Shedding new light upon circadian emergence rhythmicity in the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae)

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Abstract—The phenological behaviours of temperate insects can be highly controlled by photoperiod. Some foundational studies of the mountain pine beetle, *Dendroctonus ponderosae* (Hopkins) (Coleoptera: Curculionidae), documented a diurnal emergence rhythm that was asynchronous with maximum daily temperatures in the field and persisted under constant temperature and light conditions. In the 1970s, researchers hypothesised that this emergence rhythm was regulated by an endogenous circadian mechanism. Reflecting upon these historical data, we consider that a diurnal pattern of *D. ponderosae* emergence may result from photoperiodic entrainment of the circadian clock during the immature stages. Mechanistically, we suggest that the long-wavelength-sensitive opsin that we previously found to be expressed across *D. ponderosae* life stages could mediate, from beneath the bark, the input of light–dark cycle cues that are usually required for entrainment of the insect circadian clock.

Photoperiod is a ubiquitous regulator of insect phenology, particularly in seasonal environments (Bradshaw and Holzapfel 2007). The insect circadian clock consists of an endogenous light-independent physiological rhythm, which can be entrained to the daily light–dark cycle using light and/or temperature cues, and a molecular mechanism for photoperiodic time measurement (Saunders 1973; Emerson *et al.* 2009; Bradshaw and Holzapfel 2010). Endogenous circadian regulation in insects can influence patterns of metabolic and behavioural activity (Friedrich 2013; Tierney *et al.* 2017). For example, insect behaviours under circadian control may include eclosion, oviposition, egg hatching, and movement (summarised in Felisberti *et al.* 1997 and Lampel *et al.* 2005). Direct and circumstantial evidence from a diverse set of entomological studies indicates that a diversity of cryptic (physically concealed, see definition by Wertman *et al.* 2018) insects are capable of photoperiodic time measurement (Shintani *et al.* 1996; Doležal and Sehnal 2007; Shintani 2011; Friedrich 2013). Photoperiod is known to influence development rate, including diapause programming, in certain subcortical tree-tissue developing beetles (Coleoptera) (Shintani *et al.* 1996; Doležal and Sehnal 2007; Shintani 2011), and is implicated in the entrainment of circadian behavioural rhythms in cave beetles (Friedrich 2013).

The mountain pine beetle, *Dendroctonus ponderosae* (*Hopkins*) (Coleoptera: Curculionidae: Scolytinae), is an eruptive species of bark beetle and a severe pest of mature pine (*Pinus*: Linnaeus; Pinaceae) forests in western North America (Safranyik and Carroll 2006; Safranyik

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et al. 2010). Aside from a short period of adult dispersal, *D. ponderosae* completes its development entirely within the subcortical tissues of host trees. Life cycle and emergence synchronicity in *D. ponderosae* increases the success of mass attacks on host trees and contributes to the establishment of epidemic populations (Bentz *et al.* 1991). Accordingly, *D. ponderosae* possesses temperature-dependent mechanisms to optimise life cycle synchronisation and overwinter survival (Safranyik 1978; Bentz *et al.* 1991, 2013). In temperate univoltine populations, adult beetles emerge from their brood trees and disperse to colonise new hosts in late summer, laying eggs within the phloem (Safranyik and Carroll 2006). The timing of adult *D. ponderosae* emergence from beneath the bark is primarily determined by ambient temperature and immature development rate, which is also a function of temperature. The optimal temperature range for *D. ponderosae* emergence is 25–30 °C (Gray *et al.* 1972), and the insects do not emerge below a lower threshold of 16 °C (summarised in Safranyik and Carroll 2006).

Within this range of optimal temperatures for *D. ponderosae* emergence, there exists a rhythmic daily emergence pattern during the flight period that cannot be explained by temperature alone (Reid 1962; Gray *et al.* 1972; Billings and Gara 1975). For example, Gray *et al.* (1972) documented asynchrony between maximum daily temperatures and peak daily emergence from infested bolts maintained in the field. Over a period of nine days, the authors found that 61% (232 out of 379) of the insects emerged between 11 AM and 2 PM, despite that maximum daily temperatures were reached at 2–4 PM. These findings informed a complement of laboratory experiments that revealed the persistence of the circadian emergence rhythm in *D. ponderosae* under constant temperature and light conditions (Watson 1970; Billings and Gara 1975). Paiva and Vité (1982) observed a similar phenomenon for adult *Trypodendron lineatum* (Olivier) (Coleoptera: Curculionidae: Scolytinae), which displayed rhythmic circadian emergence patterns under constant laboratory conditions after having overwintered in the field beneath the soil.

Here we examine contemporary literature to evaluate the hypothesis presented by Watson (1970), Gray *et al.* (1972), and Billings and Gara (1975) nearly 50 years ago – that an endogenous circadian mechanism underlies the diurnal *D. ponderosae* emergence rhythm observed both in the field and in laboratory experiments. We propose that emergence rhythmicity observed in adult *D. ponderosae* may be a result of photoperiodic entrainment of the circadian clock of the immature stages developing within subcortical tree tissues. This would require that immature *D. ponderosae* can perceive and respond phenologically to photoperiod from beneath the bark.

Researchers have documented evidence of photoperiodic time measurement and circadian behavioural rhythmicity in cryptic insects. This includes both subcortical tree-tissue developing beetles (immature *Ips typographus* (Linnaeus) (Coleoptera: Curculionidae: Scolytinae) (Doležal and Sehnal 2007), *Psacothea hilaris* (Pascoe) (Shintani *et al.* 1996), and *Phytoecia rufiventris* Gautier (Coleoptera: Cerambycidae) (Shintani 2011)) and several species of cave beetle that have reduced ocular organs (Coleoptera: Carabidae) (Friedrich 2013). However, troglomorphic beetles (Coleoptera: Carabidae, Leiodidae) that lack photoreceptor organs entirely generally display arrhythmic behaviours, indicating that the degradation of circadian rhythmicity in beetle behaviour is related to the degree of subterranean adaptation (Friedrich 2013).

Photoperiod is known to influence the development rate, directly and indirectly, in *I. typographus* (Doležal and Sehnal 2007), *P. hilaris* (Shintani *et al.* 1996), and *P. rufiventris* (Shintani 2011). Doležal and Sehnal (2007) demonstrated that the exposure of immature *I. typographus* (late instars and older) to specific photoperiod treatments induced reproductive diapause in the adult stage, indicating that the insects were able to perceive light from beneath the bark and store photoperiodic information. The effects of photoperiod on diapause induction and number of instars have been described for *P. hilaris* (Shintani *et al.* 1996) and *P. rufiventris* (Shintani 2011). For example, the exposure to short day lengths at constant temperature led to the development of one or two supernumerary instars and the subsequent induction of diapause in *P. hilaris* (Shintani *et al.* 1996). *Psacothea hilaris* larval diapause was maintained under short day lengths and terminated by exposure to longer photoperiods.

Dendroctonus ponderosae larvae, like all scolytid beetle larvae, lack external ocular organs (stemmata)

(Jordal 2014), and photoperiodic information for circadian clock entrainment must therefore be received extraocularly (Wertman *et al.* 2018). The photoperiodic stimuli that reach *D. ponderosae* larvae developing beneath the bark most likely consist of long-wavelength light (480–600 nm), which is considered important to the ecology of low-light-adapted insects (Jackowska *et al.* 2007; Friedrich *et al.* 2011; Wertman *et al.* 2018). It is therefore probable that long-wavelength-sensitive opsin proteins are the photoreceptors responsible for stimulating physiological responses in cryptic insects (Wertman *et al.* 2018), including those observed in subcortical tree-tissue developing beetles (Shintani *et al.* 1996; Doležal and Sehnal 2007; Shintani 2011). We hypothesise that the photoperiodic light input for circadian entrainment could be mediated by the long-wavelength-sensitive opsin that we previously found to be expressed across *D. ponderosae* life stages and throughout the larval body (Wertman *et al.* 2018). A number of studies have implicated insect opsins, particularly those expressed in the brain, in the reception of photoperiodic information and circadian clock entrainment (Shimizu *et al.* 2001; Lampel *et al.* 2005; Spaethe and Briscoe 2005; Velarde *et al.* 2005). Additionally, deep-brain photoreceptor organs that originate from larval stemmata are widespread among endopterygote insects, and are speculated to function in circadian regulation (reviewed in Buschbeck and Friedrich 2008).

In *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), the molecular circadian clock can be entrained by two different classes of photoreceptor proteins, opsins and cryptochromes (Helfrich-Förster *et al.* 2001), the latter of which are considered to function in circadian regulation in all metazoans (Yuan *et al.* 2007). The *D. melanogaster* circadian clock is governed by a transcriptional feedback loop involving a number of clock genes and their encoding proteins, including cryptochrome 1 (reviewed in Saunders 2012). While cryptochrome 1 is known to be photosensitive, cryptochrome 2 is thought to be photo-insensitive and to function in light-independent circadian regulation (Yuan *et al.* 2007). Cryptochrome 1 is absent from the genomes of beetles, including *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Yuan *et al.* 2007), *Ptomaphagus hirtus* (Tellkampf) (Coleoptera: Leiodidae, Cholevinae) (Friedrich

et al. 2011), and *D. ponderosae* (Keeling *et al.* 2013, National Centre for Biotechnology Information Resource Coordinators 2017), which appear to encode only cryptochrome 2. Based on the absence of cryptochrome 1 and lack of additional opsin orthologs in the *D. ponderosae* genome (Wertman *et al.* 2018), we contend that the long-wavelength-sensitive opsin is the most probable candidate receptor of photoperiodic information for entrainment of the molecular clock in this species.

Daytime temperatures are generally higher than nighttime temperatures, resulting in daily thermoperiodic cycles that roughly track the photoperiod (see definition by Beck 1982). Although the photoperiod is a far more reliable environmental cue for daylength than thermoperiod and is considered a primary environmental stimulus for insect circadian regulation (Bradshaw and Holzapfel 2007), some insects can also use the thermoperiod for entrainment of the molecular clock (reviewed in Saunders 2014). For example, female *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) parasitic wasps, when reared from the egg stage in darkness, are able to differentiate short- from long-day thermoperiods and produce either diapausing or developing broods in response (Saunders 1973). Photoperiodic and thermoperiodic cues may be involved in the entrainment of the *D. ponderosae* circadian clock. This could be tested by rearing *D. ponderosae* from the egg stage under photoperiodic and sustained darkness conditions at constant temperature, and by comparing these results to those obtained under thermoperiodic conditions. For example, if photoperiodic entrainment of the *D. ponderosae* circadian clock occurs, beetles reared in constant darkness should exhibit no defined emergence rhythm, while those reared under photoperiodic laboratory conditions of stable temperature should display circadian emergence rhythmicity.

Over the last several decades, technological progress has greatly enhanced our understanding of the molecular mechanisms supporting the insect circadian clock, leading to the exploration of the physiological effects of circadian regulation (reviewed in Bradshaw and Holzapfel 2010). In the context of circadian regulation, an evaluation of existing experimental observations from across insect taxa reveals common phenological responses to the photoperiod, even among cryptic species

(Shintani *et al.* 1996; Doležal and Sehnal 2007; Shintani 2011), which can inform our interpretations of historical data. With respect to *D. ponderosae*, diurnal emergence must optimise dispersal success, as visual cues are known to be important in adult beetle navigation and host-tree location (Reid 1962; Shepherd 1966). Photoperiodic entrainment of the *D. ponderosae* circadian clock, via the long-wavelength-sensitive opsin, would ensure that adult emergence is restricted to a period of the day that is marked by both optimal temperatures and light conditions for coordinated flight.

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