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# **The evolutionary ecology of circadian rhythms in infection**

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Biological rhythms coordinate organisms' activities with daily rhythms in the environment. For parasites, this includes rhythms in both the external abiotic environment and the within-host biotic environment. Hosts exhibit rhythms in behaviours and physiologies, including immune responses, and parasites exhibit rhythms in traits underpinning virulence and transmission. Yet, the evolutionary and ecological drivers of rhythms in traits underpinning host defence and parasite offence are largely unknown. Here, we explore how hosts use rhythms to defend against infection, why parasites have rhythms, and whether parasites can manipulate host clocks to their own ends. Harnessing host rhythms or disrupting parasite rhythms could be exploited for clinical benefit; we propose an interdisciplinary effort to drive this emerging field forward.

Circadian rhythms have long been taken for granted by science. Indeed, the first observation of a clock-controlled behaviour (leaf opening and closing in *Mimosa pudica*) was not recorded until the 18<sup>th</sup> century<sup>1</sup>. Following the fundamental observation that organisms can adaptively anticipate daily rhythms in their environment, the field of "chronobiology" took off in the mid-20<sup>th</sup> century with a focus on evolutionary and ecological questions. However, the advent of genetic tools a few decades later shifted the remit to determining the molecular and genetic workings of circadian clocks. Yet, despite their assumed major impact on fitness, circadian rhythms remain overlooked in evolutionary ecology<sup>2-4</sup>. Here, we propose that the integration of chronobiology and evolutionary ecology return

36 to its roots to tackle a topic of growing and applied interest; the role of rhythms in host-parasite  
37 interactions. Note that we use the term “parasite” to collectively refer to all agents of infection (e.g.  
38 single-celled and multicellular eukaryotes, bacteria, viruses).

39  
40 One of the most fundamental ecological interactions is that between hosts and parasites. Research  
41 from diverse taxa (plants, mammals, and insects) reveals that host clocks drive daily rhythms in  
42 immune defences, disease severity and spread<sup>5,6</sup>. Parasites display daily rhythms in traits  
43 underpinning within-host survival and between-host transmission<sup>7,8</sup>. Rhythms in parasite activities  
44 and in host responses to infection could provide an advantage to parasites, hosts, both, or neither.  
45 To what extent parasites and hosts are in control of their own and/or each other’s rhythms is also  
46 poorly understood.

47  
48 Understanding the evolution (and possibly, coevolution) of rhythms may enable vaccines and drugs  
49 to take advantage of rhythmic vulnerabilities in parasites or harness host rhythms to improve  
50 efficacy and reduce drug toxicity. For such interventions to be robust to parasite evolution,  
51 understanding how host-parasite interactions shape rhythms in hosts and parasites is necessary<sup>7</sup>.  
52 Key questions include how rhythms in diverse host traits contribute to defence, how parasites cope  
53 with exposure to their host’s rhythms, and whether hosts and parasites can manipulate each other’s  
54 rhythms for their own benefit. We discuss these three scenarios, identify systems to explore them,  
55 and offer ways in which this knowledge can be exploited to improve health. An evolutionary  
56 ecologist’s introduction to chronobiology is provided in Boxes 1 and 2.

57

58 **Rhythms in host defence**

59 The most patent defence against infection is the immune response, and a wealth of evidence reveals  
60 that circadian clocks play a role in orchestrating immune defences<sup>5</sup>. Circadian clock genes are  
61 expressed in many types of immune cell, and the immune and circadian systems are connected in  
62 multiple ways<sup>9,10</sup>. For instance, the clock gene *Bmal1* mediates the balance between pro- and anti-  
63 inflammatory responses<sup>11</sup>. Rhythmic production of the pro-inflammatory cytokines TNF- $\alpha$  and IL-6  
64 by macrophages is clock controlled<sup>12</sup>, and mobilization of inflammatory monocytes is also  
65 regulated by the clock<sup>10</sup>. This phenomenon, termed “anticipatory inflammation”, appears uncoupled  
66 to metabolic rhythms and may defend against incoming parasites<sup>13</sup>. Similarly, in humans,  
67 proinflammatory cytokines peak in circulation during the day (active phase)<sup>14</sup>, whereas  
68 hematopoietic stem and progenitor cells, and most mature leukocytes, peak at night<sup>14,15</sup>. In  
69 nocturnal mammals, an inverse rhythm is often observed, with innate defences peaking at night  
70 (active phase) and repair mechanisms peaking during the day (resting phase)<sup>9</sup>.

71

72 Observations of immune rhythms have given rise to the notion that organisms invest in defence  
73 during the active phase when parasite encounter is assumed most likely, and repair during the  
74 resting phase<sup>16</sup>. Temporal segregation of immune responses may thus solve problems caused by  
75 having immune defences continually tuned to maximal (e.g. collateral damage via  
76 immunopathology<sup>17</sup>). Also, energetic demands imposed by activity and metabolism may trade-off  
77 against immune defence<sup>18</sup>. Intuitively, “defence only during the active phase” suggests the host is  
78 achieving the most “bang for the buck” by ensuring activities that are energetically costly, or likely  
79 to cause collateral damage, are only performed when most useful. However, this intuition may be  
80 naïve. First, it ignores the potential for constraints imposed by the need to temporally couple (or de-  
81 couple) certain immune rhythms with other internal rhythms<sup>7</sup>. This includes separating the timing of  
82 metabolism from defensive actions within immune cells themselves<sup>5,16</sup>. Second, it assumes that a  
83 parasite encounter is rhythmic and predictably occurs in the active phase. This is clearly the case for  
84 food-borne parasites, but ingestion is not the only route into a host. Rather, the immune system  
85 functions within a broad set of energetic demands in which parasite defence is just one of many  
86 requirements. For example, rhythmic stomatal opening for gas exchange during the day is a well-  
87 used route into plants by bacterial pathogens<sup>19</sup>. Consequently, *Arabidopsis* is better able to detect  
88 and defend against parasites in the morning than evening<sup>20,21</sup>. Given the wealth and diversity of data  
89 (illustrated in Table 1), meta-analyses are needed to test whether the timing (phase) of rhythms in  
90 immune effectors relates to nocturnal vs diurnal lifestyles and whether they function in front-line or  
91 secondary defences, or healing.

92 Infection in the active vs resting phase for diverse hosts (flies, plants, mammals) dramatically  
93 affects disease severity and mortality rates (Table 1), suggesting that the phase of immune rhythms  
94 upon infection matters. Most studies performed in plants (Table 1) point towards infection during  
95 the active phase resulting in greater resistance to infection and less damage to the plant. But the  
96 degree to which immune rhythms result in time-of-day differences in parasite control can be  
97 counter-intuitive. For example, mice mount higher clock-controlled proinflammatory responses  
98 against *Salmonella enterica* Typhimurium when challenged in their rest phase, but bacterial load is  
99 also higher and hosts have worse symptoms<sup>22</sup>. Furthermore, *Leishmania* parasites infect host  
100 neutrophils and macrophages, and the clock-controlled secretion of chemoattractants by these  
101 immune cells facilitates their infection, making parasite invasion more successful at night when  
102 immune activity is highest<sup>23</sup>. Thus, whether immune rhythms are sufficient to entirely explain  
103 divergent outcomes of time-of-day of infection is unclear (Table 1). Studies that separate the effects  
104 of immune rhythms on preventing infection from their role in dealing with ongoing infection will

105 reveal the extent to which immune rhythms are beneficial and when they should be overruled to  
106 deal with a major threat. Additionally, most time-of-day immune challenges have used either  
107 bacteria or chemicals, raising the question of whether a more diverse array of challenges are needed  
108 to establish general patterns.

109

110 That host circadian clocks impact on infection via traits other than immune responses has been  
111 largely overlooked. Rhythmicity in host activity may determine when hosts provide the best  
112 resources to their parasites and offer the most opportunities for onwards transmission<sup>24–26</sup>. For  
113 example, a recent study of the intestinal helminth *Trichuris muris* demonstrates the role of host  
114 rhythms in foraging. Mice infected in the morning (resting phase) expel worms sooner and have a  
115 stronger T-helper 2 response than dusk-infected (active phase) mice, and this effect is reversed  
116 when mice are fed only in the day, in an immune-independent manner<sup>27</sup>. Host feeding rhythms are  
117 relevant to gut microbiota, and a two-way feedback between host and microbe rhythms has been  
118 proposed<sup>28</sup>. Daily rhythms in host reproductive behaviours may make hosts vulnerable to infection.  
119 For example, the crepuscular and nocturnal singing activity of the cricket *Teleogryllus oceanicus*  
120 allows the acoustically-orienting parasitoid fly *Ormia ochracea* to locate hosts, but the flies are best  
121 able to hunt when darkness is incomplete<sup>29</sup>. A rhythmically expressed reproductive behaviour  
122 (singing) got the host into this mess, and it appears that natural selection has found two solutions  
123 (see Box 3).

124

125 In addition to immune responses, infected hosts often exhibit adaptive sickness behaviours  
126 consisting of endocrine, autonomic, and behavioural changes that perturb circadian rhythms<sup>30,31</sup>. For  
127 example, wild red colobus monkeys (*Procolobus rufomitratus tephrosceles*) decrease energetically  
128 costly activities, and rest frequently, while shedding whipworm eggs<sup>32</sup>. Fever, another common  
129 sickness behaviour, is sufficiently advantageous to offset the 10–12.5% increase in metabolic rate  
130 required for each 1°C increase in temperature<sup>33</sup> and has been conserved throughout more than 600  
131 million years of vertebrate evolution<sup>34</sup>. Fever enhances an organisms chance of survival by creating  
132 a hostile environment for parasites and a more active immune response<sup>34–37</sup>. Under normal  
133 circumstances, the so-called central (SCN) clock controls body temperature rhythms, but how the  
134 SCN and inflammation interact to control temperature is unknown. Though many behaviours  
135 altered during infection are clock-controlled during health, the extent to which organisms become  
136 too sick to maintain normal behaviour or adaptively disrupt their rhythms is unclear. Additionally,  
137 clock-control could facilitate recovery of rhythms during the return to health.

138

139 Viewing the host as a collection of traits connected by the circadian system has the potential to  
140 uncover novel strategies to resist infection and reveal the circumstance in which immune rhythms  
141 reflect constraints or adaptations. Indeed, rhythmic metabolism of xenobiotic substances (e.g. drugs  
142 and vaccines) influences efficacy and toxicity in a time-of-day dependent manner<sup>38</sup>. For example,  
143 halothane (a commonly used anaesthetic) administered to mice in the daytime results in low  
144 mortality (5%), but mortality increases (76%) if administered at night<sup>39</sup> and half of the best-selling  
145 drugs in the USA for humans target the products of genes that are rhythmically expressed (in  
146 mice)<sup>40</sup>. A better understanding of host rhythms could be harnessed to make drugs and vaccines  
147 more effective, as well as mitigating the negative effects of modern lifestyles that involve shift work  
148 and jet lag. However, for such interventions to be sustainable in the face of parasite evolution,  
149 understanding the ecology of rhythms from the perspective of parasites is also required.

150

### 151 **Rhythms in parasite offence**

152 Scheduling activities to take advantage of daily rhythms in transmission opportunities could be a  
153 general explanation for rhythms in parasites. The most well-known example concerns the  
154 transmission forms (microfilariae) of different species of filarial worms. They move from the host's  
155 organs to the capillaries during the day or night, depending on whether they are transmitted by day-  
156 or night-biting insect vectors<sup>41</sup>. In addition to the activity patterns of vectors, rhythmic interactions  
157 with hosts also matter. For example, the larval stage of the blood fluke *Schistosoma japonicum*  
158 emerge from their invertebrate host to seek a mammalian host at different times of day. Flukes  
159 emerge in the afternoon when the preferred host is nocturnal or in the morning if seeking a diurnal  
160 host<sup>42</sup>. Parasites that have free-living stages are also subject to rhythms in the abiotic environments.  
161 The coccidian parasite *Isospora* sheds from its host in the late afternoon to minimise UV exposure  
162 and desiccation risk whilst undergoing a developmental transition necessary to infect new hosts<sup>43</sup>.  
163 However, key questions remain about the adaptive nature of these rhythms. For example, why  
164 aren't microfilariae located in the peripheral capillaries all day long? Is a cost associated with this  
165 location, which is only worth paying at times of day when vectors are active?

166

167 In contrast to the role of parasite rhythms in transmission, their role in within-host survival has  
168 received less attention. Many host rhythms (in addition to immune rhythms) present opportunities  
169 and constraints for parasites. *Trypanosoma brucei* (which cause sleeping sickness) display circadian  
170 clock-driven rhythms in the expression of metabolic genes<sup>8</sup>. These rhythms correlate with time-of-  
171 day sensitivity to oxidative damage, thereby suggesting the need to cope with redox challenges  
172 caused by rhythmic digestion of food by hosts. In contrast, rhythms in the development of asexually  
173 replicating malaria parasites capitalise on daily variation in the nutritional content of blood caused

174 by host immune responses and feeding patterns<sup>44,45</sup>. Whether malaria parasites cannot complete  
175 their developmental cycle until the host makes nutrients available, and/or use nutrients rhythms as a  
176 time-of-day cue to set the pace of their development, is unknown<sup>46</sup> (see Box 3).

177

178 Clocks in parasites or hosts could have fitness consequences for one or both parties, or neither.  
179 Fitness consequences for both hosts and parasites suggests that clocks could coevolve. Clock  
180 coevolution is suspected for the plant-pollinator system *Petunia axillaris* and *Manduca sexta*<sup>47</sup>, in  
181 which nocturnal scent emission by *P. axillaris* coincides with foraging activity in the hawkmoth *M.*  
182 *sexta*. Both traits are clock-controlled, and appear so well synchronized that, even in the absence of  
183 floral scent emission, *M. sexta* exhibits a burst in foraging activity at the same time that floral scent  
184 emission is expected to be greatest. However, foraging behaviour also remains sensitive to the  
185 environment, as evidenced by absence of activity when the moth is subjected to light at night. If  
186 rhythms in different organisms do coevolve, then they should use the same Zeitgeber, but how  
187 robust should their timing systems be to fluctuations in the environment? If the rhythm of one party  
188 is more readily disrupted (masked) by environmental change, or faster at tracking seasonal changes  
189 in photoperiod, then the relationship may be disrupted to the gain of hosts or parasites. Exploring  
190 the degree and consequences of plasticity in rhythms is pertinent because climate change is  
191 interfering with the ability of interacting species to synchronise<sup>48</sup>.

192

193 The situation is further complicated when interactions between both host and parasite clocks shape  
194 disease trajectories. For example, in a plant-fungus system (*Arabidopsis thaliana* and *Botrytis*  
195 *cinerea*, respectively), when both parties are in the same photoperiod schedule, primary plant  
196 defences peak in the morning, and the fungus produces the biggest lesions when inoculated at  
197 dusk<sup>49</sup>. The authors were able to separate the contributions to pathogenicity by host and parasite  
198 clocks using reverse lighting schedules for fungus and plants: fungus at dusk produced more severe  
199 infections than fungus at dawn, regardless of time-of-day for recipient plants<sup>49</sup>. Furthermore, this  
200 suggests *B. cinerea* anticipates and exploits weaknesses in plant defence at dusk rather than  
201 attempting to overwhelm dawn defences (see section “Rhythms in host defence”). Separately  
202 assigning the contributions of rhythms in hosts/vectors and parasites to virulence and transmission  
203 is necessary to understand whose genes control which rhythms, and hence how they can be shaped  
204 by selection.

205

206 If parasite rhythms are adaptive, then disrupting them could reduce disease severity as well as  
207 transmission. However, understanding the timing mechanisms of parasite rhythms is necessary to  
208 disrupt them<sup>7</sup>. Unravelling how parasite rhythms are controlled is a considerable challenge.

209 Parasites might allow the host to inadvertently schedule their activities for them, in which case the  
210 genes encoding parasite timing mechanisms belong to hosts. Alternatively, parasites might keep  
211 time using a circadian clock (with the properties described in Box 1), as demonstrated for *T. brucei*  
212 and *B. cinerea*. Given the diversity in clock genes across taxa, searching genomes for known clock  
213 genes often yields “absence of evidence” not “evidence of absence.” Instead, round-the-clock  
214 transcriptomics or proteomics, paired with bioinformatics approaches to mine for known core  
215 clock-related functional domains and sequence patterns may find candidates. However, simpler  
216 time-keeping strategies exist, though they do not necessarily have the advantages of temperature  
217 compensation or anticipation. For example, cell division cycles are often controlled by hourglass  
218 mechanisms that rely upon threshold concentrations of substances, independently of periodic  
219 phenomena<sup>50</sup>. Alternatively, organisms can react directly (via “tracking”) to temporal changes in the  
220 environment. Note, this differs from masking, a chronobiological phenomenon in which the  
221 expression of a clock-controlled rhythm is suppressed by a change in the environment without  
222 having a direct effect on the period or phase of the underlying rhythm<sup>51</sup>. A response that directly  
223 tracks time-of-day cues may suit parasites with multi-host lifecycles if each host type provides a  
224 different time-cue.

225

226 Given that rhythms in *T. brucei* metabolism and plasticity in development during the asexual cycle  
227 of *Plasmodium spp.* enables these parasites to tolerate drugs, there is an urgent need for proximate  
228 and ultimate explanations of their rhythms. The *T. brucei* clock is entrained by temperature cycles,  
229 but if other parasites use Zeitgebers to set their clocks, or respond directly to time-of-day cues, that  
230 are readily perturbed, it should be possible to reduce parasite fitness by interfering with their  
231 rhythms. Further, reports of changes to the biting time of mosquito populations that transmit  
232 malaria suggests that insecticide-treated bed nets are imposing selection on vector rhythms<sup>8,52,53</sup>.  
233 Given that rhythms of parasites and mosquitoes each affect malaria transmission in lab  
234 experiments<sup>54,55</sup>, what are the likely epidemiological consequences? Recent work suggests that  
235 mosquitoes are more susceptible to infection when they feed in the daytime and parasites are more  
236 infectious at night<sup>54</sup>. Thus, day-biting could increase the prevalence, but not burden, of malaria in  
237 mosquitoes. However, in the longer term, if parasites evolve to invert their rhythm but mosquitoes  
238 do not, both prevalence and burden may increase.

239

240 **Parasite manipulation of host rhythms**

241 Rhythms in host processes offer opportunities that parasites could exploit. Could parasite fitness be  
242 increased by coercing hosts into altering their rhythms? Although many striking examples of



243 parasite manipulation of host phenotypes (i.e. changes to host traits that benefit parasites) are  
244 known<sup>56</sup>, the notion of “parasite manipulation of host clocks” is largely unexplored<sup>57</sup>. A pre-  
245 requisite for parasite manipulation is that a phenotypically plastic host trait is targeted; and  
246 circadian clocks are flexible. Because clocks control much of the host’s behaviour and physiology<sup>58</sup>  
247 and clocks throughout a given host involve the same players in the canonical clock (the TTFL),  
248 manipulation of the host’s time-keeping may be an efficient way to simultaneously alter many  
249 aspects of the within-host environment. Alternatively, parasites interests may be served by  
250 bolstering circadian rhythms of their hosts during sickness to ensure they forage and interact with  
251 conspecifics, as usual.

252  
253 As outlined in the section “Rhythms in host defence,” separating the effects of being sick *per se*  
254 from host defence and parasite manipulation is challenging. Recently, a combination of culture and  
255 comparison of infection models has revealed that *T. brucei* alters expression rhythms of clock genes  
256 in host mice<sup>59</sup>. Specifically, infected hosts are more active in the resting phase (phase-advanced)  
257 because the clock runs faster (shorter period). Effects at organismal, cellular, and molecular levels  
258 suggests the behaviour is not just a result of sickness<sup>59</sup>. However, it is not clear how *T. brucei*  
259 achieves this, and whether the parasite benefits from altering host rhythms. One target of circadian  
260 disruption by viral parasites is the gene *Bmall*, a core clock gene. Herpes and influenza A virus  
261 replication and dissemination within the host is enhanced in infections where *Bmall* is knocked  
262 out<sup>60</sup>. However, it remains unclear if virus replication is maximised by simply disturbing  
263 rhythmicity in host cell cycles or if this is a case of immune manipulation since *Bmall* appears  
264 involved in innate host defence<sup>60</sup>. Having observed changes to host clocks, the proceeding step is to  
265 decipher the ecological context behind these effects.

266  
267 The above examples lend proof-of-principle to the idea that parasites can manipulate host clocks  
268 and could be a general explanation for examples of host manipulation. Hairworms (Nematomorpha)  
269 are a well-known case of temporally linked behavioural manipulation. They infect various  
270 arthropods, notably crickets, and cause the host to wander in an erratic manner until a body of water  
271 is encountered. The host commits suicide by jumping in water, and the adult hairworm emerges.  
272 Infected hosts are found wandering only in the early part of the night<sup>61</sup>, and uninfected hosts are  
273 rarely motivated to jump into water. Infected crickets differentially express an array of proteins,  
274 some of which are linked to visual processes and circadian clocks<sup>62</sup>. Culturing isolated host cells  
275 with parasite products and quantifying the expression of clock genes (following Rijo-Ferreira 2018)  
276 could illuminate this case of parasite manipulation. For systems without relevant insect cells lines,  
277 or cases where manipulation is likely to be tissue/cell type specific, a transcriptomics approach may

278 be useful<sup>63</sup>. Round the clock expression data can be mined for putative core clock genes and their  
279 phase, amplitude and period assessed in control and manipulated hosts. This however, is likely to be  
280 extremely challenging for host species whose timekeeping does not rely on a canonical circadian  
281 clock.

282

283 Another putative case for clock manipulation concerns the New Zealand freshwater snail  
284 (*Potamopyrgus antipodarum*) infected with *Microphallus* trematodes<sup>64</sup> (Trematoda:  
285 Microphallidae). Uninfected adult snails forage primarily at night on the upper surfaces of rocks in  
286 the shallow-water margins of lakes. These snails retreat to under rocks at sunrise, which likely  
287 reduces their risk of predation by waterfowl, which are the definitive host for *Microphallus*.  
288 Infected snails, however, show delayed retreating, potentially making them more likely to be  
289 consumed<sup>25</sup>. Crucially, the apparent manipulation only occurs when the parasite is mature. Snails  
290 infected with immature (non-transmissible) stages exhibit the same risk-averse retreating behaviour  
291 as uninfected snails<sup>25</sup>. In addition, snails infected with other species of sterilizing trematodes, which  
292 are not trophically transmitted, do not exhibit the same risky behaviour as those infected with  
293 *Microphallus*<sup>65</sup>, thereby eliminating the possibility that the *Microphallus*-induced behavioural  
294 change is a simple artefact of parasitic castration. Finally, *Microphallus*-infected snails spend more  
295 time foraging on the top of rocks, even when food was removed whereas uninfected snails retreated  
296 to shelter<sup>65</sup>. Taken together, the data suggest that *Microphallus* induce a change in snail behaviour  
297 that increases trophic transmission, potentially via manipulation of clock-controlled activity  
298 rhythms.

299

300 There are many ways that parasites could interfere with clock-controlled host behaviours. A blunt  
301 instrument would be to alter perception/detection of the Zeitgeber that sets the time of the host's  
302 clock, which is usually light. For example, *Microphallus* could interfere with photoreception to  
303 reduce the sensitivity of snails to dawn, causing their clocks to phase delay and forage at higher  
304 light intensities than un-manipulated snails. Alternatively, parasites could induce the host to ignore  
305 its clock (mask) or alter clock regulation of hormones that relay time-of-day information around the  
306 host. For example, baculoviruses appear to perturb the circadian rhythms of their caterpillar hosts  
307 by disrupting hormones that control climbing behaviour. In the baculovirus (*Lymantria*  
308 *dispar* nucleopolyhedrovirus), a single gene inactivates 20-hydroxyecdysone<sup>66</sup> (a host hormone  
309 regulated by a circadian oscillator), motivating the caterpillar to climb high atop their host plants.  
310 Here, they liquefy and disseminate the virus to caterpillars below, as well as infecting birds who  
311 consume the corpses<sup>67</sup>. Similar to the manipulation of caterpillar hosts, many species of parasitic

312 fungi (*Ophiocordyceps spp.* and *Pandora spp.*) alter the daily behavioural rhythm of a variety of ant  
313 species<sup>68,69</sup> (See Box 3).

314

315 Parsing out whether temporal disruption is a host response or clock manipulation is nearly, if not  
316 entirely, impossible without uncovering the mechanism of manipulation. The lack of insight into the  
317 mechanisms parasites use to interfere with their hosts has stalled progress in the field of “host  
318 manipulation by parasites”<sup>70</sup>. This gap could be filled by harnessing the tools and conceptual  
319 framework developed in chronobiology. Many of the examples above have employed an ecological  
320 approach, yet a chronobiological approach can help elucidate both proximate and ultimate  
321 explanations.

322

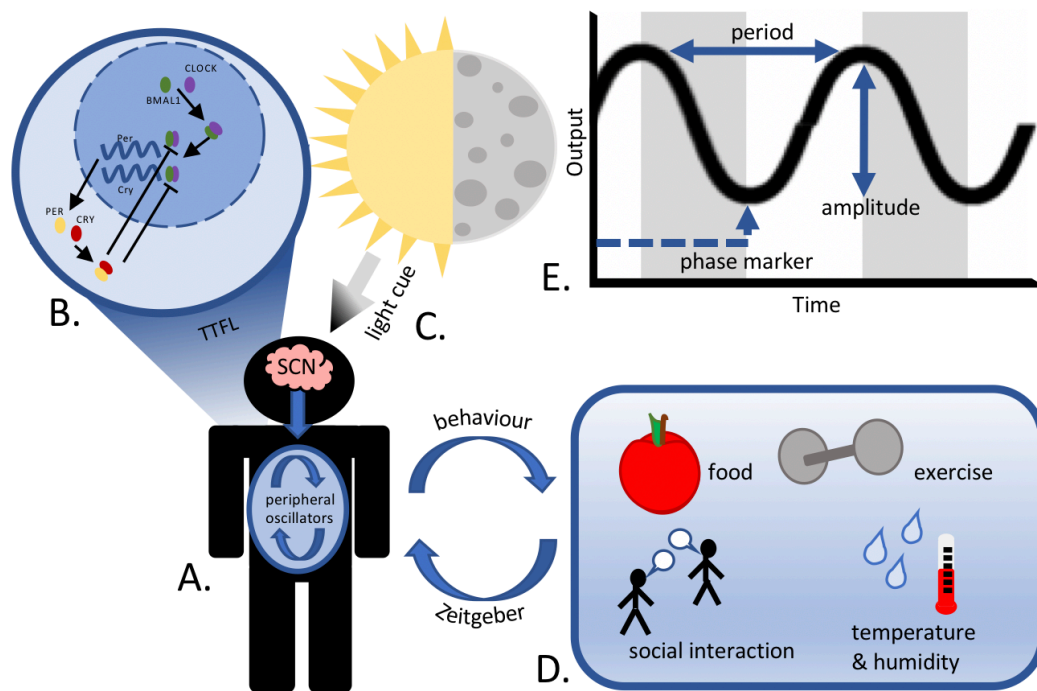
### 323 **Conclusion**

324 Over the past few decades, the focus of chronobiology has been to elucidate the mechanistic  
325 underpinnings of biological rhythms. We propose that now is the time to integrate this knowledge  
326 into parasitology, evolutionary ecology, and immunology (see Box 2). Indeed, the role of biological  
327 rhythms in infectious disease is a growing topic that holds promise for improving human and  
328 animal health. History clearly illustrates that attempts to control parasites are usually met with  
329 counter-evolution (in the form of drug resistance, vaccine escape, and host shifts). A comprehensive  
330 understanding of how rhythms affect parasite invasion and exploitation of a host (or vector) offers  
331 novel ways to disrupt the chain of transmission and treat disease. Further, clock coevolution may  
332 occur in host-parasite-vector interactions, resulting in complex arms races best understood through  
333 the lens of chronobiology coupled with evolutionary ecology. Chronobiology supplies a myriad of  
334 tools to help elucidate rhythmic phenotypes and reveal to what extent host and parasite genes are  
335 responsible for rhythms in disease phenotypes. Adding an evolutionary ecology framework will  
336 ensure this information is generalisable and used to make interventions as evolution-proof as  
337 possible.

**Box 1. What are circadian rhythms?** Biological rhythms are deemed to be controlled by circadian clocks if they meet several criteria<sup>71</sup>. First, their duration (period) must be approximately 24 hours. Second, they must persist (free-run) in conditions without time-of-day cues, which is usually assessed by observation in constant light or dark. Third, the phase of the oscillator or outputs are set (entrained) by a time-of-day cue (Zeitgeber) which is usually light. Fourth, unlike the rate of many chemical reactions, the speed of a circadian clock varies little over a biologically realistic range of environmental temperatures (temperature compensation). Together, these criteria allow organisms to fulfil a key feature of circadian rhythms: anticipatory, rather than reactionary, behaviour. For instance, plants ready photosynthetic machinery in anticipation of sunlight<sup>72,73</sup> and animals exhibit food-anticipatory activity (e.g. increases in core temperature, activity, serum corticosterone, and duodenal disaccharides) prior to foraging<sup>74</sup>. The workings of circadian clocks are sufficiently flexible to allow organisms to cope with gradual changes in photoperiod across seasons, but not flexible enough to instantly cope with changes in time zones (which is why travellers experience jet lag).

The mammalian circadian system is composed of the “central” clock in the brain (suprachiasmatic nucleus; SCN) and “peripheral clocks” in other organs and tissues (A). Clocks in nucleated cells are run by transcription-translation feedback loops (TTFL). For example, in animals the proteins CLOCK and BMAL1 act as activators and members of the PER and CRY families are repressors<sup>75</sup> (B). Retinal photoreceptors receive light cues which are carried through the hypothalamic optic tract and transmitted to the SCN, resulting in its synchronization/entrainment (C). Clocks in organs and tissues (peripheral clocks) can be entrained by feeding rhythms, and in taxa other than mammals, exercise, social cues, and abiotic rhythms in temperature and humidity may entrain clocks (D). Rhythms are often characterised by their period, amplitude, and markers for phase (E; grey bars illustrate night time for a rhythmic trait measured over 48 hours). They are described in relation to the time since the Zeitgeber (ZT) occurred (e.g. ZT6 refers to 6 hours after dawn) which usually differs from the actual time-of-day (Circadian Time; CT).

\*we suggest that the image [Box\_1] be placed here.



Box 1 image.

**Box 2. Why have circadian rhythms evolved?** Circadian clocks appear so advantageous that nearly all eukaryotes have a circadian system in most cells<sup>76</sup>. Circadian clocks may confer two kinds of fitness benefit: coordinating behaviours with rhythms in the external environment (extrinsic adaptive value), and temporally compartmentalising incompatible processes (intrinsic adaptive value)<sup>2</sup>. For instance, intrinsic benefits are conferred when cell division in yeast is temporally constrained to the reductive phase of metabolism, minimising rates of genetic mutation<sup>77</sup>. However, most studies of the fitness consequences of circadian rhythms have focussed on the benefits of synchronizing activities with rhythms in the abiotic environment: matching the period of day-night rhythms enables cyanobacteria to outcompete strains whose clocks run faster or slower<sup>78</sup> and enhances the survival of *Arabidopsis*<sup>73</sup>. Rhythms in the biotic environment<sup>2</sup> matter too. For example, the sea urchin *Centrostephanus coronatus* avoids predatory sheephead wrasse (*Pimelometopon pulchrum*) by foraging at night and retreating to shelter prior to the onset of wrasse activity<sup>79</sup>.

Despite the diversity of extrinsic rhythms that could select for the scheduling of diverse processes, there are surprisingly few demonstrations that circadian clocks actually affect fitness. For example, fitness is greater in wild-type mice than mutant mice with shortened periods<sup>80</sup>, flies with clock mutations die more rapidly than wild types after infection with bacteria<sup>81,82</sup>, and circadian knockout plants flower later and are less viable than wild-type plants<sup>3</sup>. However, depending on ecological context, rigidly scheduling activities according to day and night is not always the best strategy. For example, nocturnal mice boost energy efficiency by switching to diurnality when challenged with cold and hunger<sup>83</sup>. Nursing honeybees, that remain in the hive are arrhythmic, because round-the-clock care is necessary for larvae; and, if needed, diurnal foraging bees can revert to arrhythmic nursing behaviour<sup>84</sup>. Shorebirds also display considerable plasticity in activity rhythms during breeding, likely explained by predator avoidance strategies<sup>85</sup>.

The above examples illustrate the gains to be made from integrating chronobiology with evolutionary ecology in general<sup>4</sup>. We propose that such an approach offers a novel advance to the study of host-parasite interactions and coevolution. Coupling the well-developed conceptual frameworks for unravelling how circadian oscillators operate, and probing the costs and benefits of phenotypically plastic traits that are relevant to infection, will explain why rhythms in immune defences and parasite traits occur.

**Box 3. Case studies illustrating the role of circadian rhythms in parasite offence, host defence, and host manipulation**

**Host-parasite system:** *Teleogryllus oceanicus* (Pacific field cricket) & *Ormia ochracea* (parasitoid fly)

**What we know:** *O. ochracea* deposit larvae which burrow into the host and emerge 7-10 days later, resulting in host death. A flatwing morph that is physically incapable of calling has evolved to evade the risk of parasitism by acting as a silent, satellite male<sup>24</sup>.

**A more nuanced form of parasite evasion?** In addition to the flatwing morph, natural selection may have found another solution. Some males condense singing activity to the darkest part of the night<sup>29</sup> which may hamper the fly's ability to use visual cues to home in on hosts. Parasite evasion (via a flatwing phenotype or phase-shifted calling) trades off against attracting females, potentially constraining selection on these strategies. Moreover, multiple activities need to be coordinated for successful reproduction (e.g. locomotion, foraging, spermatophore production). Given that many of these traits are clock-controlled, could altering the timing outputs of the clock be a streamlined way of phase-shifting all related activities and minimizing the costs of parasite evasion? [associated image = cricket\_fly.png] Photo credit: Norman Lee



**Host-parasite system:** Carpenter ants & *Ophiocordyceps* spp. and *Pandora* spp. (fungi)

**What we know:** *O. unilateralis* s.l. induces workers of its carpenter ant host, ordinarily active during the night-time, to wander out of the ant nest during the day-time. Hosts then summit vegetation and adopt a mandibular death-grip in elevated positions. This manipulated behaviour is highly time-of-day and species-specific and occurs within a 3-hour window at dawn or in the mid-late morning, depending on the species<sup>68,86</sup>. Clinging to vegetation, the ant dies whilst the fungus completes its life cycle by growing a spore-producing stalk out of the dorsal region of the ant's thorax<sup>86</sup>.

**A case for coevolution and ecosystem specificity?** The jigsaw puzzle of how the fungus controls the ant is still being pieced together. Clocks may play a central role because infection alters the expression of host clock homologues *period* and *cycle*<sup>68</sup>. Host manipulation also appears to involve altering host chemosensory abilities, potentially via rhythmic secretion of enterotoxins<sup>87</sup>, all achieved from the fungus's primary location in muscle tissues<sup>88</sup>. [associated image = ant\_fungi.png] Photo credit: Miles Zhang



**Host-parasite system:** Mammals & *Plasmodium* spp. (*malaria parasites*)

**What we know:** Malaria parasites synchronously burst from the host's blood cells every 24, 48, or 72 hours depending on the parasite species<sup>89</sup>. When out of synch with the host's circadian rhythms, parasites incur an approximately 50 percent reduction in the densities of both asexual stages (necessary for in-host survival), and sexual stages (responsible for transmission)<sup>90</sup> before they become rescheduled to be in synch with host feeding rhythms<sup>44,45</sup>.

**Three worlds collide: a complex system of interactions?** Why aligning the phase of parasite rhythms with the host's rhythms is important remains mysterious, but recent work suggests that parasites are also selected to coordinate with the time-of-day their mosquito vectors are active<sup>54,55</sup> (see Rund et al. 2011 for information on *Anopheles* circadian rhythms). If differently phased rhythms for asexual replication are required to provide the best matches to host and vector rhythms, parasites face a trade-off between maximizing in-host survival and between-host transmission. Such a tension could be exploited by novel drug treatments to coerce parasites into a loss of fitness. Further, mosquito nets have induced a shift in *Anopheles gambiae* biting activity, ultimately resulting in a change in host-parasite timing<sup>8,52,53</sup>. The epidemiological consequences of this are unknown. [associated image = mosquito\_malaria.png] Photo credit: Sinclair Stammers





535 **Table 1. Impact of immune challenge during the rest and active phases of hosts.** A selection of studies  
536 identified as time-of-day immune challenges from PubMed searches for “time of day” plus “immune and  
537 infection” and “circadian rhythm” plus “immune and infection”. Articles were included if the study involved a  
538 time-of-day immune challenge; those without a time-of-day immune challenge were not included in the table.  
539 Time-of-day (ToD) is given as hours since lights on (ZT) for organisms in entrainment conditions, and as  
540 subjective day/night for those in constant light or dark conditions (i.e. corresponding to the light or dark portion  
541 of the cycle before experiencing constant conditions). Unless otherwise stated, entrainment conditions are 12  
542 hour light:dark. Outcomes of challenge in the rest phase (daytime for nocturnal organisms, nighttime for  
543 diurnal organisms) are compared to challenge in the active phase in terms of virulence metrics and immune  
544 effectors measured.  
545

Host spp.	Challenge	ToD	Outcome in rest versus active phase	Ref
<i>Mus musculus</i> – house mouse (nocturnal)	<i>Salmonella typhmuri</i>	ZT4/16	Greater inflammation and bacterial load when infected in the rest phase	22
	<i>Leishmania major</i>	Subjective day/night	Lower parasite burden and lower severity when infected in the rest phase	23
	Lipopolysaccharide (LPS) endotoxin	Subjective day/night	Lower concentrations of cytokines when infected in the rest phase	91
		ZT11/19	Higher mortality when challenged in the rest phase	92
		Subjective day/night	Greater inflammatory responses and lower bacterial burden when challenged/infected in the rest phase	93
	<i>Streptococcus pneumoniae</i>	ZT0/12		
	Murid Herpesvirus 4	ZT0/10	Greater viral replication when infected in the rest phase	60
	<i>Helicobacter pylori</i>	ZT1/7/13	Lower lymphocyte numbers when infected in the rest phase	94
	Vesicular stomatitis virus	ZT0/12	Higher mortality when infected in the rest phase	95
<i>Drosophila melanogaster</i> – fruit fly (diurnal)	<i>Pseudomonas aeruginosa</i>	ZT1/5/9/13/17/21/1	Lowest mortality when infected in the rest phase (especially ZT21)	82
		Subjective day/night	Lowest bacterial burden when infected in the rest phase	
	<i>Streptococcus pneumoniae</i>	ZT7/19	Slowest rate of mortality when infected in the rest phase	81
	<i>Escherichia coli</i>	ZT0/6/12/18	Infection at all ZT induces sleep the morning after infection and sleep was more prolonged after infection in the rest phase	96
<i>Anopheles stephensi</i> - Asian malaria mosquito (nocturnal)	<i>Escherichia coli</i>	Morning/evening	Lower bacterial growth and lower mortality when infected in the rest phase	97
<i>Arabidopsis thaliana</i> – thale cress (diurnal)	<i>Pseudomonas syringae</i>	ZT0/4/10/16	Immune defences are highest when inoculation occurs early in the active phase Note photoperiod is 9 hours light:15 hours dark	98
	<i>Botrytis cinerea</i>	Dawn/dusk	Larger lesions when inoculated in the rest phase	49
		ZT0/3/6/9/12/15/18/21/24	Greater susceptibility when inoculated in the rest phase	21
	<i>Pseudomonas syringae</i>	Subjective day/night	Lower infiltration of bacteria when infected in the rest phase	99

		Subjective morning /evening	Greater suppression of bacterial growth at the start of the rest phase when spray-inoculated, and greater suppression of bacterial growth at the start of the active phase when syringe-infiltrated	20
	<i>Hyaloperonospora arabidopsidis</i>	Dawn/dusk	Highest percentage of leaves with sporangiophores when infected in the start of the rest phase	100
<i>Danio rerio</i> zebrafish (diurnal)	<i>Salmonella typhimurium</i>	ZT4/16	Lower survival when infected in the rest phase	101
<i>Oreochromis niloticus</i> – Nile tilapia (mostly diurnal)	LPS	ZT3/15	Greater humoral immune response when infected in the rest phase	102
<i>Phodopus sungorus</i> - Siberian hamster (nocturnal)	LPS	ZT1/16	Shorter febrile response and more persistent locomotor activity when infected in the rest phase. Note, photoperiod is 16 hours light:8 hours dark	103

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863 SER conceived the study, MLW and SER drafted the manuscript, and all authors provided  
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866 **Competing interests**  
867 The authors declare no competing interests.  
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