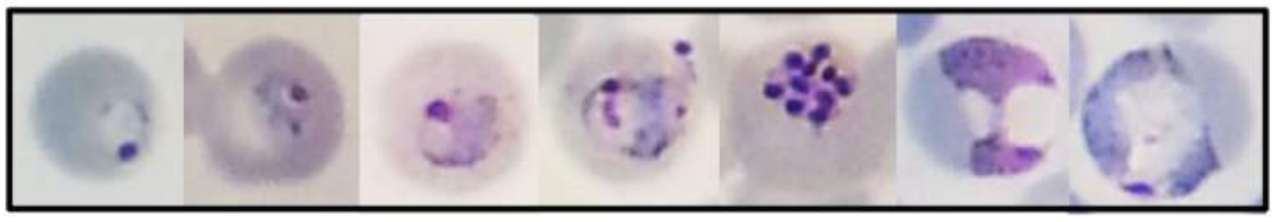
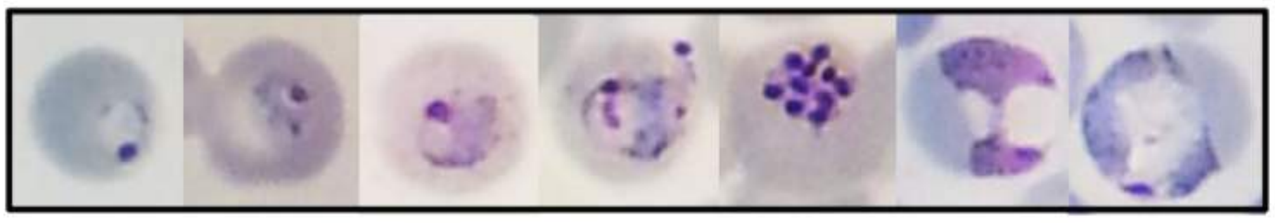




Circadian rhythms in malaria parasites?

Kimberley F. Prior, Aidan J. O'Donnell, Nicholas J. Savill, Sarah E. Reece

Institutes of Evolution, Immunology and Infection Research, University of Edinburgh

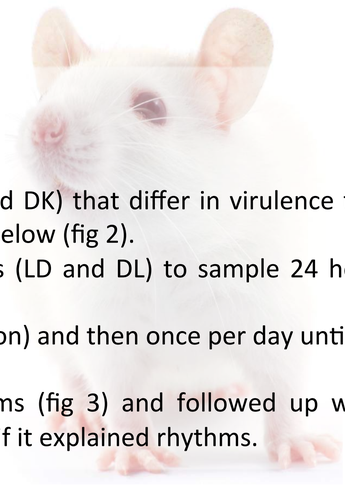
Circadian rhythms maximise fitness by allowing organisms to anticipate and exploit daily environmental fluctuations. Circadian rhythms in disease is generally an understudied area. Rhythms appear important for hosts to cope with infections, e.g. there is a time of day effect of controlling bacterial infection in mice [1]. Rhythms are also important for parasite fitness e.g. we see a 50% reduction in parasite density when malaria parasites are jetlagged [2].

Malaria parasites display 24 hours rhythms (or multiples of) in their cell cycle duration (see fig 1).
I want to ask 3 questions:

1. What is driving parasite rhythms?
2. Are rhythms adaptive for parasites/hosts?
3. Are parasites or hosts in control?

However, before we can answer these questions we need to better characterise parasite rhythms.

Methods and Results



Mice were infected with 3 *P. chabaudi* strains (AJ, AS and DK) that differ in virulence to investigate variation in rhythms. Results for the DK strain are shown below (fig 2).
A split entrainment protocol was used by using 2 rooms (LD and DL) to sample 24 hours during a working day.
Mice were sampled every 3 hours days 3-5 pi (post infection) and then once per day until day 14 pi.

We modelled the infections to quantify parasite rhythms (fig 3) and followed up with a second experiment looking at the host environment (fig 4) to see if it explained rhythms.

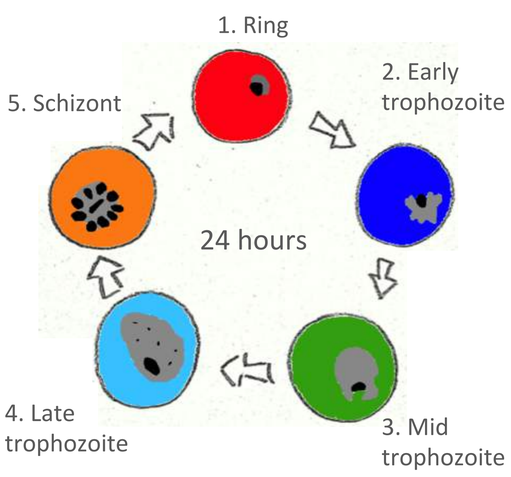


Figure 1. The asexual malaria parasite cell cycle separated into 5 stages based on morphological characteristics. Colours correspond to the stage proportions in fig 2.

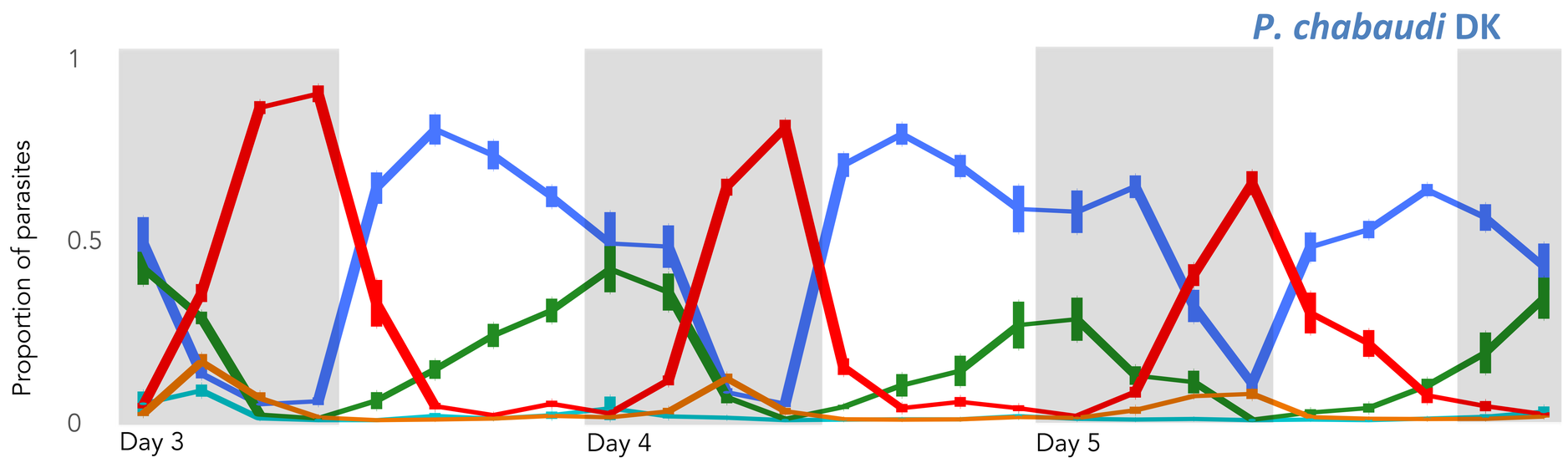


Figure 2. Stage proportions of the *P. chabaudi* DK parasite. Three day time series plotted every 3 hours displaying the proportion of each parasite stage present in the blood, see fig 1 for colour key. We see each stage peaking sequentially in the blood and this is repeatable each day.

Created shift working LD mice?

Modelling the infections to quantify the parasite asexual cycle reveals a difference in parasite dynamics between LD and DL. We see a shift in the timing of parasite schizogony in LD mice that we do not see in DL mice. Parasites in DL maintain a 24h cell cycle (see fig 3 DL plot) and time of schizogony stays the same throughout the infection. Parasites in LD lengthen their cell cycle duration from around 24h to around 25.5h (see fig 3 LD plot). This shifts the time of schizogony from night to day. Parasites then shorten their cell cycle duration to around 23h, which shifts schizogony back into the night.

We are sampling the LD mice in the day, during their rest phase. The DL mice are sampled in the night, during their active phase. Sampling LD mice during their rest phase might have caused an unintentional disruption in host rhythm, analogous to shiftwork, which might have generated a shift in parasite rhythm. As DL mice were sampled during their active phase this disruption may not have occurred.

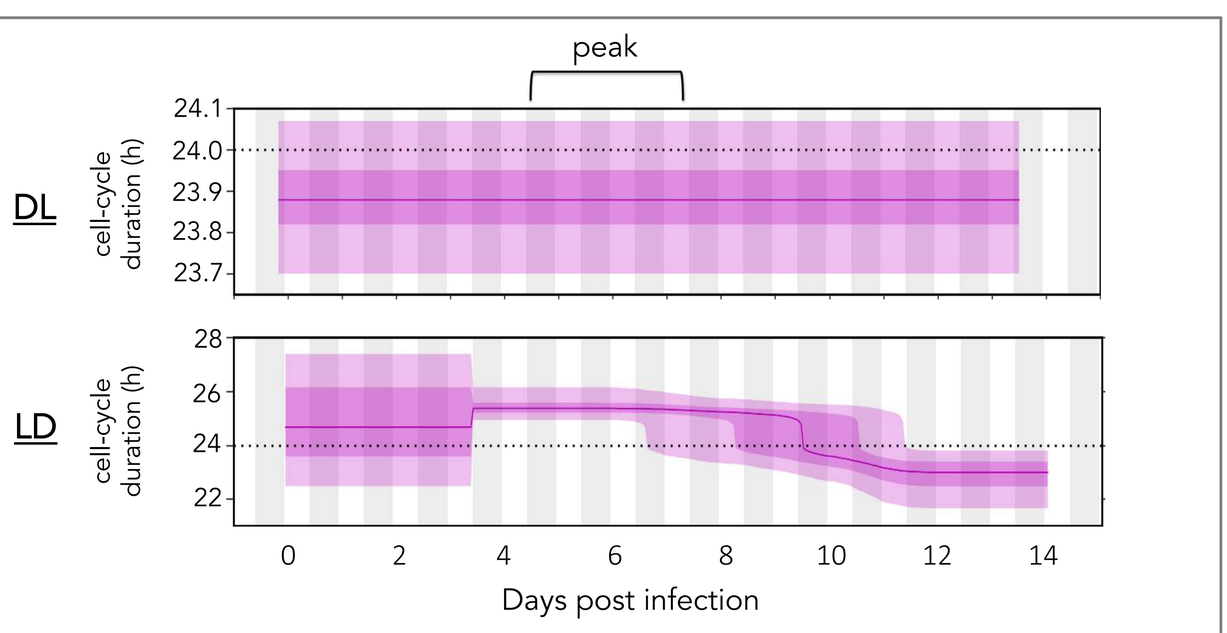


Figure 3. When we model the infections there appears to be a room effect. Parasite rhythms appear more stable in DL when we sampled mice during their active phase. We see the cell cycle duration stay the same at 24h across the course of the infection. Parasite rhythms change in LD when we sample mice during their rest phase. The cell cycle duration lengthens to around 25.5h which shifts the timing of schizogony to the day time. This lengthening appears to happen some time before the peak parasite density (indicated by 'peak' bracket – approx. day 5-7 pi). The cell cycle duration then shortens to around 23h and schizogony moves back to the night. This occurs around the time the host is the sickest (approx. day 7-10 pi).

We monitored host rhythms (temperature/activity) during a normal infection (without sampling/ shiftwork) to characterise the host environment before investigating what happens to host rhythms when hosts undergo shiftwork.

Host environment

Figure 4 shows rhythms in host body temperature (which follow activity) being lost during infection. If DL mice from fig 3 (sampled during active phase – no shift work) could be tenuously compared to mice from fig 4 (not sampled – no shift work) then parasites appear to maintain their 24h rhythms when the hosts lose their rhythms during a normal infection.

The time series will be repeated for the LD mice (fig 3) to see if changes in shift working mouse rhythms might explain variation/breakdown in parasite rhythms.

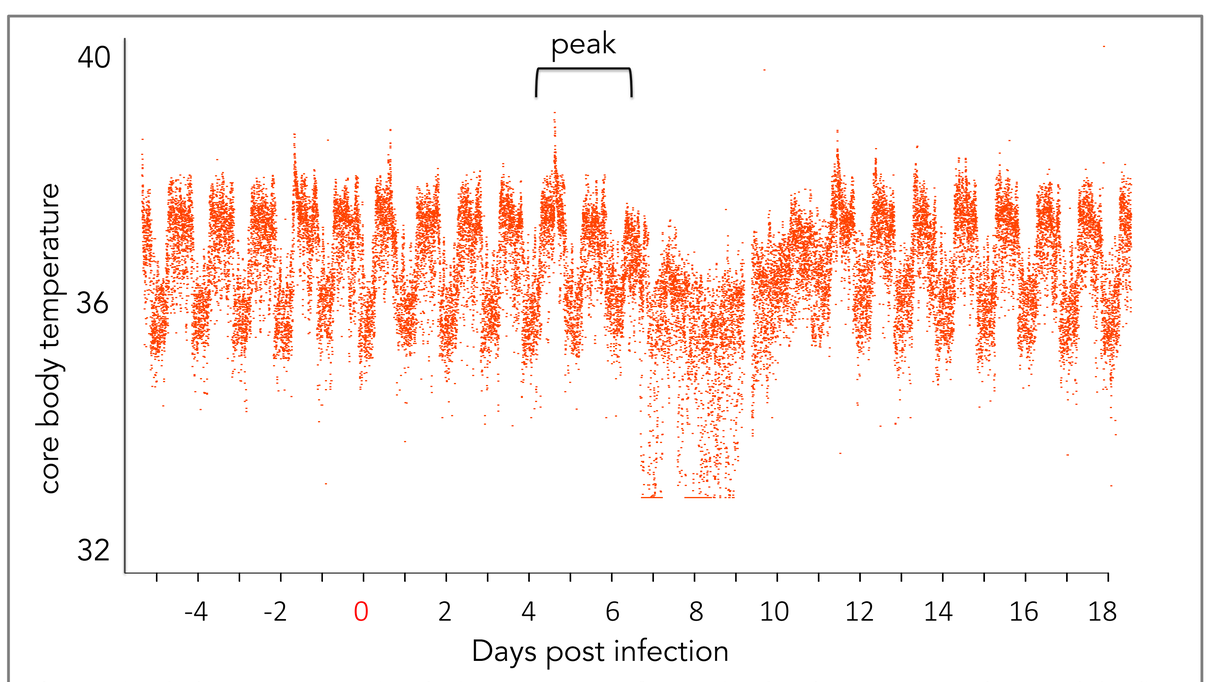


Figure 4. Core body temperature monitored using RFID tags. Data from 5 mice plotted every 5 minutes during *P. chabaudi* DK infection. Mice were not sampled so could be comparable with the DL mice in fig 3. We see a breakdown in mouse temperature/ activity rhythms that occurs after peak parasite density (indicated by 'peak' bracket) during a normal infection. Any change in parasite rhythm in the LD mice in fig 3 is unlikely to be the result of normal sickness behaviour.

Summary

The drivers of rhythms in malaria parasites remains unknown. The host is likely to be important, however the explanation is more complex than parasites following normal host temperature/activity rhythms. A change in parasite rhythms when hosts are disturbed more suggests that parasites are responding to their environment. When considering if rhythms are adaptive it appears malaria parasites undergo a cost when mismatched from the host, indicating that being in sync with the host is important. This can be explored further by investigating changes in host rhythm when mice are sampled during their rest phase and seeing if parasites are following a change in the mice.

Implications and future work

Investigating which parts of the host physiology are important to the timing of malaria parasites may offer new targets for malaria control.

- can shiftwork explain the change in parasite rhythms?
- how do host circadian rhythms help parasites keep time?
- how do parasites reschedule after jet lag?

[1] Bellet et al 2013 PNAS; [2] O'Donnell et al 2011 Proc Roy Soc B