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Homeostatic and circadian mechanisms of bioluminescence regulation differ between a forest and a facultative cave species of glowworm, *Arachnocampa*

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ABSTRACT

Glowworms, members of the keroplatid fly genus, *Arachnocampa,* glow to attract prey. Here we describe substantial differences in the bioluminescence regulatory systems of two species; one is a troglophile with populations both in caves and outside of caves in wet forest (*Arachnocampa tasmaniensis*) and the other has no known cave populations (*Arachnocampa flava*). We find that *A. tasmaniensis* is ready to initiate bioluminescence at any time darkness is encountered. In contrast, *A. flava* shows a homeostatic control of bioluminescence; it is unlikely to initiate bioluminescence when exposed to dark pulses during the photophase and it does so with a long latency. Another difference between the two species is that *A. tasmaniensis* individuals synchronize their bioluminescence in the dark zone of caves under the control of the circadian system and *A. flava* individuals do not synchronize to each other, rather their circadian control system entrains to the light:dark cycle to promote nocturnal bioluminescence. Consequently, we produced a phase-response curve in response to photic entrainment under constant darkness for both species. The shape of the phase-response curves differs between the two species as does the overall sensitivity to the identical entrainment conditions. The phase-response curve of *A. tasmaniensis* facilitates synchronization whereas that of *A. flava* facilitates nocturnal glowing. The two-species comparison highlights possible pathways of divergence of circadian control of physiological functions that could be associated with the extreme ecological differences experienced in cave and surface habitats.

1. Introduction

The most potent and ubiquitous synchronizer of the physiology and behavior of organisms is the light:dark (LD) cycle. While organisms can and do react directly to the LD cycle, endogenous circadian control of physiological processes is widespread. Numerous reviews have examined the adaptive value of placing key physiological and behavioural processes under circadian control, including the ability to prepare for upcoming events, buffering against natural variations in light intensity and coordinating internal physiological processes (Friedrich, 2013; Kronfeld-Schor and Dayan, 2003; Sharma, 2003; Yerushalmi and Green, 2009). Arguably, circadian control enhances social synchronization of the time of sleep, locomotion, mating and foraging, which can be advantageous in animals living in groups. While entrainment to a common set of external signals can synchronize behaviours within a population, organisms might also show direct social entrainment, where social interactions over-ride the influence of the LD cycle (Bloch et al., 2013). Bats and bees provide examples of social synchronization where the home microhabitat (a cave or a bee-hive) has minimal if any light and where individuals sample the outside regime and communicate information back to the colony (Fuchikawa et al., 2016; Marimuthu et al., 1981). In the absence of an ability to sample light, what happens in organisms that are restricted to arrhythmic environments such as the dark zone of caves? Such environments are seen as a test of the adaptive value of circadian rhythms (Beale et al., 2016; Friedrich, 2013). True cave dwellers (troglobites) can retain rhythmicity but its expression is often weak or variable (Koilraj et al., 2000; Soriano-Morales et al., 2013). On the other hand, eutroglophiles (facultative cave-dwellers that can survive and reproduce in both epigean and hypogean environments (sensu Sket, 2008)) would be expected to retain a consistent circadian control system because of the presumed value of circadian control in the epigean environment.

Here we define the substantial differences in the circadian systems of a eutroglophilic species of glowworm that synchronizes the bioluminescence cycle within caves, and a related non-cave-dwelling species. The glowworms of Australia and New Zealand are fly larvae that produce light to attract prey to their webs (Baker and Merritt, 2003; Meyer-Rochow, 2007). They are members of *Arachnocampa*, a genus of

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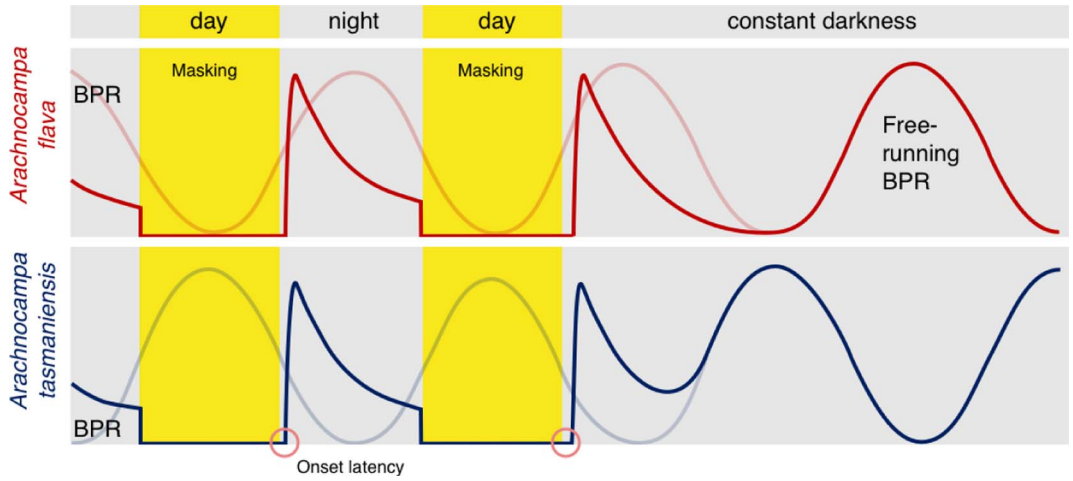

Fig. 1. Diagram of the pattern of light output of two species, *Arachnocampa tasmaniensis* and *Arachnocampa flava*, under day/night conditions and constant darkness along with the underlying bioluminescence propensity rhythm (BPR). Under daylight, bioluminescence output is masked. At nightfall, there is a delay to the onset of bioluminescence, followed by a peak in bioluminescence and a decline through the night. Under constant darkness, the realized light output matches the underlying BPR; however, the BPR of the two species is in antiphase.

keroplatid flies. In any one species, individuals that are reared in caves show less body pigmentation and larger size (Baker, 2010) but they do not show other troglomorphisms such as a reduction of eyes, most likely because they need to detect light when in their epigean habitat. The sub-tropical, forest-dwelling species, *Arachnocampa flava*, has no known cave populations. Bioluminescence is emitted from dusk to dawn, it comes under circadian control, showing free-running cycles under constant darkness (DD) and can be entrained by light and temperature cycles (Merritt and Aotani, 2008). The bioluminescence propensity rhythm (BPR), seen under constant darkness, is sinusoidal in shape with an acrophase around midnight (Fig. 1). The epigean bioluminescence pattern is saw-tooth in shape with a sharp rise to peak after dusk followed by a gradual decline through the night (Merritt and Patterson, 2017). Under laboratory conditions, when exposed to a sharp transition from lights on to off, there is an approximately 20 min latency before glowing starts, possibly needed for air supply to reach the cells of the light organ or production of metabolites associated with the luciferin–luciferase system (Mills et al., 2016). The peak occurring soon after darkness under natural conditions is lost when larvae are exposed to extended dark periods, suggesting that an approximately 12-h period of inhibition of bioluminescence under exposure to light is required to sensitize larvae to produce the post-dusk bioluminescence peak (Merritt and Clarke, 2009).

The second species, *Arachnocampa tasmaniensis*, has large populations in caves as well as being found in wet forests. In caves, populations can be present around the cave mouth as well as deeper, consequently they experience extremely variable light:dark (LD) ratios, ranging from complete darkness to the typical epigean seasonal LD cycle (Merritt and Clarke, 2011). Wherever colonies are located in a cave, the larvae appear to glow in unison. Like *A. flava*, *A. tasmaniensis* shows true circadian regulation of bioluminescence; under DD, *A. tasmaniensis* also show a sinusoidal pattern of glowing but, unlike *A. flava*, their BPR shows a maximum during the subjective day and a minimum at night (Merritt and Clarke, 2011). The opposite phase-setting of the BPR in reaction to light in this species (Fig. 1) was paradoxical: why have a circadian system that promotes bioluminescence at a time when the reflexive dousing response, known as masking, would turn off bioluminescence? Numerous experimental approaches confirmed the unusual entrainment pattern and offered an explanation: the circadian system enables glowing synchronization in caves. Under laboratory conditions, light exposures followed by DD shift the phase of the BPR to be at a maximum during the subjective photophase of the circadian cycle (Merritt and Clarke, 2011; Merritt et al., 2012). An in-situ light exposure experiment in a cave confirmed the entrainment pattern (Maynard and Merritt, 2013); a spot of light was focused onto a subgroup of larvae in a large colony on a cave ceiling with daily 3 h pulses over 5 days. The larvae in the focal population progressively delayed the phase of their bioluminescence cycle so that the time of the acrophase matched the time of the pulse and was out of phase with the surrounding population. After the pulses were terminated, the exposed population progressively phase-advanced over the next 10 days to re-synchronize with the surrounding colony. Also, laboratory exposure of out-of-phase, free-running larvae to each other in an otherwise DD environment produced synchronization through progressive phase adjustment (Maynard and Merritt, 2013). The entrainment pattern in this species—having a BPR that promotes maximal bioluminescence at a time when it would be masked by daylight—was attributed to the fact that *A. tasmaniensis* populations located in the dark zone of caves synchronize their collective daily glowing cycles, and that synchronization comes about by detection of the bioluminescence cycle of surrounding individuals—not bright enough to induce the masking response—that then entrains the BPR (Merritt et al., 2012). Despite this incongruous circadian control system, *A. tasmaniensis* larvae in epigean environments glow only at night, due to the over-riding inhibition of bioluminescence upon detection of light. Simply put, masking by light in LD environments produces the nocturnal glowing pattern seen in epigean environments and the circadian system produces the synchronization seen in caves. We hypothesize that, in epigean populations of this species, the release of bioluminescence at night when the BPR is at a low could be promoted through a homeostatic mechanism: if light exposure prevents bioluminescence when the endogenous drive to bioluminesce is high then it is possible that a homeostatic process would shift glowing into the dark period.

Here we use a comparative approach to identify adaptations of the circadian/bioluminescence system to living in dual environments vs. a purely epigean environment. We confirm that bioluminescence comes under circadian regulation in both species, then show that the species with cave populations is ready and able to initiate bioluminescence at the onset of darkness at any part of the circadian cycle; whereas, the species that is known to inhabit only epigean habitats is restricted to glowing only at night through a homeostatic mechanism. We then establish each species' phase-response curve (PRC) to light exposure, finding substantial differences in the degree of phase change induced by the same entraining light pulse. The species with cave populations shows a PRC that promotes synchronization of bioluminescence while the epigean species' PRC promotes nocturnal bioluminescence. The