ARTICLE

Bioluminescence in the ghost fungus *Omphalotus nidiformis* does not attract potential spore dispersing insects

Philip Weinstein[1], Steven Delean[1], Tom Wood[1], and Andrew D Austin[2]

[1] Department of Ecology and Environmental Sciences, School of Biological Sciences, The University of Adelaide, Adelaide SA 5005, Australia; corresponding author e-mail: philip.weinstein@adelaide.edu.au

[2] Australian Centre for Evolutionary Biology and Biodiversity, and Department of Genetics and Evolution, School of Biological Sciences, The University of Adelaide, Adelaide SA 5005, Australia

Abstract: Bioluminescence has been known from fungi since ancient times, but little work has been done to establish its potential role. There is evidence that some bioluminescent fungi differentially attract potential spore-dispersing insects, and we aimed to establish if this was the case for the ghost fungus, *Omphalotus nidiformis* (*Agaricales, Marasmiaceae*), a widespread Australian temperate zone species. We examined three corroborative lines of evidence: circadian rhythmicity of bioluminescence; field-recorded insect abundance at the time of basidiome production; and attractiveness of glowing fungi to flying insects. Basidiomes glowed continuously day and night, and were present in winter (June-July) when insect abundance was low. To assess attractiveness, we deployed sticky-traps in open woodland in the absence of light pollution, in Treatment (baited with fresh bioluminescent *O. nidiformis*) and Control pairs, for 480 trap-hours on moonless nights. There was no statistical difference in mean insect abundance between Treatment and Control traps (mean 0.33 and 0.54 individuals per trap night, respectively). To interpret these results, we provide a brief review of competing hypotheses for fungal bioluminescence, and conclude that for some fungi, bioluminescence may be an incidental by-product of metabolism rather than conferring any selective advantage. It is possible that the role of bioluminescence differs among evolutionary lineages of fungi and/or with attributes of their growth environments that could affect spore dispersal, such as wind and insect abundance.

INTRODUCTION

Bioluminescence has been known since ancient times, with bioluminescent fungi being documented by Aristotle (384-322 BC) as emitting light "which differed from that of fire" from a rotten log (Harvey 1952, Desjardin *et al*. 2008). The phenomenon is most common in marine environments, and a number of theories have been put forward to account for its apparent selective advantage in the dark of the deep ocean (Rees *et al*. 1998). Hastings (1983) reviewed the potential ecological roles of bioluminescence, listing defence, offence, and communication, but did not discuss fungal bioluminescence. Of the 150,000 or so described species of fungi, bioluminescence is known from only 71, with 40 % of these records being documented since 2001 (Desjardin *et al*. 2010).

For bioluminescent fungi, it has been suggested that the attraction of potentially spore dispersing insects can account for the selective advantage conferred by glowing, and this is supported by the recent experimental work in the Neotropics by Oliveira *et al*. (2015) for *Neonothopanus garderni* (*Agaricales, Marasmiaceae*). Bioluminescence could nevertheless come with a thermodynamic cost and

potential of attracting fungivores, so alternative hypotheses could include fungivore deterrence, fungivore predator attraction, warning signalling, and as an incidental by-product of detoxification or other metabolic reactions.

To our knowledge, no studies on Australian fungi have focused on the ecological role of bioluminescence, despite the occurrence of several bioluminescent species, including the widespread *Omphalotus nidiformis* (ghost fungus; *Agaricales, Marasmiaceae*) in the temperate south of the continent. In this study, we capitalized on the presence of this fungus to test the hypothesis that ITS bioluminescence would be attractive to insects at night.

MATERIALS AND METHODS

Fungus and field site

Omphalotus nidiformis is an Australian temperate-zone basidiomycete, generally found at or near the base of *Eucalyptus* trees, with variable cream to brown basidomes, most often in the range of 20-40 cm diam. It produces basidomes in the Southern Hemisphere winter (June-July) and emits a continuous, faint white-bluish glow (Fig. 1B).

Fig 1. **A.** The Australian ghost fungus *Omphalotus nidiformis* in natural daylight and **B.** Under long exposure using its own light. Note that to the naked eye, the glow is very faint with a more bluish hue. **C.** The Kangaroo Island study site consisting of sclerophyll woodland dominated by *Eucalyptus fasciculosa*, and **D.** A yellow sticky trap in situ, baited with a cut piece of fresh fungus. Images A, C, and D are by PW, and B is courtesy of Australian Museum/Ray Kearney.

Fresh, glowing specimens were located in Flinders Chase National Park, Kangaroo Island, South Australia, in open eucalypt woodland approximately 1 km NE of the University of Adelaide Field Station at Rocky River. Large (approx. 5 cm across), glowing (visually checked) pieces of these fungi were used to bait 10 x17 cm two-sided sticky traps (Australian Entomological Supplies Catalogue reference E95101A; these are yellow cards coated with non-volatile, non-drying adhesive, designed for visual attractiveness in day use only; Fig. 1D).

The site was deliberately chosen to be free from any possible light pollution, and sampling was undertaken over two completely moonless nights (19 and 20 June 2015).

These restrictive conditions eliminated any possibility of confounding or dilution from other light sources, but at the same time limited the number of catch nights possible. Nocturnal trapping was undertaken on clear nights (intermittent light cloud, no rain) with no wind, and with ambient temperatures around 6 °C. Traps were deployed and retrieved in darkness, approximately 1 h after sunset and 1 h before sunrise, respectively, to eliminate any attraction of day-flying insects to the traps. Diurnal trapping was undertaken on the intervening days with intermittent light cloud and no wind, with ambient temperatures around 12 °C. Traps were deployed and retrieved during the day, approximately 1 h

Table 1. Trap catches by type of trap (sticky, malaise, and pan) and time (day/night) for each (sub)order represented.

	Both nights combined (20 h)			Day (10 h)		
	Sticky traps	Malaise	Pan	Sticky traps	Malaise	Pan
	480 trap-hrs	20 trap-hrs	200 trap-hrs	120 trap-hrs	10 trap-hrs	100 trap-hrs
Collembola			2			16
Blatodea				1		
Homoptera					1	
Heteroptera		1	1			
Coleoptera						3
Diptera: *Nematocera*	15	14		39	49	61
Diptera (other)	4			30	22	18
Lepidoptera				1	1	
Hymenoptera: *Formicidae*	1	2	3			
Hymenoptera (other)		1	2	10	7	71
Araneae	1					
Total	**21**	**18**	**8**	**81**	**80**	**169**

after sunrise and one hour before sunset, respectively, to eliminate any attraction of night-flying insects to the traps. All work was carried out under natural field conditions.

Study design and analysis

We checked basidiomes for bioluminescence regularly in the field, both day and night, and both attached to and detached from the mycelium, by using a heavy coat to create a 'dark room'. They were present in the field throughout June, corroborating records of winter basidiome production from the State Herbarium of South Australia (Pam Catcheside, pers com.).

To assess the nature and abundance of flying insects, we sampled four sites separated by approx. 50 m. At each site, three types of traps were deployed: sticky (see below), malaise (1), and pan (10) traps. These were used to assess both nocturnal and diurnal abundance as above, and catches were identified to (sub)order and recorded against the total number of trap hours by trap type.

To assess the attractiveness of bioluminescence to flying insects at night, three replicates of treatment-control pairs of sticky traps were used at each of the four sites, with the treatment trap in each pair having a piece of basidiome attached. All traps were separated by approximately 2 m and were well away from any naturally occurring fungi, to avoid any potential interference. A total of 24 traps were deployed for at least 10 h each on each of two nights, giving 48 trap-nights. The total catch for each trap was counted for each night, and all insects were included in the analysis as potential spore-dispersers. Differences in the abundance of insects between Treatment and Control traps were examined using generalized linear mixed models. Site and Trap-pair within Site were included as random effects to account for the two
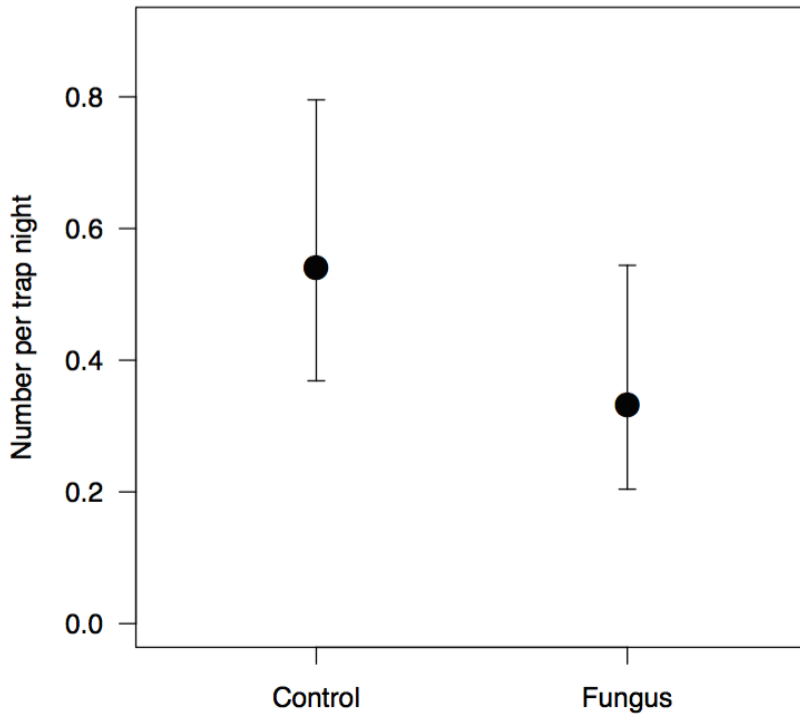
scales of sampling variation. We assumed Poisson variation in the abundance counts and used a log link function. Model fitting was undertaken using the *lme4* package (Bates *et al*. 2014) in *R*-3.2.0 (Team 2015).

Due to low catch numbers in both Treatment and Control sticky traps at night, we summed the abundances across the two sampling nights for analysis; exploratory analysis indicated no statistically significant differences between nights. There was also no statistically significant variation in catch abundances between sites or between trap-pairs within sites; these variance components were estimated to be close to zero in the analysis. Consequently, model estimates were equal to those from a generalised linear model (Poisson variance and log link) that did not include the random effects. There was also no evidence for extra-Poisson variation.

RESULTS

We observed basidiomes glowing continuously day and night under natural field conditions, irrespective of attachment to mycelium (which did not glow). Flying insects were, as expected, not abundant at this time of year, with catch numbers low for all trapping methods (Table 1), and particularly low at night (about 6 %, 10 %, and 2 % of diurnal catch numbers per trap-hour for sticky, malaise, and pan trapping respectively). No taxonomic bias was evident between day and night catches (Table 1), with *Diptera* (flies) and their hymenopteran parasitoids (*Diapriidae*, *Braconidae*; data not shown) dominating the fauna.

Sticky traps at night collected 21 insects, 13 in Controls and 8 in Treatments, with a sampling effort of over 480 trap-hours. There was no statistical evidence for a difference

Fig. 2. Estimated catch abundance in Control and Treatment (fungus baited) traps. Error bars are 95 % confidence intervals.

in mean insect abundance between Treatment and Control traps (x^2_1 = 1.17, *P* = 0.28). Mean insect abundance in the Control was 0.54 individuals per trap night (95 % confidence interval = 0.37, 0.80), and 0.33 (CI = 0.20, 0.54) individuals in the Treatment (Fig. 2).

DISCUSSION

Our results provide three corroborative pieces of evidence suggesting that bioluminescence in *Omphalotus nidiformis* does not attract potential spore dispersing insects. Firstly, glowing only at night is suggestive of a thermodynamic trade-off between the cost of bioluminescence and the fitness gain resulting from improved spore dispersal (Oliveira *et al.* 2015). That *O. nidiformis* glows continuously suggests that there is no such trade-off, and that there is no fitness gain through attraction of potential spore dispersing insects in this species. Secondly, the abundance of potential spore-dispersing insects was very low, as would be expected in mid-winter. This was particularly the case at night, because nocturnal sticky, malaise and pan-trapping produced less than 10 % of the number of diurnal catches per trap-hour (Table 1). For nocturnal sticky traps, catches per fungus-baited trap-night averaged fewer than 0.33 individuals, despite ideal conditions for attracting positively phototactic flying insects to the bioluminescent fungi with no competing light sources or adverse weather. Thirdly, there was no difference in attractiveness between Treatment (bioluminescent) and Control sticky traps. For bioluminescence to confer a significant selective advantage under conditions of low insect abundance would require a strong attraction, to draw the few available flying insects to the spore-carrying basidiomes, and one would expect that the strength of such an attraction would swamp any potential change in volatiles that might have resulted from cutting the fungi (Faldt *et al.* 1999). No attraction at all was detected,

again suggesting that the attraction of spore dispersing insects is not a driver of bioluminescence in *O. nidiformis*. Our three lines of evidence – lack of circadian rhythmicity, low insect abundance at the time of basidiome production, and no difference in attractiveness of bioluminescent treatments over controls – are mutually reinforcing. Further, our work was carried out under ideal natural field conditions using fresh fungi in the complete absence of competing light sources or adverse weather conditions, thereby strengthening the significance of our findings.

To explain the presence of bioluminescence in *O. nidiformis*, it is therefore necessary to explore competing hypotheses for fungal bioluminescence, which we briefly review below.

Attraction of spore-dispersers

Bermudes *et al.* (1992) suggested this mechanism, supported by the observation that the gills or spores of some bioluminescent species glow more strongly than other parts (although this is not obvious in *Omphalotus nidiformis*). Sivinski (1981), working in Florida, enclosed forest floor litter in glass test-tubes and found that more arthropods were attracted to luminous forest litter containing mycelia of a *Mycena* species than to non-luminous forest floor litter, suggesting that the visual cue was the attractant. In a more sophisticated version of the same approach, Oliveira *et al.* (2015) used acrylic models of the Neotropical *Neonothopanus garderni* covered with a non-volatile glue emitting LED light at 530 nm. They compared catches with lights on and off, and found that significantly more insects were trapped with the lights on. It is also know that some fungivorous insects are positively phototactic to low emissions of light in the wavelength range of 300–650 nm (Jess & Bingham 2004), and although this might seem counterintuitive in the context of fitness, it is possible that a trade-off operates between basidiome damage and spore dispersal (cf. pollen consumption *v*. pollination

services to plants by bees and wasps). If bioluminescence does serve to attract spore-dispersers, it is likely that this strategy would be most successful in fungi that grow in relatively wind-still conditions (dense lower canopy or ground cover fungi). Further, several species have both luminous and non-luminous strains (Desjardin *et al*, 2008), possibly reflecting phenotypic plasticity in different climatic and floristic environments. We could find no published accounts relating the abundance of bioluminescent species to vegetation type or insect abundance, and although more bioluminescent species occur in tropical rainforests, it is unclear if their frequency is proportional to the richer diversity in such (low wind and high insect abundance) environments. We suggest that an examination of potential relationships of this nature could be a productive area of further research.

By-product of metabolism

An alternative hypothesis is that bioluminescence in fungi is a by-product of detoxification reactions, and provides no particular selective advantage. For example, Weitz (2004) suggests that light is emitted instead of heat as an energy by-product of enzyme-mediated oxidation reactions, as is the case in some bioluminescent bacteria (where bioluminescence is continuously produced as a by-product of respiration). In *O. nidiformis*, bioluminescence is continuous (supporting this hypothesis), but in species of other lineages, bioluminescence is regulated by a circadian clock so as to produce light only at night (which would be more consistent with expectation if the attraction of spore-dispersers was involved, such as in *N. garderni* (Oliveira *et al.* 2015). The metabolic by-product hypothesis could also be consistent with an evolutionary origin of bioluminescence as a by-product, which may subsequently have been adapted to play a significant ecological role in some lineages.

Fungal bioluminescence uses an enzymatic pathway where a 'luciferin' is oxidised in the presence of 'luciferase' (Airth & Foerster 1962, Desjardin *et al*. 2008). This process has been tested in several extract-based experiments (Desjardin *et al*. 2008), with Oliveira *et al*. (2012) mixing extracts from a range of species to demonstrate that different evolutionary lineages of luminescent fungi share similar luciferin-luciferase mechanisms of bioluminescence. All fungi that are known to exhibit bioluminescence are basidiomycetes (Herring 1994, Matheny *et al*. 2006), but belong to three distinct lineages; the *Omphalotus*-, *Armillaria*- and the mycenoid lineages (Desjardin *et al*. 2008). Thus, it is possible that bioluminescence serves different ecological roles in different lineages, even though the molecular mechanisms are similar.

CONCLUSION

From the experiments of Oliveira *et al.* (2015) and earlier workers, it is edvident that bioluminescence does attract insects to at least some species of fungi, and that these fungi are likely to have the advantage of increased spore dispersal. Characteristics of such fungi may include the emission of a bright light with a nocturnal circadian rhythm, a growth environment where wind dispersal of spores is relatively less likely, and an abundance of flying insects. Significantly,

Omphalotus nidiformis displays none of these characteristics: it emits a very faint light which is present continuously; it occurs in open eucalypt woodland where wind dispersal of spores is relatively more likely, and it produces basidiomes at a time of year when the abundance of potential spore-dispersing insects is low.

In addition, we could find no difference in attractiveness between catches from control traps and those baited with actively glowing fungus. We therefore conclude that the attraction of spore-dispersing insects and the incidental by-product of metabolism hypotheses for bioluminescence in fungi are both supported, but by evidence from different lineages of fungi and possibly also differing growth environments. One possible explanation for such a situation would be that bioluminescence in fungi arose as an incidental by-product of metabolism, and that some fungi such as *O. nidiformis* remained in this condition. Others, such as *N. garderni*, acquired an advantage in fitness as a result of adapting bioluminescence to maximise their attractiveness to potentially spore-dispersing insects. It would be a worthwhile exercise to map the ecological correlates of fungal bioluminescence onto a phylogeny, once sufficient new data become available for this purpose.

ACKNOWLEDGEMENTS

REFERENCES

Airth R, Foerster GE (1962) The isolation of catalytic components required for cell-free fungal bioluminescence. *Archives of Biochemistry and Biophysic,* **97**: 567–573.

Bates D, Maechler M, Bolker B, Walker S (2014) *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-7: http://CRAN. R-project. org/package= lme4.

Bermudes D, Petersen RH, Nealson KH (1992) Low-level bio-luminescence detected in *Mycena haematopus* basidiocarps. *Mycologia* **84**: 799–802.

Desjardin DE, Oliveira AG, Stevani CV (2008) Fungi bioluminescence revisited. *Photochemical and Photobiological Science* **7**: 170–182.

Desjardin DE, Perry BA, Lodge DJ, Stevani CV, Nagasawa E (2010) Luminescent *Mycena*: new and noteworthy species. *Mycologia* **102**: 459–477.

Fäldt J, Jonsell M, Norlanderg G, Borg-Karlson A-K (1999) Volatiles of bracket fungi *Fomitopsis pinicola* and *Fomes fomentarius* and their functions as insect attractants. *Journal of Chemical Ecology* **25**: 567–590.

Harvey EN (1952) *Bioluminescence.* New York: Academic Press.

Hasting J (1983) Biological diversity, chemical mechanisms, and the evolutionary origins of bioluminescent systems. *Journal of*

Molecular Evolution **19**: 309–321.

Herring PJ (1994) Luminous fungi. *Mycologist* **8**: 181–183.

Jess S, Bingham J (2004) The spectral specific responses of *Lycoriella ingenua* and *Megaselia halterata* during mushroom cultivation. *Journal of Agricultural Science* **142**: 421–430.

Matheny PB, Curtis J M, Hofstetter V, Aime MC, Moncalvo J-M, *et al.* (2006) Major clades of *Agaricales*: a multilocus phylogenetic overview. *Mycologia* **98**: 982–995.

Moncalvo J-M, Vilgalys R, Redhead SA, Johnson JE, James TY, *et al.* (2002) One hundred and seventeen clades of euagarics. *Molecular Phylogenetics and Evolution* **23**: 357–400.

Oliveira AG, Desjardin DE, Perry BA, Stevani CV (2012) Evidence that a single bioluminescent system is shared by all known bioluminescent fungal lineages. *Photochemical and Photobiological Sciences* **11**: 848–852.

Oliviera AG, Stevani CV, Waldenmaier HE, Viviani V, Emerson JM, *et al*. (2015) Circadian control sheds light on fungal bioluminescence. *Current Biology* **25**: 964–968.

Rees J-F, de Wergifosse B, Noiset O, Dubuisson M, Janssens B, Thompson EM (1998) The origins of marine bioluminescence: turning oxygen defence mechanisms into deep-sea communication tools. *Journal of Experimental Biology* **201**: 1211–1221.

Sivinski J (1981) Arthropods attracted to luminous fungi. *Psyche: a Journal of Entomology* **88**: 383–390.

Team RC (2015) *R: A language and environment for statistical computing.* Vienna: Foundation for Statistical Computing; http://www.R-project.org/.

Weitz WHJ (2004) Naturally bioluminescent fungi. *Mycologist* **18**: 4–5.

ARTICLE