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

2

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5

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15

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17

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

27

28 Circadian rhythms have long been taken for granted by science. Indeed, the first observation of a
29 clock-controlled behaviour (leaf opening and closing in *Mimosa pudica*) was not recorded until the
30 18th century¹. Following the fundamental observation that organisms can adaptively anticipate daily
31 rhythms in their environment, the field of "chronobiology" took off in the mid-20th century with a
32 focus on evolutionary and ecological questions. However, the advent of genetic tools a few decades
33 later shifted the remit to determining the molecular and genetic workings of circadian clocks. Yet,
34 despite their assumed major impact on fitness, circadian rhythms remain overlooked in evolutionary
35 ecology²⁻⁴. Here, we propose that the integration²⁻⁴ 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^{5,6}. Parasites display daily rhythms in traits
43 underpinning within-host survival and between-host transmission^{7,8}. 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 necessary⁷.
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 defences⁵. Circadian clock genes are
61 expressed in many types of immune cell, and the immune and circadian systems are connected in
62 multiple ways^{9,10}. For instance, the clock gene *Bmal1* mediates the balance between pro- and anti-
63 inflammatory responses¹¹. Rhythmic production of the pro-inflammatory cytokines TNF- α and IL-6
64 by macrophages is clock controlled¹², and mobilization of inflammatory monocytes is also
65 regulated by the clock¹⁰. This phenomenon, termed “anticipatory inflammation”, appears uncoupled
66 to metabolic rhythms and may defend against incoming parasites¹³. Similarly, in humans,
67 proinflammatory cytokines peak in circulation during the day (active phase)¹⁴, whereas
68 hematopoietic stem and progenitor cells, and most mature leukocytes, peak at night^{14,15}. 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)⁹.

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 phase¹⁶. 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 immunopathology¹⁷). Also, energetic demands imposed by activity and metabolism may trade-off
77 against immune defence¹⁸. 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 rhythms⁷. This includes separating the timing of
82 metabolism from defensive actions within immune cells themselves^{5,16}. 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 pathogens¹⁹. Consequently, *Arabidopsis* is better able to detect
88 and defend against parasites in the morning than evening^{20,21}. 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 symptoms²². 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 highest²³. 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^{24–26}. 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 manner²⁷. Host feeding rhythms are
117 relevant to gut microbiota, and a two-way feedback between host and microbe rhythms has been
118 proposed²⁸. 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 incomplete²⁹. 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^{30,31}. For
127 example, wild red colobus monkeys (*Procolobus rufomitratu tephrosceles*) decrease energetically
128 costly activities, and rest frequently, while shedding whipworm eggs³². 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 temperature³³ and has been conserved throughout more than 600
131 million years of vertebrate evolution³⁴. Fever enhances an organisms chance of survival by creating
132 a hostile environment for parasites and a more active immune response^{34–37}. 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 manner³⁸. 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 night³⁹ 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)⁴⁰. 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 vectors⁴¹. 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 host⁴². 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 hosts⁴³.
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 genes⁸. 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^{44,45}. 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 unknown⁴⁶ (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*⁴⁷, 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 synchronise⁴⁸.

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 dusk⁴⁹. 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 plants⁴⁹. 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 them⁷. 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 phenomena⁵⁰. 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 rhythm⁵¹. 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^{8,52,53}.
233 Given that rhythms of parasites and mosquitoes each affect malaria transmission in lab
234 experiments^{54,55}, 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 night⁵⁴. 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 known⁵⁶, the notion of “parasite manipulation of host clocks” is largely unexplored⁵⁷. 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 physiology⁵⁸
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 mice⁵⁹. 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 sickness⁵⁹. 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 out⁶⁰. 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 defence⁶⁰. 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 night⁶¹, 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 clocks⁶². 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 useful⁶³. 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* trematodes⁶⁴ (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 consumed²⁵. 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 snails²⁵. 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*⁶⁵, 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 shelter⁶⁵. 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-hydroxyecdysone⁶⁶ (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 corpses⁶⁷. 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^{68,69} (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”⁷⁰. 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

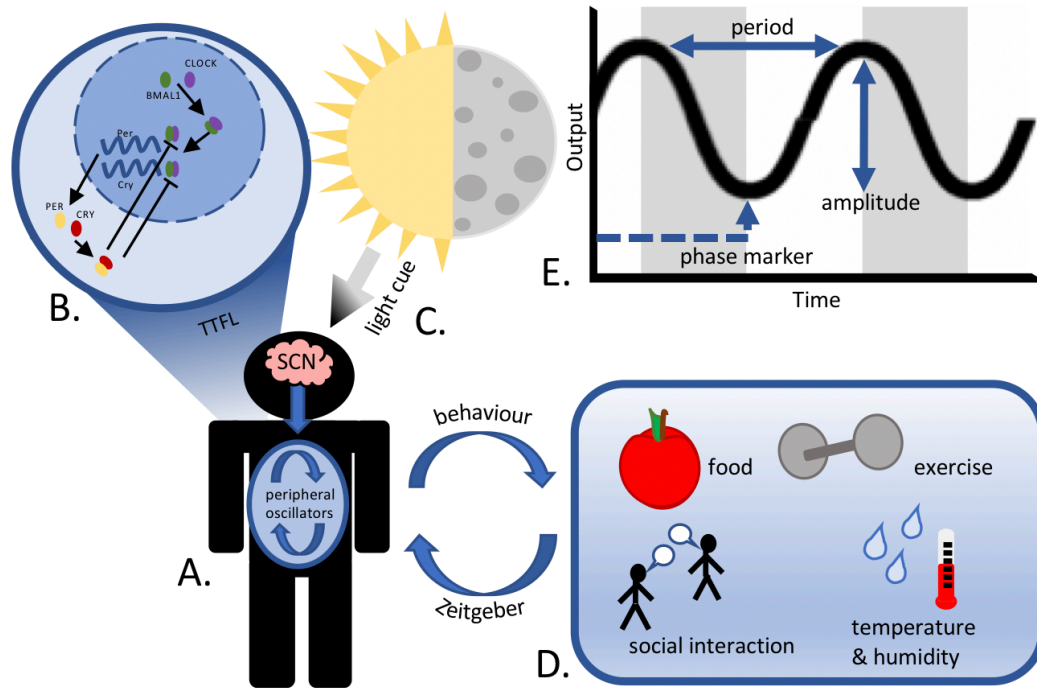
323 **Conclusion**

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.

338 **Box 1. What are circadian rhythms?** Biological rhythms are deemed to be controlled by circadian
339 clocks if they meet several criteria⁷¹. First, their duration (period) must be approximately 24 hours.
340 Second, they must persist (free-run) in conditions without time-of-day cues, which is usually
341 assessed by observation in constant light or dark. Third, the phase of the oscillator or outputs are set
342 (entrained) by a time-of-day cue (Zeitgeber) which is usually light. Fourth, unlike the rate of many
343 chemical reactions, the speed of a circadian clock varies little over a biologically realistic range of
344 environmental temperatures (temperature compensation). Together, these criteria allow organisms
345 to fulfil a key feature of circadian rhythms: anticipatory, rather than reactionary, behaviour. For
346 instance, plants ready photosynthetic machinery in anticipation of sunlight^{72,73} and animals exhibit
347 food-anticipatory activity (e.g. increases in core temperature, activity, serum corticosterone, and
348 duodenal disaccharides) prior to foraging⁷⁴. The workings of circadian clocks are sufficiently
349 flexible to allow organisms to cope with gradual changes in photoperiod across seasons, but not
350 flexible enough to instantly cope with changes in time zones (which is why travellers experience jet
351 lag).

352
353 The mammalian circadian system is composed of the “central” clock in the brain (suprachiasmatic
354 nucleus; SCN) and “peripheral clocks” in other organs and tissues (A). Clocks in nucleated cells are
355 run by transcription-translation feedback loops (TTFL). For example, in animals the proteins
356 CLOCK and BMAL1 act as activators and members of the PER and CRY families are repressors⁷⁵
357 (B). Retinal photoreceptors receive light cues which are carried through the hypothalamic optic tract
358 and transmitted to the SCN, resulting in its synchronization/entrainment (C). Clocks in organs and
359 tissues (peripheral clocks) can be entrained by feeding rhythms, and in
360 taxa other than mammals, exercise, social cues, and abiotic rhythms in temperature and humidity
361 may entrain clocks (D). Rhythms are often characterised by their period, amplitude, and markers for
362 phase (E; grey bars illustrate night time for a rhythmic trait measured over 48 hours). They are
363 described in relation to the time since the Zeitgeber (ZT) occurred (e.g. ZT6 refers to 6 hours after
364 dawn) which usually differs from the actual time-of-day (Circadian Time; CT).

365
366 *we suggest that the image [Box_1] be placed here.
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Box 1 image.

403 **Box 2. Why have circadian rhythms evolved?** Circadian clocks appear so advantageous that
404 nearly all eukaryotes have a circadian system in most cells⁷⁶. Circadian clocks may confer two
405 kinds of fitness benefit: coordinating behaviours with rhythms in the external environment
406 (extrinsic adaptive value), and temporally compartmentalising incompatible processes (intrinsic
407 adaptive value)². For instance, intrinsic benefits are conferred when cell division in yeast is
408 temporally constrained to the reductive phase of metabolism, minimising rates of genetic
409 mutation⁷⁷. However, most studies of the fitness consequences of circadian rhythms have focussed
410 on the benefits of synchronizing activities with rhythms in the abiotic environment: matching the
411 period of day-night rhythms enables cyanobacteria to outcompete strains whose clocks run faster or
412 slower⁷⁸ and enhances the survival of *Arabidopsis*⁷³. Rhythms in the biotic environment² matter too.
413 For example, the sea urchin *Centrostephanus coronatus* avoids predatory sheephead wrasse
414 (*Pimelometopon pulchrum*) by foraging at night and retreating to shelter prior to the onset of wrasse
415 activity⁷⁹.

416
417 Despite the diversity of extrinsic rhythms that could select for the scheduling of diverse processes,
418 there are surprisingly few demonstrations that circadian clocks actually affect fitness. For example,
419 fitness is greater in wild-type mice than mutant mice with shortened periods⁸⁰, flies with clock
420 mutations die more rapidly than wild types after infection with bacteria^{81,82}, and circadian knockout
421 plants flower later and are less viable than wild-type plants³. However, depending on ecological
422 context, rigidly scheduling activities according to day and night is not always the best strategy. For
423 example, nocturnal mice boost energy efficiency by switching to diurnality when challenged with
424 cold and hunger⁸³. Nursing honeybees, that remain in the hive are arrhythmic, because round-the-
425 clock care is necessary for larvae; and, if needed, diurnal foraging bees can revert to arrhythmic
426 nursing behaviour⁸⁴. Shorebirds also display considerable plasticity in activity rhythms during
427 breeding, likely explained by predator avoidance strategies⁸⁵.

428
429 The above examples illustrate the gains to be made from integrating chronobiology with
430 evolutionary ecology in general⁴. We propose that such an approach offers a novel advance to the
431 study of host-parasite interactions and coevolution. Coupling the well-developed conceptual
432 frameworks for unravelling how circadian oscillators operate, and probing the costs and benefits of
433 phenotypically plastic traits that are relevant to infection, will explain why rhythms in immune
434 defences and parasite traits occur.

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

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

459 **What we know:** *O. ochracea* deposit larvae which burrow into the host and emerge 7-10 days later,
460 resulting in host death. A flatwing morph that is physically incapable of calling has evolved to
461 evade the risk of parasitism by acting as a silent, satellite male²⁴.

462 **A more nuanced form of parasite evasion?** In addition to the flatwing morph, natural selection
463 may have found another solution. Some males condense singing activity to the darkest part of the
464 night²⁹ which may hamper the fly's ability to use visual cues to home in on hosts. Parasite evasion
465 (via a flatwing phenotype or phase-shifted calling) trades off against attracting females, potentially
466 constraining selection on these strategies. Moreover, multiple activities need to be coordinated for
467 successful reproduction (e.g. locomotion, foraging, spermatophore production). Given that many of
468 these traits are clock-controlled, could altering the timing outputs of the clock be a streamlined way
469 of phase-shifting all related activities and minimizing the costs of parasite evasion? [associated
470 image = cricket_fly.png] Photo credit: Norman Lee
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474 **Host-parasite system:** Carpenter ants & *Ophiocordyceps* spp. and *Pandora* spp. (fungi)

475 **What we know:** *O. unilateralis s.l.* induces workers of its carpenter ant host, ordinarily active
476 during the night-time, to wander out of the ant nest during the day-time. Hosts then summit
477 vegetation and adopt a mandibular death-grip in elevated positions. This manipulated behaviour is
478 highly time-of-day and species-specific and occurs within a 3-hour window at dawn or in the mid-
479 late morning, depending on the species^{68,86}. Clinging to vegetation, the ant dies whilst the fungus
480 completes its life cycle by growing a spore-producing stalk out of the dorsal region of the ant's
481 thorax⁸⁶.

482 **A case for coevolution and ecosystem specificity?** The jigsaw puzzle of how the fungus controls
483 the ant is still being pieced together. Clocks may play a central role because infection alters the
484 expression of host clock homologues *period* and *cycle*⁶⁸. Host manipulation also appears to involve
485 altering host chemosensory abilities, potentially via rhythmic secretion of enterotoxins⁸⁷, all
486 achieved from the fungus's primary location in muscle tissues⁸⁸. [associated image = ant_fungi.png]
487 Photo credit: Miles Zhang
488



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

492 **What we know:** Malaria parasites synchronously burst from the host's blood cells every 24, 48, or
493 72 hours depending on the parasite species⁸⁹. When out of synch with the host's circadian rhythms,
494 parasites incur an approximately 50 percent reduction in the densities of both asexual stages
495 (necessary for in-host survival), and sexual stages (responsible for transmission)⁹⁰ before they
496 become rescheduled to be in synch with host feeding rhythms^{44,45}.

497 **Three worlds collide: a complex system of interactions?** Why aligning the phase of parasite
498 rhythms with the host's rhythms is important remains mysterious, but recent work suggests that
499 parasites are also selected to coordinate with the time-of-day their mosquito vectors are active^{54,55}
500 (see Rund et al. 2011 for information on *Anopheles* circadian rhythms). If differently phased
501 rhythms for asexual replication are required to provide the best matches to host and vector rhythms,
502 parasites face a trade-off between maximizing in-host survival and between-host transmission. Such
503 a tension could be exploited by novel drug treatments to coerce parasites into a loss of fitness.
504 Further, mosquito nets have induced a shift in *Anopheles gambiae* biting activity, ultimately
505 resulting in a change in host-parasite timing^{8,52,53}. The epidemiological consequences of this are
506 unknown. [associated image = mosquito_malaria.png] Photo credit: Sinclair Stammers
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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	98
			Note photoperiod is 9 hours light:15 hours dark	
	<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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582 **References**

- 583
- 584 1. de Mairan, J. Observation botanique. *Hist. l'Academie R. des Sci. Paris* (1729).
- 585 2. Sharma, V. K. Adaptive Significance of Circadian Clocks. *Chronobiol. Int.* **20**, 901–919
- 586 (2003).
- 587 3. Green, R. M., Tingay, S., Wang, Z.-Y. & Tobin, E. M. Circadian Rhythms Confer a Higher
- 588 Level of Fitness to Arabidopsis Plants. *Plant Physiol.* **129**, 576 LP-584 (2002).
- 589 4. Helm, B. *et al.* Two sides of a coin: ecological and chronobiological perspectives of timing in
- 590 the wild. *Philos. Trans. R. Soc. B Biol. Sci.* **372**, (2017).
- 591 5. Scheiermann, C., Gibbs, J., Ince, L. & Loudon, A. Clocking in to immunity. *Nat. Rev.*
- 592 *Immunol.* **18**, 423–437 (2018).
- 593 6. Martinez-Bakker, M. & Helm, B. The influence of biological rhythms on host–parasite
- 594 interactions. *Trends Ecol. Evol.* **30**, 314–326 (2015).
- 595 7. Reece, S. E., Prior, K. F. & Mideo, N. The Life and Times of Parasites: Rhythms in
- 596 Strategies for Within-host Survival and Between-host Transmission. *J. Biol. Rhythms* **32**,
- 597 516–533 (2017).
- 598 8. Rijo-Ferreira, F., Pinto-Neves, D., Barbosa-Morais, N. L., Takahashi, J. S. & Figueiredo, L.
- 599 M. Trypanosoma brucei metabolism is under circadian control. *Nat. Microbiol.* **2**, 17032
- 600 (2017).
- 601 9. Scheiermann, C., Kunisaki, Y. & Frenette, P. S. Circadian control of the immune system.
- 602 *Nat. Rev. Immunol.* **13**, 190 (2013).
- 603 10. Curtis, A. M., Bellet, M. M., Sassone-Corsi, P. & O'Neill, L. A. J. Circadian Clock Proteins
- 604 and Immunity. *Immunity* **40**, 178–186 (2014).
- 605 11. Zaslona, Z. *et al.* The circadian protein BMAL1 in myeloid cells is a negative regulator of
- 606 allergic asthma. *Am. J. Physiol. Cell. Mol. Physiol.* **312**, L855–L860 (2017).
- 607 12. Keller, M. *et al.* A circadian clock in macrophages controls inflammatory immune responses.
- 608 *Proc. Natl. Acad. Sci.* **106**, 21407 LP-21412 (2009).
- 609 13. Nguyen, K. D. *et al.* Circadian Gene *Bmal1*; Regulates Diurnal
- 610 Oscillations of Ly6C^{hi}; Inflammatory Monocytes. *Science* (80-.).
- 611 **341**, 1483 LP-1488 (2013).
- 612 14. Haus, E. & Smolensky, M. H. Biologic Rhythms in the Immune System. *Chronobiol. Int.* **16**,
- 613 581–622 (1999).
- 614 15. Haus, E., Lakatua, D. J., Swoyer, J. & Sackett-Lundeen, L. Chronobiology in hematology
- 615 and immunology. *Am. J. Anat.* **168**, 467–517 (2018).
- 616 16. Labrecque, N. & Cermakian, N. Circadian Clocks in the Immune System. *J. Biol. Rhythms*
- 617 **30**, 277–290 (2015).
- 618 17. Graham, A. L., Allen, J. E. & Read, A. F. Evolutionary Causes and Consequences of
- 619 Immunopathology. *Annu. Rev. Ecol. Evol. Syst.* **36**, 373–397 (2005).
- 620 18. Kerr, A. M., Gershman, S. N. & Sakaluk, S. K. Experimentally induced spermatophore
- 621 production and immune responses reveal a trade-off in crickets. *Behav. Ecol.* **21**, 647–654
- 622 (2010).
- 623 19. Roden, L. C. & Ingle, R. A. Lights, Rhythms, Infection: The Role of Light and the Circadian
- 624 Clock in Determining the Outcome of Plant–Pathogen Interactions. *Plant Cell* **21**, 2546 LP-
- 625 2552 (2009).
- 626 20. Bhardwaj, V., Meier, S., Petersen, L. N., Ingle, R. A. & Roden, L. C. Defence Responses of
- 627 Arabidopsis thaliana to Infection by Pseudomonas syringae Are Regulated by the Circadian
- 628 Clock. *PLoS One* **6**, e26968 (2011).
- 629 21. Ingle, R. A. *et al.* Jasmonate signalling drives time-of-day differences in susceptibility of
- 630 Arabidopsis to the fungal pathogen Botrytis cinerea. *Plant J.* **84**, 937–948 (2015).
- 631 22. Bellet, M. M. *et al.* Circadian clock regulates the host response to Salmonella. *Proc. Natl.*
- 632 *Acad. Sci.* **110**, 9897 LP-9902 (2013).
- 633 23. Kiessling, S. *et al.* The circadian clock in immune cells controls the magnitude of

- 634 Leishmania parasite infection. *Sci. Rep.* **7**, 10892 (2017).
- 635 24. Zuk, M., Rotenberry, J. T. & Tinghitella, R. M. Silent night: adaptive disappearance of a
636 sexual signal in a parasitized population of field crickets. *Biol. Lett.* **2**, 521 LP-524 (2006).
- 637 25. Levri, E. P. & Lively, C. M. The effects of size, reproductive condition, and parasitism on
638 foraging behaviour in a freshwater snail, *Potamopyrgus antipodarum*. *Anim. Behav.* **51**, 891–
639 901 (1996).
- 640 26. Ponton, F. *et al.* Water-seeking behavior in worm-infected crickets and reversibility of
641 parasitic manipulation. *Behav. Ecol.* **22**, 392–400 (2011).
- 642 27. Hopwood, T. W. *et al.* The circadian regulator BMAL1 programmes responses to parasitic
643 worm infection via a dendritic cell clock. *Sci. Rep.* **8**, 3782 (2018).
- 644 28. Johnson, C. H., Zhao, C., Xu, Y. & Mori, T. Timing the day: what makes bacterial clocks
645 tick? *Nat. Rev. Microbiol.* **15**, 232 (2017).
- 646 29. Zuk, M., Simmons, L. & Cupp, L. Calling characteristics of parasitized and unparasitized
647 populations of the field cricket *Teleogryllus oceanicus*. *Behav. Ecol. Sociobiol.* **33**, 339–343
648 (1993).
- 649 30. Clark, I. A., Budd, A. C. & Alleva, L. M. Sickness behaviour pushed too far—the basis of the
650 syndrome seen in severe protozoal, bacterial and viral diseases and post-trauma. *Malar. J.* **7**,
651 208 (2008).
- 652 31. Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W. & Kelley, K. W. From
653 inflammation to sickness and depression: when the immune system subjugates the brain. *Nat.*
654 *Rev. Neurosci.* **9**, 46 (2008).
- 655 32. Ghai, R. R., Fugere, V., Chapman, C. A., Goldberg, T. L. & Davies, T. J. Sickness behaviour
656 associated with non-lethal infections in wild primates. *Proc. R. Soc. B* **282**, 20151436 (2015).
- 657 33. Kluger, M. J. Phylogeny of fever. in *Federation Proceedings* **38**, 30–34 (1979).
- 658 34. Evans, S. S., Repasky, E. A. & Fisher, D. T. Fever and the thermal regulation of immunity:
659 the immune system feels the heat. *Nat. Rev. Immunol.* **15**, 335 (2015).
- 660 35. Kluger, M. J., Ringler, D. H. & Anver, M. R. Fever and survival. *Science (80-.)*. **188**, 166–
661 168 (1975).
- 662 36. Schulman, C. I. *et al.* The effect of antipyretic therapy upon outcomes in critically ill
663 patients: a randomized, prospective study. *Surg. Infect. (Larchmt)*. **6**, 369–375 (2005).
- 664 37. Earn, D. J. D., Andrews, P. W. & Bolker, B. M. Population-level effects of suppressing
665 fever. *Proc. R. Soc. B* **281**, 20132570 (2014).
- 666 38. Levi, F. & Schibler, U. Circadian rhythms: mechanisms and therapeutic implications. *Annu.*
667 *Rev. Pharmacol. Toxicol.* **47**, 593–628 (2007).
- 668 39. Matthews, J. H., Marte, E. & Halberg, F. A Circadian susceptibility-resistance cycle to
669 fluothane in male B 1 mice. *Can. Anaesth. Soc. J.* **11**, 280 (1964).
- 670 40. Zhang, R., Lahens, N. F., Ballance, H. I., Hughes, M. E. & Hogenesch, J. B. A circadian
671 gene expression atlas in mammals: implications for biology and medicine. *Proc. Natl. Acad.*
672 *Sci.* **111**, 16219–16224 (2014).
- 673 41. Hawking, F. The 24-hour periodicity of microfilariae: biological mechanisms responsible for
674 its production and control. *Proc. R. Soc. Lond. B* **169**, 59–76 (1967).
- 675 42. Mouahid, G. *et al.* A new chronotype of *Schistosoma mansoni*: adaptive significance. *Trop.*
676 *Med. Int. Heal.* **17**, 727–732 (2012).
- 677 43. Martinaud, G., Billaudelle, M. & Moreau, J. Circadian variation in shedding of the oocysts of
678 *Isospora turdi* (Apicomplexa) in blackbirds (*Turdus merula*): An adaptative trait against
679 desiccation and ultraviolet radiation. *Int. J. Parasitol.* **39**, 735–739 (2009).
- 680 44. Prior, K. F. *et al.* Timing of host feeding drives rhythms in parasite replication. *PLoS Pathog.*
681 **14**, e1006900 (2018).
- 682 45. Hirako, I. C. *et al.* Daily Rhythms of TNF α Expression and Food Intake Regulate Synchrony
683 of Plasmodium Stages with the Host Circadian Cycle. *Cell Host Microbe* (2018).
- 684 46. Reece, S. E. & Prior, K. F. Malaria Makes the Most of Mealtimes. *Cell Host Microbe* **23**,
685 695–697 (2018).

- 686 47. Fenske, M. P., Nguyen, L. P., Horn, E. K., Riffell, J. A. & Imaizumi, T. Circadian clocks of
687 both plants and pollinators influence flower seeking behavior of the pollinator hawkmoth
688 *Manduca sexta*. *Sci. Rep.* **8**, 2842 (2018).
- 689 48. Kharouba, H. M. *et al.* Global shifts in the phenological synchrony of species interactions
690 over recent decades. *Proc. Natl. Acad. Sci.* **115**, 5211–5216 (2018).
- 691 49. Hevia, M. A., Canessa, P., Müller-Esparza, H. & Larrondo, L. F. A circadian oscillator in the
692 fungus *Botrytis cinerea* regulates virulence when infecting *Arabidopsis thaliana*. *Proc. Natl.*
693 *Acad. Sci.* **112**, 8744–8749 (2015).
- 694 50. Rensing, L., Meyer-Grahe, U. & Ruoff, P. Biological timing and the clock metaphor:
695 oscillatory and hourglass mechanisms. *Chronobiol. Int.* **18**, 329–369 (2001).
- 696 51. Mrosovsky, N. Masking: history, definitions, and measurement. *Chronobiol. Int.* **16**, 415–
697 429 (1999).
- 698 52. Sougoufara, S. *et al.* Biting by *Anopheles funestus* in broad daylight after use of long-lasting
699 insecticidal nets: a new challenge to malaria elimination. *Malar. J.* **13**, 125 (2014).
- 700 53. Rund, S. S. C., O'Donnell, A. J., Gentile, J. E. & Reece, S. E. Daily rhythms in mosquitoes
701 and their consequences for malaria transmission. *Insects* **7**, 14 (2016).
- 702 54. Schneider, P. *et al.* Adaptive periodicity in the infectivity of malaria gametocytes to
703 mosquitoes. *bioRxiv* 294942 (2018).
- 704 55. Pigeault, R., Caudron, Q., Nicot, A., Rivero, A. & Gandon, S. Timing malaria transmission
705 with mosquito fluctuations. *Evol. Lett.* (2018).
- 706 56. Thomas, F., Rigaud, T. & Brodeur, J. Evolution of parasite-induced behavioral alterations.
707 (2010).
- 708 57. De Bekker, C., Mellow, M. & Hughes, D. P. From behavior to mechanisms: an integrative
709 approach to the manipulation by a parasitic fungus (*Ophiocordyceps unilateralis* sl) of its
710 host ants (*Camponotus* spp.). *Integr. Comp. Biol.* **54**, 166–176 (2014).
- 711 58. Ko, C. H. & Takahashi, J. S. Molecular components of the mammalian circadian clock. *Hum.*
712 *Mol. Genet.* **15**, R271–R277 (2006).
- 713 59. Rijo-Ferreira, F. *et al.* Sleeping sickness is a circadian disorder. *Nat. Commun.* **9**, 62 (2018).
- 714 60. Edgar, R. S. *et al.* Cell autonomous regulation of herpes and influenza virus infection by the
715 circadian clock. *Proc. Natl. Acad. Sci.* **113**, 10085–10090 (2016).
- 716 61. Thomas, F. *et al.* Do hairworms (Nematomorpha) manipulate the water seeking behaviour of
717 their terrestrial hosts? *J. Evol. Biol.* **15**, 356–361 (2002).
- 718 62. Biron, D. G. *et al.* 'Suicide' of crickets harbouring hairworms: a proteomics investigation.
719 *Insect Mol. Biol.* **15**, 731–742 (2006).
- 720 63. Hughes, M. E. *et al.* Guidelines for genome-scale analysis of biological rhythms. *J. Biol.*
721 *Rhythms* **32**, 380–393 (2017).
- 722 64. Lively, C. M. Evidence from a New Zealand snail for the maintenance of sex by parasitism.
723 *Nature* **328**, 519 (1987).
- 724 65. Levri, E. P. Parasite-induced change in host behavior of a freshwater snail: parasitic
725 manipulation or byproduct of infection? *Behav. Ecol.* **10**, 234–241 (1999).
- 726 66. Hoover, K. *et al.* A gene for an extended phenotype. *Science (80-)*. **333**, 1401 (2011).
- 727 67. Goulson, D. Wipfelkrankheit: modification of host behaviour during baculoviral infection.
728 *Oecologia* **109**, 219–228 (1997).
- 729 68. de Bekker, C. *et al.* Gene expression during zombie ant biting behavior reflects the
730 complexity underlying fungal parasitic behavioral manipulation. *BMC Genomics* **16**, 620
731 (2015).
- 732 69. de Bekker, C., Will, I., Das, B. & Adams, R. M. M. The ants (Hymenoptera: Formicidae) and
733 their parasites: effects of parasitic manipulations and host responses on ant behavioral
734 ecology. *Myrmecological News* **28**, 1–24 (2018).
- 735 70. Herbison, R., Lagrue, C. & Poulin, R. The missing link in parasite manipulation of host
736 behaviour. *Parasit. Vectors* **11**, 222 (2018).
- 737 71. Johnson, C. H., Elliott, J., Foster, R., Honma, K. & Kronauer, R. *Chronobiology: Biological*

- 738 *Timekeeping*. (2004).
- 739 72. Michael, T. P. *et al.* Enhanced Fitness Conferred by Naturally Occurring Variation in the
740 Circadian Clock. *Science (80-.)*. **302**, 1049 LP-1053 (2003).
- 741 73. Dodd, A. N. *et al.* Plant Circadian Clocks Increase Photosynthesis, Growth, Survival, and
742 Competitive Advantage. *Science (80-.)*. **309**, 630 LP-633 (2005).
- 743 74. Stephan, F. K. The “other” circadian system: food as a Zeitgeber. *J. Biol. Rhythms* **17**, 284–
744 292 (2002).
- 745 75. Young, M. W. & Kay, S. A. Time zones: a comparative genetics of circadian clocks. *Nat.*
746 *Rev. Genet.* **2**, 702 (2001).
- 747 76. Dunlap, J. C. Molecular bases for circadian clocks. *Cell* **96**, 271–290 (1999).
- 748 77. Chen, Z., Odstreil, E. A., Tu, B. P. & McKnight, S. L. Restriction of DNA Replication to the
749 Reductive Phase of the Metabolic Cycle Protects Genome Integrity. *Science (80-.)*. **316**,
750 1916 LP-1919 (2007).
- 751 78. Ouyang, Y., Andersson, C. R., Kondo, T., Golden, S. S. & Johnson, C. H. Resonating
752 circadian clocks enhance fitness in cyanobacteria. *Proc. Natl. Acad. Sci.* **95**, 8660–8664
753 (1998).
- 754 79. Nelson, B.V. & Vance, R. R. Diel foraging patterns of the sea urchin *Centrostephanus*
755 *coronatus* as a predator avoidance strategy. *Mar. Biol.* **51**, 251–258 (1979).
- 756 80. Spoelstra, K., Wikelski, M., Daan, S., Loudon, A. S. I. & Hau, M. Natural selection against a
757 circadian clock gene mutation in mice. *Proc. Natl. Acad. Sci.* **113**, 686–691 (2016).
- 758 81. Stone, E. F. *et al.* The circadian clock protein timeless regulates phagocytosis of bacteria in
759 *Drosophila*. *PLoS Pathog.* **8**, e1002445 (2012).
- 760 82. Lee, J.-E. & Edery, I. Circadian regulation in the ability of *Drosophila* to combat pathogenic
761 infections. *Curr. Biol.* **18**, 195–199 (2008).
- 762 83. van der Vinne, V. *et al.* Cold and hunger induce diurnality in a nocturnal mammal. *Proc.*
763 *Natl. Acad. Sci.* **111**, 15256–15260 (2014).
- 764 84. Bloch, G. & Robinson, G. E. Chronobiology: reversal of honeybee behavioural rhythms.
765 *Nature* **410**, 1048 (2001).
- 766 85. Bulla, M. *et al.* Unexpected diversity in socially synchronized rhythms of shorebirds. *Nature*
767 **540**, 109 (2016).
- 768 86. Hughes, D. P. *et al.* Behavioral mechanisms and morphological symptoms of zombie ants
769 dying from fungal infection. *BMC Ecol.* **11**, 13 (2011).
- 770 87. De Bekker, C., Ohm, R. A., Evans, H. C., Brachmann, A. & Hughes, D. P. Ant-infecting
771 *Ophiocordyceps* genomes reveal a high diversity of potential behavioral manipulation genes
772 and a possible major role for enterotoxins. *Sci. Rep.* **7**, 12508 (2017).
- 773 88. Fredericksen, M. A. *et al.* Three-dimensional visualization and a deep-learning model reveal
774 complex fungal parasite networks in behaviorally manipulated ants. *Proc. Natl. Acad. Sci.*
775 201711673 (2017).
- 776 89. Garcia, C. R. S., Markus, R. P. & Madeira, L. Tertian and quartan fevers: temporal regulation
777 in malarial infection. *J. Biol. Rhythms* **16**, 436–443 (2001).
- 778 90. Donnell, A. J. O., Schneider, P., Mcwatters, H. G. & Reece, S. E. Fitness costs of disrupting
779 circadian rhythms in malaria parasites Fitness costs of disrupting circadian rhythms in
780 malaria parasites. *Proc. R. Soc. B Biol. Sci.* 2429–2436 (2011). doi:10.1098/rspb.2010.2457
- 781 91. Gibbs, J. E. *et al.* The nuclear receptor REV-ERB α mediates circadian regulation of innate
782 immunity through selective regulation of inflammatory cytokines. *Proc. Natl. Acad. Sci.* **109**,
783 582–587 (2012).
- 784 92. Marpegan, L. *et al.* Diurnal variation in endotoxin-induced mortality in mice: correlation
785 with proinflammatory factors. *Chronobiol. Int.* **26**, 1430–1442 (2009).
- 786 93. Gibbs, J. *et al.* An epithelial circadian clock controls pulmonary inflammation and
787 glucocorticoid action. *Nat. Med.* **20**, 919 (2014).
- 788 94. Druzd, D. *et al.* Lymphocyte circadian clocks control lymph node trafficking and adaptive
789 immune responses. *Immunity* **46**, 120–132 (2017).

- 790 95. Gagnidze, K. *et al.* Nuclear receptor REV-ERB α mediates circadian sensitivity to mortality
791 in murine vesicular stomatitis virus-induced encephalitis. *Proc. Natl. Acad. Sci.* 201520489
792 (2016).
- 793 96. Kuo, T.-H., Pike, D. H., Beizaeipour, Z. & Williams, J. A. Sleep triggered by an immune
794 response in *Drosophila* is regulated by the circadian clock and requires the NF κ B Relish.
795 *BMC Neurosci.* **11**, 17 (2010).
- 796 97. Murdock, C. C., Moller-Jacobs, L. L. & Thomas, M. B. Complex environmental drivers of
797 immunity and resistance in malaria mosquitoes. *Proc. R. Soc. B* **280**, 20132030 (2013).
- 798 98. Griebel, T. & Zeier, J. Light regulation and daytime dependency of inducible plant defenses
799 in *Arabidopsis*: phytochrome signaling controls systemic acquired resistance rather than local
800 defense. *Plant Physiol.* **147**, 790–801 (2008).
- 801 99. Korneli, C., Danisman, S. & Staiger, D. Differential control of pre-invasive and post-invasive
802 antibacterial defense by the *Arabidopsis* circadian clock. *Plant Cell Physiol.* **55**, 1613–1622
803 (2014).
- 804 100. Wang, W. *et al.* Timing of plant immune responses by a central circadian regulator. *Nature*
805 **470**, 110 (2011).
- 806 101. Du, L. Y. *et al.* The innate immune cell response to bacterial infection in larval zebrafish is
807 light-regulated. *Sci. Rep.* **7**, 12657 (2017).
- 808 102. Lazado, C. C., Skov, P. V. & Pedersen, P. B. Innate immune defenses exhibit circadian
809 rhythmicity and differential temporal sensitivity to a bacterial endotoxin in Nile tilapia
810 (*Oreochromis niloticus*). *Fish Shellfish Immunol.* **55**, 613–622 (2016).
- 811 103. Prendergast, B. J. *et al.* Circadian disruption alters the effects of lipopolysaccharide treatment
812 on circadian and ultradian locomotor activity and body temperature rhythms of female
813 Siberian hamsters. *J. Biol. Rhythms* **30**, 543–556 (2015).
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862 **Contributions**

863 SER conceived the study, MLW and SER drafted the manuscript, and all authors provided
864 substantial input into ideas and the writing of subsequent drafts.

865

866 **Competing interests**

867 The authors declare no competing interests.

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