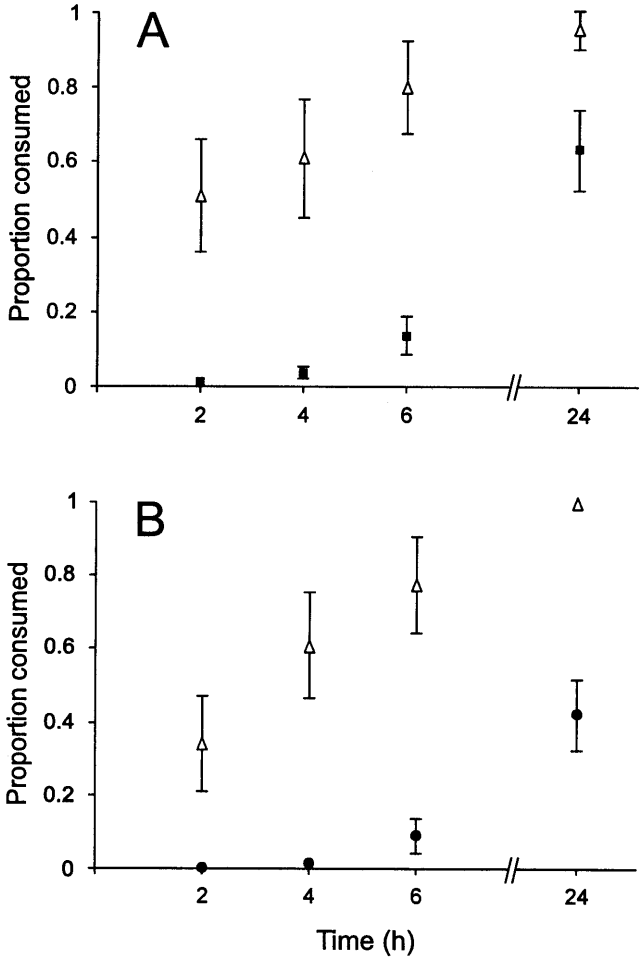


**Fig. 7** Predation rates in two-choice experiments with *H. axyridis* larvae. The larvae were offered a choice of two *U. ornatrix* egg clusters, one of which had been treated with (**A**) lucibufagin (solid squares, N = 10) or (**B**) betaine (solid circles, N = 10). Open triangles represent solvent-treated control eggs. Error bars give standard errors

earwigs rejected some eggs and accepted others within the same cluster, suggesting that there may be variation in the palatability of the eggs laid by a given *Photuris* female.

*b. Field experiment*. The results after 10 days showed equal predation rates for (+) and (−) *Photuris* eggs (Fig. 6A) (Wilcoxon signed-rank test, $P = 0.29$).

2. Predation on lucibufagin and betaine-treated food items (*Utetheisa* eggs)

*a. Laboratory experiments*. Both lucibufagin- and betaine-treated eggs were unpalatable to *H. axyridis* larvae ($P < 0.001$ for both cases) (Fig. 7A and 7B). The larvae consumed all control eggs, and only thereafter started to feed on some of the experimental eggs. Similarly, tests with *L. longispinosus* showed that both compounds were significantly deterrent (lucibufagin, $P < 0.005$; betaine, $P < 0.002$) (Fig. 8A and 8B).

*F. auricularia* earwigs, however, showed preference for control eggs only when these were tested against lucibufagin-treated eggs ($P < 0.001$) (Fig. 9A). They did not discriminate against the betaine-treated eggs ($P > 0.90$) (Fig. 9B).

*b. Field experiment*. The results after 10 days (Fig 6B) showed that the control clusters were missing significantly more eggs than the clusters treated with either lucibufagin or the betaine (two-way ANOVA followed by one-tailed contrasts, $P < 0.03$ for both chemical treatments).

## Discussion

*Photuris* females endow their eggs with a combination of lucibufagin and the betaine. While the lucibufagin is available only opportunistically to the females and is acquired (almost entirely) from *Photinus* prey, the betaine appears to be an endogenous staple, present in *Photuris* eggs, larvae, and adults alike. It was to be expected, therefore, that field-collected *Photuris* females and their eggs should contain lucibufagin on occasion only and in variable amounts, but betaine consistently. This was borne out by our data.

The analyses of field-collected females and their eggs showed further that the eggs receive lucibufagin at higher concentration than is present in the females themselves, with no such enrichment occuring for the

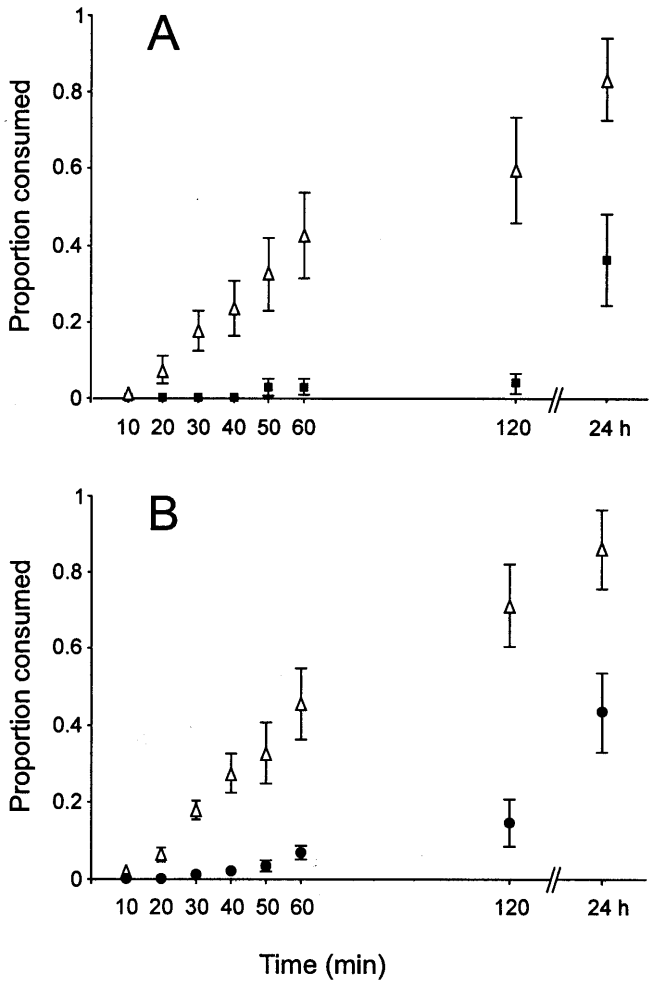


**Fig. 8** Predation rates in single-choice experiments with *L. longispinosus* ants. Each colony was offered a single *U. ornatrix* egg cluster that had been treated with (**A**) lucibufagin (solid squares, N = 8), (**B**) betaine (solid circles, N = 11), or (**A**, **B**) methanol (control clusters, open triangles, N = 11 in **A**, N = 10 in **B**). Error bars give standard errors

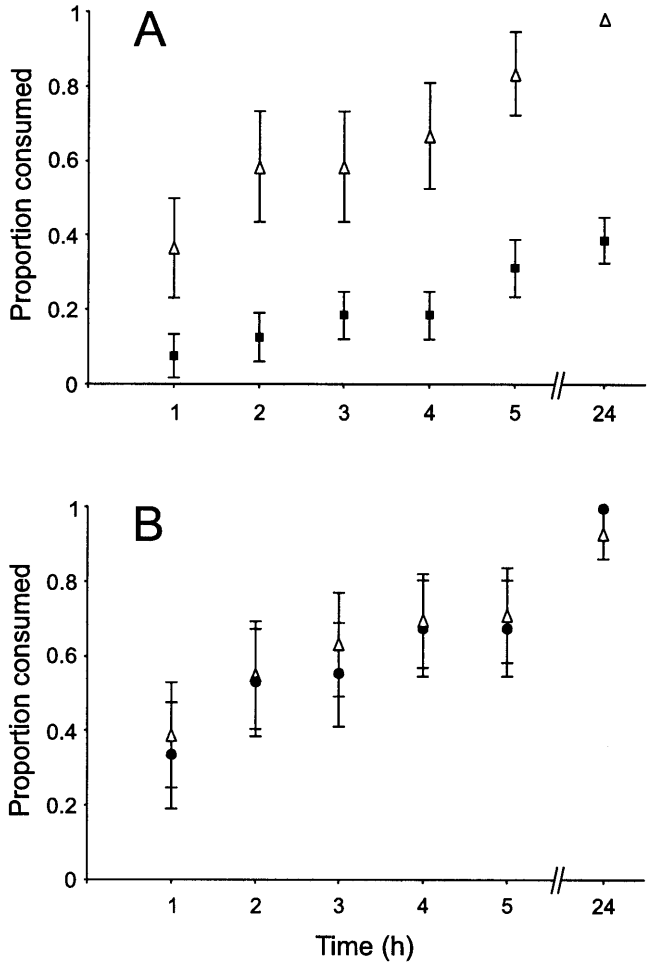


**Fig. 9** Predation rates in two-choice experiments with *F. auricularia* earwigs. The earwigs were offered a choice of two *U. ornatrix* egg clusters, one of which had been treated with (**A**) lucibufagin (solid squares, N = 12) or (**B**) betaine (solid circles, N = 12). Open triangles represent solvent-treated control eggs. Error bars give standard error

betaine. One possible explanation for this is that the female "overloads" the eggs with lucibufagin to provide for protection of the emerging larvae as well. The female could be "exempt" from similarly overloading the eggs with betaine, since – as we know from precursor-incorporation studies (González *et al.*, unpublished results)—the larvae themselves are able to biosynthesize the compound.

Our laboratory predation tests with *Photuris* eggs indicate clearly that both lucibufagin and the betaine can contribute to the distastefulness of the eggs. The results were clear-cut with the coccinellid larva and the ant. Both these predators rejected the (+) and (−) eggs, and both discriminated against *U. ornatrix* eggs treated with either of the compounds. With the earwig there was an indication of differential sensitivity to the two compounds. While the betaine, tested by itself (on *U. ornatrix* eggs), showed no deterrency, the lucibufagin proved potently antifeedant. It would follow from this difference in sensitivity that (−) eggs should be more acceptable than (+) eggs to the earwig. The data provided an indication to that effect (Fig. 5B), but none that held up statistically. The relative acceptability of the (+) eggs to the earwig was somewhat unexpected. It should be noted, however, that in the experiment with (+) and (−) eggs, the earwig was not given these items as a choice, but was presented with them in separate tests. Perhaps, in the absence of choice, *F. auricularia* is driven to accept food that it would otherwise reject.

The field tests on the whole supported what might have been expected from the laboratory tests. The (+) and (−) eggs proved equally vulnerable to predation, and the lucibufagin and betaine, tested as additives to *U. ornatrix* eggs, both reduced the rate at which these eggs disappeared.

Our overall conclusion from these findings is that lucibufagin and the betaine are both effective as defensive agents, but not necessarily equally so against all predators.

Insects use a wide range of compounds, both of endogenous origin and acquired, in defense of their

eggs. Although most examples are from Lepidoptera and Coleoptera, there are also documented cases from Hemiptera (Duffey & Scudder 1974; von Euw *et al*. 1971), Orthoptera (von Euw *et al*. 1967), Neuroptera (Eisner *et al*. 1996a; Henry 1972), and Diptera (Hinton 1968). The coleopteran examples include Coccinellidae (Pasteels *et al*. 1973), Pyrochroidae (Eisner *et al*. 1996b; Holz *et al*. 1994), Meloidae (McCormick and Carrel 1987), Oedemeridae (Holz *et al*. 1994), and Chrysomelidae. Interestingly, some chrysomelids protect their eggs exclusively with compounds of seemingly endogenous origin (Daloze & Pasteels 1979, Hilker & Schulz 1991, Howard *et al*. 1982, Pasteels & Daloze 1977, Pasteels *et al*. 1986), while others do so with chemicals acquired from the diet (Ferguson *et al*. 1985, Pasteels *et al*. 1986). Moreover, several species of *Chrysomela*, and a species of *Phratora* (*P. vitellinae*), protect their eggs with a combination of dietary salicin, and isoxazolinone glucosides that they themselves produce (Pasteels *et al*. 1986). *Photuris* is evidently not alone in relying on a diversified chemical strategy for the protection of its eggs.

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