ABSTRACT

Bioluminescence in cave glow-worms

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Larval fungus gnats of the genus *Arachnocampa* are sit-and-lure predators that use bioluminescence to attract flying prey to their silk webs. Nine species are described in Australia and New Zealand. Some species are most common in rainforest habitat and others inhabit both caves and rainforest.

Time-lapse recording of light output in rainforest has shown that the light intensity varies through the night in a characteristic pattern with maximum brightness soon after dusk. The intensity of the colony and number of individuals glowing varies seasonally with the brightest displays in summer. Larvae are sensitive to rainfall, increasing their brightness when rain starts falling and are sensitive to moonlight, reducing their brightness on clear, moonlit nights. Laboratory experiments show that larvae douse in response to ultraviolet, blue and green light and are insensitive to red, suggesting they do not see red light. They are also sensitive to vibration, brightening when they sense physical disturbance.

The biological clock influences bioluminescence cycles, but with major differences between two of the studied species; one found in Queensland subtropical rainforest with no known cave populations (*Arachnocampa flava*), the other found in temperate rainforest with large populations in caves (*Arachnocampa tasmaniensis*). Larvae of *A. tasmaniensis* in the cave dark zone synchronize to each other, creating a daily sinusoidal rhythm of bioluminescence intensity in the many thousands of individuals making up a colony. This synchronization could provide a group-foraging advantage, allowing the colony to glow most brightly when the prey are most likely to be active. This is a novel adaptation of the circadian system to living in the constant darkness of caves. The New Zealand glow-worm (*Arachnocampa luminosa*) is also likely to be a synchronizing species: photographic monitoring of the population in the Glowworm Grotto of Waitomo Glowworm Cave has been ongoing since 2011. The daily synchronized cycle is similar to that of *A. tasmaniensis*.

Future research directions are also outlined.

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Introduction

The insects known as glow-worms in Australia and New Zealand are larval fungus gnats of the genus *Arachnocampa*. They are members of the Order Diptera, family Keroplatidae, subfamily Arachnocampinae (Matile 1981). They are sit-and-lure predators that use bioluminescence to attract flying prey to their silk webs.

Larvae are extremely susceptible to desiccation so even in rainforest habitats they are usually confined to moist areas near streams. From hatching to pupation (6–12 months) the larvae lie suspended from the substrate in a mucous tube from which they hang silk lines dotted with sticky mucus droplets to capture prey attracted by the larval bioluminescence. Adults are short lived (3–14 days) and do not feed (Richards 1960; Baker and Merritt 2003; Meyer-Rochow 2007).

The bioluminescence display produced by the high density of larvae in some suitable locations is a major tourist attraction at sites in Australia and New Zealand, making *Arachnocampa* a commercially valued insect associated with nature-based tourism (Baker 2002). Some sites where visitors experience glow-worm colonies in caves or rainforest include Natural Bridge at Springbrook National Park, Queensland, with approximately 100,000 visits per year (Baker 2002), Marakoopa Cave, Tasmania, and Waitomo Glowworm Cave in New Zealand.

Distribution and evolutionary history

Glow-worms are found only in Australia and New Zealand. In Australia, they are distributed through the wet forests of the Great Dividing Range and in Tasmania and southern Victoria. In New Zealand, the single species, *Arachnocampa luminosa*, is found in wet forests and caves of both the north and south islands.

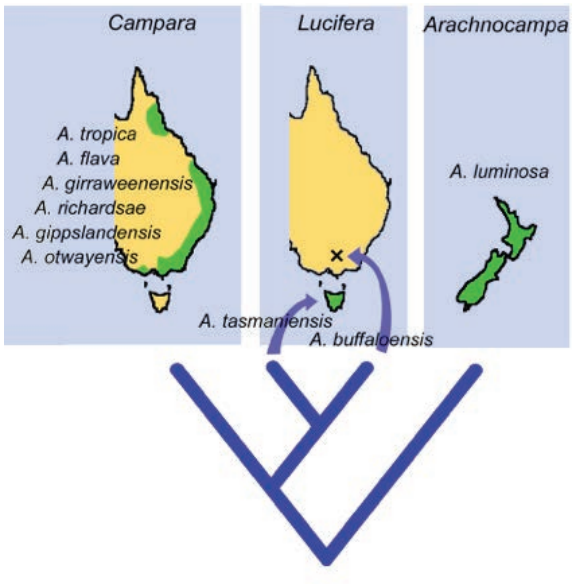


Figure 1. The distributions of glow-worm species and their phylogenetic relationships. The subgenus name is shown at the top of each panel.

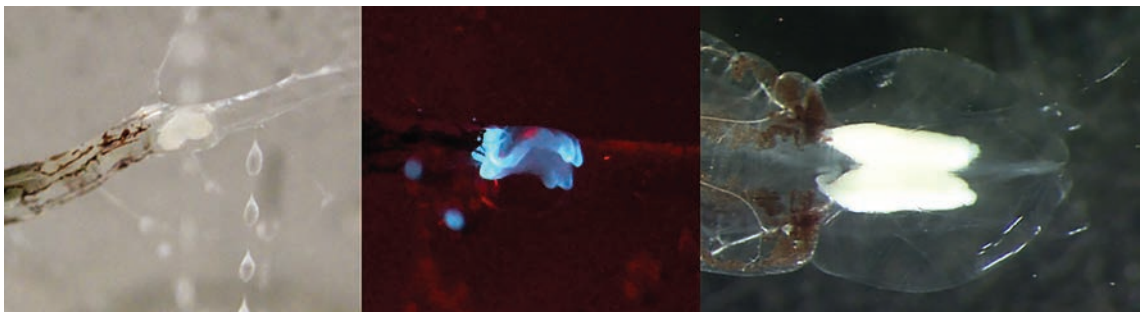


Figure 2. The light organ of *Arachnocampa flava*. The left panel shows the posterior region with the tracheal reflector appearing as a white mass. The central panel is a photograph of the light organ when it is releasing light. The right panel is a picture of the light organ region viewed from above, showing the tracheal mass that sits adjacent to the light-producing cells.

An Australia-wide examination of glow-worm species identity resulted in the naming of five new species (Baker 2010), bringing the total number of species to nine. Species tend to be clustered into geographic groups. The evolutionary relationships of the species were determined based upon mitochondrial gene sequence (Baker et al. 2008). Regarding phylogenetic relationships, the New Zealand species (*A. luminosa*) was placed as sister to all of the Australian taxa (Fig. 1). Within the Australian taxa, the Tasmanian species (*A. tasmaniensis*) was shown to be sister to a species from Mt Buffalo in Victoria (*A. buffaloensis*). A new subgenus (*A.* subgen. *Lucifera*) was named to encompass *A. tasmaniensis* and *A. buffaloensis* (Baker 2010). The remaining Australian species are placed within subgenus Campara.

Light production

The larval light organ, composed of modified cells of the four Malpighian tubules (Wheeler and Williams 1915; Gatenby 1959) is located in a swollen posterior segment. The air-filled trachea form a dense mass covering the light-producing photocytes (Green 1979). Externally, the tracheal mass is visible through the cuticle (Fig. 2). An ultrastructural investigation reported fine nerves containing opaque and clear synaptic vesicles, characteristic of the release of biogenic amines, running alongside the cells of the light organ (Green 1979).

Cave and rainforest populations

Rainforest glow-worm populations tend to be found in colonies in the vicinity of vegetation-sheltered streams, especially those with steep, excised stream banks; thus, they are present throughout much of the rainforests of the Great Dividing Range of eastern Australia, and wet forests of New Zealand (Baker 2010). The most likely dispersal scenario is that under favourable conditions of consistent rainfall (Merritt and Patterson 2017), populations grow and expand along such forest corridors. In rare cases, dispersing females encounter caves with stream inflow that are good habitat due to the sheltered, persistent, humid conditions. Under drying climates, such as the Pleistocene glacial cycles, fragmentation of populations through loss of favourable sites could have left relictual populations in isolated refugia such as caves. The sluggish flight behaviour and short lifespan of adults suggests that they do not disperse long distances under normal circumstances (Richards 1960; Baker et al. 2008) so it is possible that long-standing cave populations are genetically distinct. Morphologically, cave populations show reduced pigmentation of the epidermal cells, tend to make much longer snares than rainforest populations and produce larger adults than nearby forest populations (Richards 1960); however, these traits emerge from responses to different environments rather than being due to genetic differences. The population genetics of glow-worms is currently being studied by comparing nuclear and mitochondrial DNA sequence from many individuals in adjacent caves and forests using high through-put DNA sequencing.

Natural bioluminescence cycles in forest

To see how larvae respond to natural environmental conditions, time-lapse digital photography using an SLR camera was set up in a rainforest gully in Springbrook National Park to image a glow-worm colony at 10 min intervals over x months of a year, at the same time recording temperature and rainfall with dataloggers (Merritt and Patterson 2017). On a typical night, larvae start glowing soon after dusk and rapidly rise to a peak intensity before their intensity decreases through the night (Fig. 3). At dawn they douse in response to the increase in ambient light levels. The intensity of larvae varies night-to-night. In winter, they generally glow more dimly than in summer. Rainfall has a large influence on glowing intensity. If rain fell through the night, the number of larvae glowing increased immediately and an increase in light intensity was seen among larvae in the field of view. After several days of rainfall the colony's bioluminescence output will remain elevated for several days. In forest settings, the best times to see glow-worms is after recent rainfall. The larvae are quite sensitive to low levels of ambient light, decreasing their own light output on strongly moon-lit, clear nights. Moonlight around the time of a full moon inhibited larval bioluminescence output (Fig. 3).

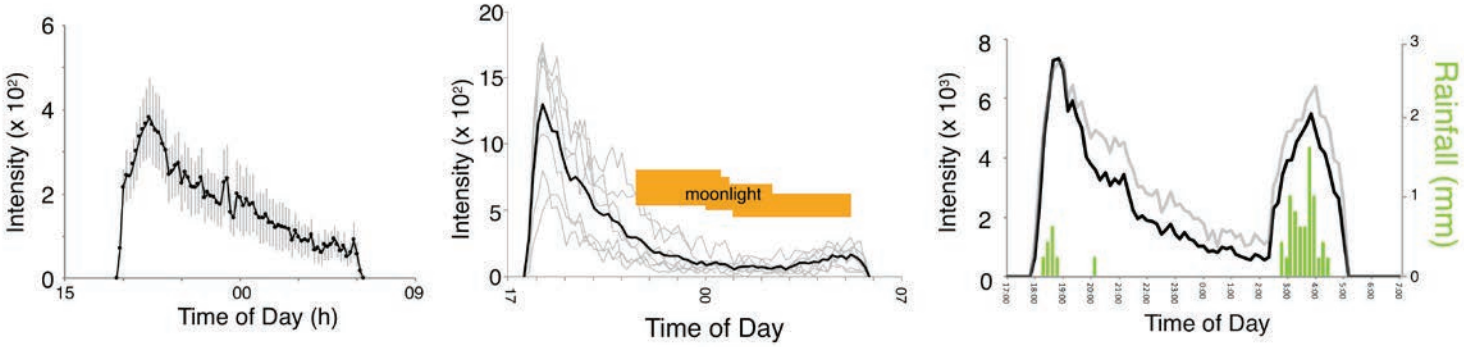


Figure 3. The light intensity profile of *Arachnocampa flava* in forest conditions The left panel shows the light output of larvae on a typical night. The central panel shows the light output over a period of exposure to a full moon. The black line is the average of the grey lines. The right panel is light output on a single night when rain fell after dusk and again at about 3 am.

It is well-known that larvae in forest glow only at night. Light suppresses bioluminescence; that is why visitors at tourism sites are requested not to shine torch-light directly on the glow-worm colonies. The dimming response was quantified in the laboratory (Mills et al. 2016). Exposure to a 5-min light pulse in the laboratory causes larvae to exponentially decrease their light output over 5–10 minutes until they completely switch off. Recovery of bioluminescence after light exposure is slow: once they have switched off, larvae took an average of 33 ± 6 min to resume bioluminescence and took an average of 70 ± 8 min to reach the pre-exposure level (Mills et al. 2016).

To obtain an idea of their spectral sensitivity, glowing larvae were exposed to different coloured light at equivalent photon fluxes (Mills et al. 2016). Larvae proved to be most sensitive to ultraviolet light, followed by blue, green and red. Like many insects, they are quite insensitive to red light so red LED sources are an alternative for safe lighting at night that allows visitors to see their way around without causing larvae to douse their own light. In caves, use of a red LED light source allows us to see the glow-worms' glows as well as the cave surroundings.

Response to vibration and disturbance

So far, we have seen how larvae can modulate their light according to detection of moonlight and artificial light. Another form of light modulation has long been recognised — the ability to rapidly increase light levels on exposure to loud sounds, vibration or other disturbances.

A long-standing display used by tour operators in quiet cave chambers is the "inner tube slam" brightening response where glow-worms in a quiet chamber visibly brighten after an inflated rubber inner tube is slammed onto the rock or water surface. In other observations, pupae or larvae of some species brighten when their container is tapped (Sivinski 1998; Baker and Merritt 2003; Broadley 2012) and female *A. luminosa* pupae glow brightly when a male adult alights on them (Richards 1960). Lee (1976) reported that slight vibration would induce an increase in bioluminescence that falls back to a lower level over 10 minutes. *A. luminosa* larvae increase their glow intensity when prey is caught in their web (Stringer 1967) and when they aggressively fight with each other if their webs encroach in a densely-packed colony. In laboratory tests of controlled stimuli, *A. flava* larvae were found to respond to vibration of a single silk line at 100 Hz with an average 3-fold increase in light output over 20–30 sec, followed by a slow return to pre-exposure levels. Using haptic motors taped to an aquarium, vibration of many larvae individually housed in the aquarium resulted in a several-fold increase in bioluminescence over 20–30 sec, followed by an exponential return to pre-exposure levels over 1–30 min (Mills et al. 2016).

Biological clocks

All nine species of glow-worm are morphologically similar, with minor differences between them in size, pigmentation and other cuticular features. Consequently, we expected that their physiology and behavior would also be similar but were surprised to discover a fundamental difference in how the biological clock controls bioluminescence in two representative species. The background to this discovery was our testing of whether bioluminescence comes under the control of a biological clock in *Arachnocampa flava*, the south-east Queensland rainforest species. We found that the rhythm of glowing continues even when larvae are held in constant darkness for many weeks. In addition, individuals show different periodicities of the on-going cycle, meaning that they drift out of phase with each other over long periods in total darkness (Merritt and Aotani 2008). The dual control system — an internal clock and a switch-off response to light — was not, in itself, surprising because many animals combine direct response to lighting conditions with the internal clock to modulate the time of activity (Aschoff 1960; Rensing 1989; Mrosovsky 1999).

The between-species difference became apparent when we investigated another species, *A. tasmaniensis*. We wanted to know whether bioluminescence rhythms persist in caves in the dark zone where light:dark cycles are absent: caves could be seen as a natural experiment, replicating how we placed *A. flava* in constant darkness in the lab. Because *A. flava* does not have any known cave populations, *A. tasmaniensis* was chosen to test the question. Time-lapse imaging in caves showed that the intensity of larvae waxes and wanes in synchrony, with a period of close to 24 h and the peak occurring in late afternoon to early evening (Merritt and Clarke 2011; Merritt et al. 2012). This went against our prediction, so a series of cave- and laboratory-based experiments were undertaken to compare the clock control of bioluminescence in the two species.

But first, we analysed the synchronization phenomenon in more detail. The within-colony synchronization suggested that larvae detect and match others' bioluminescence cycles (Merritt and Clarke 2011). To confirm, experiments in caves showed that a cluster of individuals within a colony shifted phase in response to exposure to an artificial light pulse each day for several days and once the daily stimuli were stopped, the phase-shifted larvae gradually returned to synchrony with the rest of the colony (Maynard and Merritt 2013). In the laboratory, exposure of larvae to each other under controlled conditions also produced synchronization, confirming that individuals are able to detect the glows of others and change the phase of their daily cycle to synchronize.

A. flava do the opposite; they do not synchronize to each other, they just synchronise to the light:dark cycle. Thus, the two species respond differently to the same entrainment cues. In *A. flava,* entrainment to light reinforces the nocturnal glowing rhythm while in

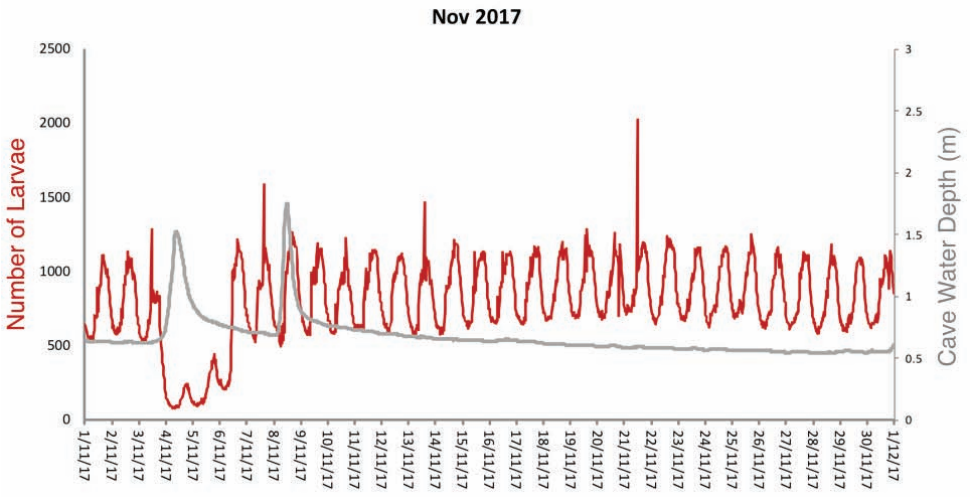
A. tasmaniensis entrainment induces synchronization to the low light levels of other larvae. *A. tasmaniensis* are also found in forest colonies, so it seems that the synchronization response is overwhelmed by the switch-off response to ambient light, meaning that they switch off under daylight and switch on at night (Berry et al. 2017).

The masking response is the same in both species of *Arachnocampa* so the phase response to entrainment appears to be key (Berry et al. 2017). A phase-response curve (PRC) to photic entrainment is a graphical depiction of the degree and direction of phase change shown by individuals exposed to a light pulse according to the subject's phase when the stimulus is applied (Johnson 1992). Analysis of the published PRCs of a diverse group of nocturnal and diurnal animals shows that their PRCs are similar, with slight but consistent differences (Refinetti 2016). This, along with comparisons of the PRCs of related species with different nocturnal/diurnal tendencies, indicates that differences between nocturnal and diurnal animals lie not so much in the nature of their phase-responses as through processes downstream of the clock (Refinetti 2016). However, *A. tasmaniensis* and *A. flava* do not fit this scenario: they show very different PRCs (Berry et al. 2017).

There are indications that the New Zealand glow-worm, *A. luminosa*, is also a synchronizing species. Time-lapse monitoring cameras installed in the Glowworm Grotto in 2011 have provided a continuous record of the bioluminescence intensity and number of glowing larvae since that time. They show ongoing, synchronized, daily cycles in the cave (Fig. 4), just like *A. tasmaniensis*. The cycles show some disruption due to floods and occasional, momentary increases in the count due to noise disturbance (Fig. 4). They also show strong seasonal cycles, with the most intense display and greatest population density in December and January. While it has not been experimentally proven, *A. luminosa* appears to have the same synchronization capability as *A. tasmaniensis*, suggesting that it shares the same type of biological clock and PRC.

The substantial difference between two species of same genus is very unusual. One hypothesis is that the biological clock of *A. tasmaniensis* is representative of species adapted to cave environments and that of *A. flava* to forest-adapted species. We hope to follow up the species differences through several avenues; (1) establish the synchronization ability of all species and see if the *flava*-like and *tasmaniensis*-like traits can be matched against the evolutionary tree of Fig. 1, (2) sequence some of the clock-associated genes in both species to see if there are obvious differences and (3) carry out a population genetics study of representative species covering both forest and cave habitats where available, looking for signs of genetic isolation by distance or isolation by habitat.

Figure 4. The number of *Arachnocampa tasmaniensis* larvae visible in the field of view of a monitoring camera in Waitomo Glowworm Cave through November 2017. Images were recorded at 30 min intervals. The cave water depth shows floods on 4 and 9 November.



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