Going gentle into that pathogen-induced goodnight

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A B S T R A C T

A diverse set of pathogens have evolved extended phenotypes that manipulate the moribund behavior of their various insect hosts. By elevating host positioning at death, a phenomenon called “summit disease”, these pathogens have been shown to have higher fitness. Though a few summit disease systems have been intensively characterized, in particular the Ophiocordyceps-ant system, summit diseases lack an overarching theory for the underlying mechanisms of this complex behavioral manipulation. In this article, we combine the gamut of summiting systems into a cohesive framework: we propose two types of summit disease (juvenile and adult), which both exploit natural insect behaviors during periods of quiescence. We place this framework in the context of available literature and propose investigations that follow from this comprehensive understanding of summit disease in insects.

1. Introduction

Summit disease is a pathogen-induced behavior that sends an infected insect to an elevated position prior to death (Roy et al., 2006). This moribund journey can be highly stereotyped, and in some insect-pathogen systems, the stages of this ascent are named and currently under intensive study (Hughes et al., 2011). Among these are the famous “zombie ants”, which display a remarkable behavior-manipulating extended phenotype when infected with a specialist Ophiocordyceps fungus (Andersen et al., 2009). Depending on the ant species, zombie ants that are nearing death make their macabre march upward into the canopy at dawn or at solar noon, then secure themselves in a death grip before succumbing to the pathogen, after which a fruiting body emerges from their head to rain down spores (Lovett and St. Leger, 2017). The positioning of this cadaver is clearly advantageous to the fungus in that 1) the environmental conditions are conducive to the growth of the fruiting body and 2) the summiting ensures widespread dispersal of spores (Hughes et al., 2016). These benefits contribute to the requisite selective pressure for evolution of this phenotype.

Indeed, similar phenotypes are observed in other common summit disease systems. However, what is striking is how foundationally dissimilar these systems are. Ants summit on grass when infected with a trematode (Esch et al., 2002), caterpillars will summit when infected with a baculovirus (Hoover et al., 2011), unrelated fungi can cause millipedes (Hodge et al., 2017), house flies (De Fine Licht et al., 2017), snipe flies (Kramer, 1981), ants (Evans et al., 2011; Malagocka et al., 2017), soldier beetles (Steinkraus et al., 2017), fruit flies (Elya et al., 2018) and crickets (Carruthers et al., 1997) to summit. How can phylogenetically diverse pathogens induce such a similar extended phenotype in phylogenetically diverse hosts? It could be that each pathogen has evolved to independently pull sequential behavioral levers of their respective host, and, from there, have converged on this evolutionarily advantageous set of stereotyped behaviors. A more parsimonious explanation may be that these pathogens have evolved to exploit a single ancestral behavior shared by their respective hosts.

Considering the etiology of summit disease, we propose that insect pathogens are zombifying their hosts by exploiting periods of quiescence in insect biology: specifically, molting in immature insects and sleep in adult insects. These two behaviors reproduce the gamut of stereotyped summit disease traits, and, importantly, these behaviors are highly conserved across a broad range of insects (Helfrich-Förster, 2018). A defining feature of insects is molting, and the need for sleep is so ancient that it is necessary for the survival of humans and insects alike. This framework for understanding summit disease offers testable hypotheses about both insects and pathogens involved in this behavioral manipulation, and it exposes our shadowy understanding of the basic natural history of most insects: namely, where they sleep and what induces this behavior.
2. Juvenile summit disease

We propose a separate category of summit diseases may affect juvenile insect stages. Adult and juvenile summit diseases are linked by the exploitation of periods of quiescence, but for juvenile insects this period coincides with molting between life stages. Molting is a synapomorphy of all arthropods, and the process is incredibly taxing physically and metabolically. A symphony of hormones and genes are required for an insect to successfully shed its previous exoskeleton and transition to its new one (Nijhout, 1981; Truman, 2005). The subsequent hardening of the new exoskeleton is necessary because its initial softness allows the insect to emerge from the previous exoskeleton. Insects are vulnerable during this period because many processes must occur in concert for successful molting, and, similar to sleep, insects have a reduced capacity to respond to their surroundings. Consequently, insects have evolved behaviors that protect them from threats during molting, which are near indistinguishable from summiting behaviors. Wandering is both a symptom of summit disease in caterpillars and a natural behavioral stage prior to pupation (Dominick and Truman, 1986; Kamitani et al., 2005; Kataoka et al., 2012): a process that is notably linked to the circadian rhythm.

In the foliage pest Lymantria dispar, summiting caused by a baculovirus has been characterized in fine-detail (also called tree-top disease or Wipfelkrankheit). Somewhat surprisingly, a single gene has been implicated in the summiting of these moths: ecdysoneroid UDP-glucosyltransferase (egt) (Hoover et al., 2011). As the name implies, this gene is involved in the regulation of molting, and the expression of this gene by baculoviruses unbalances molting homeostasis (O’Reilly and Miller, 1989). Recent studies have established that the lengthened feeding period (delayed pupation) is mediated by repression of 20-hydroxyecdysone (Shen et al., 2018), which normally binds to dopamine receptors to repress feeding and promote pupation (Kang et al., 2019). This baculovirus-moth interaction has been demonstrated to be critical for summiting in the preferred host, but investigation of this interaction across non-optimal hosts led to the conclusion that “an effect of egt on tree-top disease can be observed through indirect effects of egt on molting-related climbing behavior” (Rox et al., 2015). This fits neatly into our larger paradigm of summit diseases exploiting periods of quiescence, the timing of which can be fine-tuned as the pathogen specializes to its preferred host to maximize pathogen fitness.

Somewhat paradoxically, this summiting paradigm extends down into the soil. Cordyceps militaris kills pupae of caterpillars that “summit” to the soil surface in order to pupate (Zheng et al., 2011). Naturally, this saves the adult insect an arduous dig to the surface: a daunting task immediately after eclosion. During fungal infection, the timing of this behavior allows the pathogen to form fruiting bodies that extend just above the ground. A similar “summit” is observed with the caterpillar fungus Ophiocordyceps sinensis (Hu et al., 2013): the caterpillar travels to a few centimeters below the soil surface where it is killed by the fungus prior to pupation. Though this summit is underground, it relates to other juvenile summiting because it exploits periods of quiescence circa ecdisis.

A recent comprehensive phylogenetic reconstruction of the fungal genus Ophiocordyceps, which contains many behavior-manipulating, insect-pathogenic fungi, also suggests an evolutionary link between juvenile and adult manipulation (Araújo and Hughes, 2019). The Ophiocordyceps-ant infecting fungi appear to have evolved from Ophiocordyceps species that infect beetle larvae inside wooden logs and belowground in the soil. Assuming, as we propose here, that these fungi exploited periods of larval quiescence, this provides a plausible evolutionary trajectory linking larval and adult behavioral manipulation via modification of periods of quiescence and circadian rhythms. Indeed, the molecular genetics linking neuroendocrine regulation of quiescence periods, including a state of “molting-sleep”, are already under investigation in Manduca sexta (MacWilliam et al., 2015).

3. When insects fall asleep, where do they go?

Sleep is a behavior which is closely tied to the circadian rhythm, but these two phenomena are not synonymous (Helfrich-Förster, 2018). The circadian rhythm is a delicate orchestration of proteins that is conducted by environmental cues (zeitgebers) (Eban-Rothschild et al., 2017). Under normal conditions, circadian proteins allow insects to interpret the time of day and metabolize appropriately (Tomiska and Matsumoto, 2010). In summit disease systems involving an insect and a fungus, each organism maintains their circadian clock (Westwood et al., 2019). Intensive genetic and molecular characterization of Ophiocordyceps-ant summit disease has revealed that the circadian clocks of both pathogen and host intervene in the execution of summiting behavior (De Bekker et al., 2017). Given that the timing of summiting behavior is often predictable (de Bekker et al., 2015; Elya et al., 2018), it is also predictable that circadian clocks will be found to play a role in coordinating summit disease in other systems. The link between summit disease and the circadian rhythm is analogous to the link between sleep and circadian rhythm (Bhattarai et al., 2018; De Bekker et al., 2017), but many other behaviors, including molting, are also tied closely to the circadian rhythm (Helfrich-Förster, 2018).

However, the links between summit disease in adult insects and sleep behavior do not stop there. As an example, many genera of solitary wasps and bees sleep while grasping onto foliage with their mandibles (Fig. 1), perhaps to avoid predators (Mahmoud et al., 2017; Pinheiro et al., 2017; Rau, 1938; Rau and Rau, 1916). A defining feature of insect sleep is an increased threshold of arousal (Helfrich-Förster, 2018), and insects must grip to prevent falling from their perch in their vulnerable state. The increased vulnerability of sleep governs insect sleeping location, orientation and timing. For some unfortunate insects, these traits may be exploited to increase the fitness of a pathogen with a high level of host adaption. In this sense, the various death grips (primarily using mandibles or legs) and various elevated locations (e.g. on flowers, leaves, twigs, blades of grass, etc.) may reflect differences in sleep behavior, not differences in the mode of manipulation (Loreto et al., 2018). Similarly, phototaxis observed in the ant summit disease system may reflect the role of light in early morning sleep positioning for nocturnal ants (Andrioli et al., 2019). It would be much simpler for an insect pathogen to signal physiologically (either...
directly or indirectly) that it is time to sleep, than to methodically send an insect up the appropriate perch and coordinate fungal-specific gripping behavior. Here, natural selection alone could explain the evolution of this behavioral manipulation: pathogens that induce insect sleep or kill opportunistically during insect sleep would be more fit because the insect cadaver is located advantageously. Increased attention to the natural history of summing insects could quickly test this hypothesis by revealing where and when summing insects may sleep. Similarly, increased attention to the natural history of all insects may elucidate which insects are predisposed to summit disease, namely those that display natural periods of quiescent in microclimatic conditions that are conducive to the fitness of a pathogen.

If adult summing is a matter of inducing or exploiting natural sleep behaviors, this would go far to explain how multiple unrelated pathogens have stumbled upon the same extended phenotype in multiple unrelated insects that are connected by their innate need for sleep. While our knowledge of how sleep is regulated in insects is far from complete, it is clear there could be many avenues to induce sleep and its associated stereotyped behaviors (i.e., by altering the circadian rhythm via sleep-associated neurotransmitters) (Helfrich-Förster, 2018). Quiescent periods are dictated by homeostasis of hormones and neuromodulators that would offer multiple opportunities for insect pathogens to acquire the means to induce sleep, perhaps by secreting proteins or molecules functionally similar to those produced by the insect itself. The necessary genes could be acquired through gene exaptation, de-novo gene production or horizontal gene transfer. All of these could reasonably result in summit disease. As our knowledge of the molecular genetics of sleep in insects increases, we can expect to identify more candidate genes behind summit disease.

To highlight basic gaps in our understanding of normal sleep behaviors in insects, we can use a model insect: the fruit fly Drosophila melanogaster. A strain of Entomaphthora muscae was recently isolated and optimized for infection of fruit flies in the lab (Elya et al., 2018). A key innovation of this system was the introduction of a small dowel into the traditional fruit fly rearing tube to supply a perch for observing summing behavior. In addition to providing a summing perch, this small dowel provided a more natural landscape than the flat food at the bottom of a plastic cylinder that is the typical laboratory environment. When such a dowel is inserted into a tube of uninfected flies, they display a marked preference for sleeping near the top of the dowel (Fig. 2). Thus, there are commonalities between sleep behavior in healthy flies and the summing of infected flies. Of course, there is variation in the sleep location in healthy flies, but variation in “summit” locations was also observed in this Entomaphthora-Drosophila system (Elya et al., 2018). Our understanding of when and how this model organism sleeps, which is being developed as a sleep model in its own right (Axelrod et al., 2015; Cirelli et al., 2005; Hendricks, 2003; Huber et al., 2004; Tononi, 2000), is hindered by a dearth of sleep-focused natural history studies and by simplified lab conditions that prioritize ease of rearing. The possible correlation of sleep position and summing position would make Drosophila a powerful system to test the hypothesis that sleep and summing are underpinned by similar underlying biology. Natural variation in sleep positioning is already present in laboratory fly lines (personal observation). Similarly, the many sleep mutants available for the Drosophila system may also furnish a wealth of interesting summit disease mutants for unraveling this host-pathogen interaction (Harbison et al., 2017).

Variation in summit disease etiology in eusocial insects, like ants, is complicated further by the progressive change in behavior as these insects mature (Eban-Rothschild and Bloch, 2012; Fourcassié et al., 1999). Eusocial insects become increasingly isolated as they age (Fujikai et al., 2019; Korczyńska et al., 2014; Stroeymeyt et al., 2018): for many ant (Mildner and Roces, 2017) and bee (Bloch, 2010; Eban-Rothschild and Bloch, 2008) species there is a marked transition from early-life behavior (within the nest) to senescent foraging behavior (outside the nest or extranidal), which involves adoption of circadian rhythms (Fuchikawa et al., 2016). Mature eusocial insects spend more time outside of the nest and so are more likely to be exposed to behavior manipulating pathogens. The extent of this transition during infection and the differential evolutionary loss of solitary behaviors, such as sleep positioning, among eusocial insects may go a long way to explain the complex variation in behavior manipulations, which has been reviewed comprehensively in ants (De Bekker et al., 2018).

4. Future implications

Recognizing the similarities in summit diseases across stages and systems will facilitate interdisciplinary collaboration to develop a unifying theory of extended, behavior-manipulating phenotypes (Westwood et al., 2019). Though it is clear that pathogens that manipulate host behavior are able to recognize and specifically interact with the brain of their preferred host (de Bekker et al., 2014), host-specific neuromodulators may play a supporting role to enzymes or metabolites that alter homeostasis to exploit already existing behaviors. This may explain why the role of circadian rhythms in summit disease symptoms has gained prominence as molecular characterization of the Ophiocordyceps-ant system has intensified (De Bekker et al., 2017).

Functional genetics and metabolomics hold the potential to elucidate whether similar proteins or pathways are affected in different summing insects. Further, comparative techniques may reveal an overlap between the physiological states of summing insects and sleeping insects. A complication to identification of these interactions is that by exploiting host processes, a pathogen could alter behavior simply by producing precursors which the host machinery further processes to elicit a behavioral change.

Practically, this theory means that laboratory investigations of summit disease would benefit from providing spaces for insect host quiescence. In many insects, this may require investigation into their natural history to identify these stages (i.e., where and when they occur). These investigations may require revision of the definition of insect sleep, which currently may include specifications that make observations in nature or on multiple individuals impractical: for example, the Drosophila field requires observation of inactivity for five minutes to identify sleeping flies. By unifying summit diseases under natural periods of insect quiescence, the disparate investigations into
tree-top disease, terminal disease, and various arthropods “zombies” can be synthesized into a cohesive understanding of the ecology and evolution of pathogens that manipulate their hosts.

References


