

Who turned out the lights: the shortcomings and successes of a Boolean network model for circadian processes[†]

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The purpose of this paper is to stress test a Boolean network representing circadian rhythms of *Arabidopsis thaliana*. We reproduced a Boolean model for these rhythms obtained from [O. E. Akman, Journal of The Royal Society, 2012.], and subjected the model to situations outside of the standard conditions under which it was designed. We tested it with a variety of photoperiods and submitted it to asynchronous behavior from the light inputs. We found that this particular model doesn't respond well to large changes in photoperiods, but does respond fairly well to asynchronous light node behavior. For a model so small (in terms of number of nodes) it is difficult to rectify these issues, however there are more complex models of the same system that might be more robust to change.

1 Introduction

Biology is comprised of many processes regulated by the 24hr period of the day, temperature, or other environmental factors. These systems, circadian systems, typically stand on the back of gene regulatory networks (GRNs). A GRN is a group of genes linked by excitatory and inhibitory pathways which, in the circadian case, produce regulated, periodic outputs. These networks can be, and frequently are, modeled using ordinary differential equations (ODEs); however, it has been shown that Boolean networks can perform just as well under certain conditions¹.

A Boolean network is a way to model systems with binary operators. The vertices (with values 0 or 1) are connected by edges and the corresponding logic gates. The model then uses the values of the connected vertices to generate values at the next time step. A basic 3 node model is shown below along with the accompanying table of values.

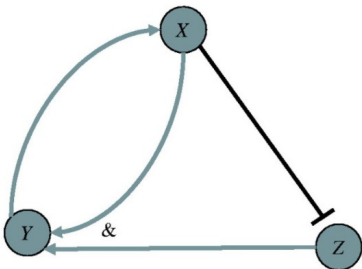


Fig. 1 A simple 3 node Boolean network with no signal delay

Table 1 The values of X,Y and Z at t+1 given XYZ(t)

XYZ(t)	XYZ(t+1)
000	001
001	001
010	101
011	101
100	000
101	010
110	100
111	110

The paper put forward by Akman et al. compares the Boolean model to data under rather ideal conditions (synchronous, physically relevant photoperiods), where it performs rather well. This purpose of this paper is to stress test one of these Boolean models put forth under more extreme conditions and parameterizations to determine the model's limitations to further understand the Boolean model's place in GRN modeling.

2 Methods

We built a codebase in Matlab to represent and evaluate boolean networks, utilizing a recursive class and evaluation function to represent complex Boolean gates. A matrix was used to represent and store the states of the nodes for each time point, which allowed data from different time points to be accessed during simulation - a necessary piece for signalling delays. Light nodes were implemented to update based on their absolute time step, rather than basing their outputs on stimulus. At the start of any simulation, all states were assumed to be off, and states prior to the start of the simulation (accessed via signalling delays), were assumed to be off.

Once this system had been built, it was fairly trivial to implement and simulate a variety of boolean networks. The network we settled on was the 2-loop *Arabidopsis* model (Figure 2) given by Akman et al¹. We utilized the logic configuration and signaling

[†] Electronic Supplementary Information (ESI) available: <https://github.com/gnorthrup/BooleanControl>

delays presented, with a little bit of fiddling to ensure that the model produced the results expected by the paper. Once this model was established it was fairly easy to make changes to the parameters in order to experiment with the model's response to change.

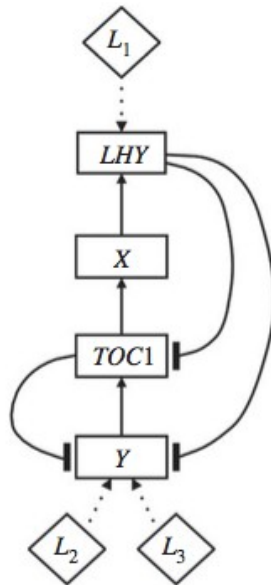


Fig. 2 A simple 3 node Boolean network with no signal delay

In order to stress the model, we tested all the possible photoperiods that totalled to 24 hours (24hr day, 0 hr night; 23hr day, 1 hr night; etc.) and all the possible photoperiods that totalled to 48 hours. Additionally we ran the model with subsets of lights entirely off (either L_1 , or L_2 & L_3), and tried turning off those subsets of lights in the middle of a simulation. We also tried running the subsets of lights with different periods to examine how the model responded.

3 Results

3.1 Photoperiods

One of the things we checked was how the model performed when the lights were entirely on or entirely off. When the lights were entirely off, the entire system remained off. When the lights were entirely on, the system produced a fairly simple, biologically plausible cycle, with a period of 28 hours (Figure 6). For photoperiods totalling to 24 hours within a reasonable window of twelve hours dark, twelve hours light, the model performed about as we expected. The behavior was pretty similar to the general behavior of the model under 12-12 (Figure 3), and we were able to fairly easily understand the causes of any peculiarities. However, for any 24hr period with 18 or more hours of light, the model performed in ways that were clearly not biologically sound (Figure 5). At all times the nodes were constantly switching back and forth between on and off.

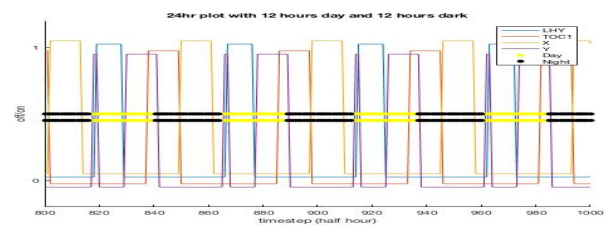


Fig. 3 12 hour day

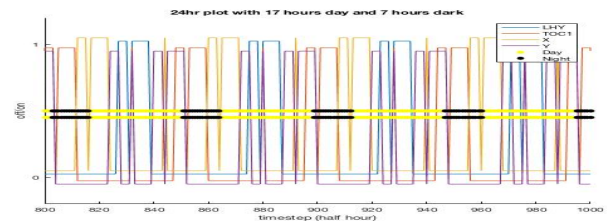


Fig. 4 17 hour day

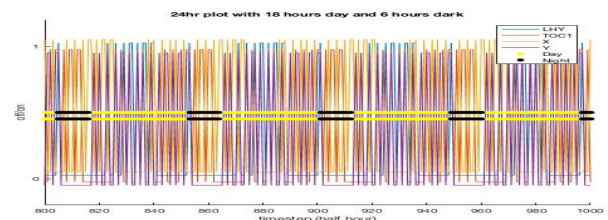


Fig. 5 18 hour day

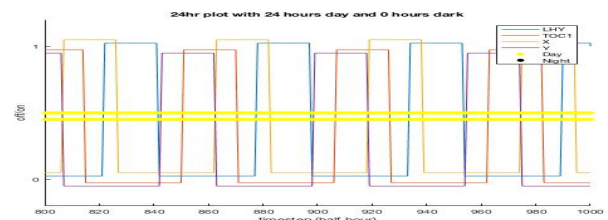


Fig. 6 24 hour day

For photoperiods totalling to 48 hours, we saw some similar behaviors to that of 24hr periods as well as some more unusual things. We noticed that 20 hours was the maximum amount of dark that could occur where genes would be active during the night (Figure 7). Additionally, with a photoperiod of 42 hours light and 6 hours dark, we discovered a situation where the model acted non-periodically, and noticed a peculiar rapid change in behavior around 430 hours (Figure 8).

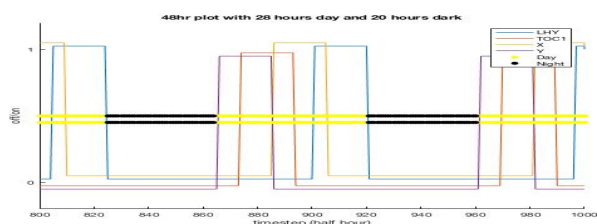


Fig. 7 28hr day, 20 hr night

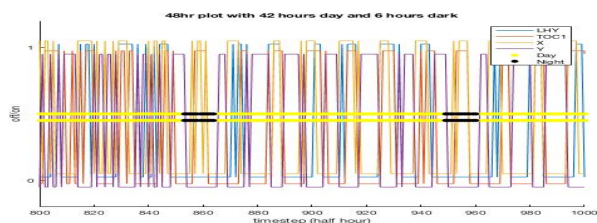


Fig. 8 42hr day, 6 hr night

3.2 Asynchronous Lights

For the purposes of this section we grouped L_1 as a group of lights and L_2 with L_3 as a second group of lights. This is because the second group act on the same gene with roughly the same signal delay. We saw in both cases turning off L_1 resulted in a new periodic behavior (still 24 hr periodic), one without LHY (Figure 9). When we turned off the other group of lights (L_2 and L_3), this resulted in a completely inactive model (Figure 10).

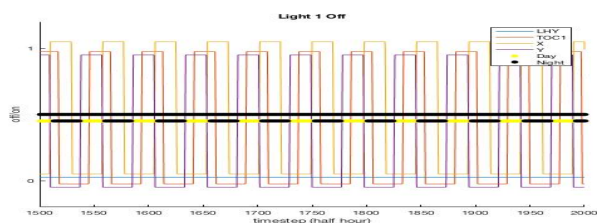


Fig. 9 Never turning on L1

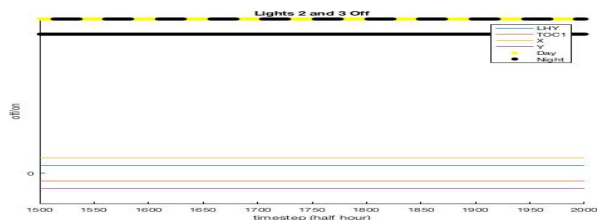


Fig. 10 Never turning on L2 or L3

We then ran these groups of lights concurrently, but with different photo periods. In this case we saw the model still produced periodic behavior, where the period is exactly the least common multiple of the two photoperiods used by the light groups. This claim comes without proper mathematical derivation to show this

is always true; however we experimented with many varied photoperiods and each time the resulting period was the LCM of the light photoperiods.

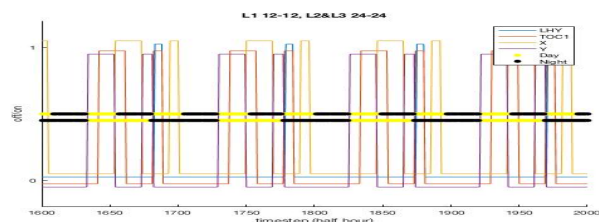


Fig. 11 24hr period + 48hr period = 48hr period

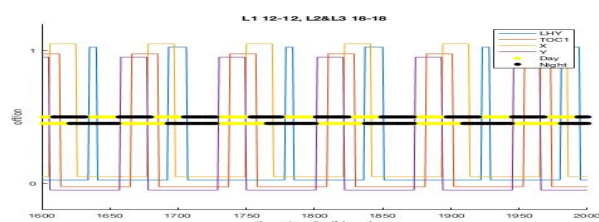


Fig. 12 24hr period + 36hr period = 72hr period

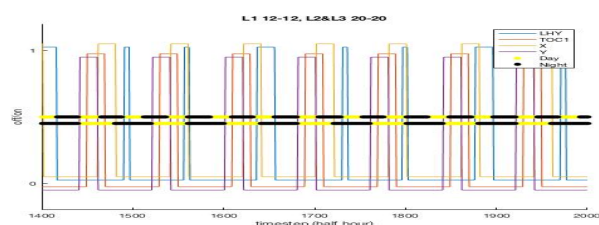


Fig. 13 24hr period + 40hr period = 120hr period

4 Discussion

When thinking about the photoperiod, some of the results were expected, and some of them were not. It is easy to extrapolate that without light, none of the genes would turn on. Additionally, the constant light produced a fairly reasonable solution that matched what could be expected by a biological equivalent. This isn't particularly surprising given the model was generated using data produced with lights entirely on. However, the results from 24hr photoperiods with more than 18 hours of light were unexpected. It is difficult to trace why they might be occurring, and as such it is difficult to understand why the change happens so starkly at 18 hours, when at 17 hours the response is fairly normal.

We noticed that the 28 hours of day which paired with 20 hours of night at which no genes turn on during the night coincided with the 'natural' period of the system when the lights were just left on. It is unclear at this time whether the 28 hours is more significant than the 20 for dictating if genes remain off during the night. The unusual results produced by 42 hours of light and 6 hours of light are as yet not understood. We were unable to discern why this particular combination might result in chaos, while

many similar photoperiods remained periodic.

While the model appeared to have trouble working with non-biological photoperiods, the model held up well in the face of asynchronous light behaviors. In the case of turning off L_1 , we see there still is periodic behavior. This can be expected after seeing that L_1 only excites LHY, an inhibitory gene, while gene Y (the main excitatory gene) still activates. Similarly when we turn off the other light group the model produces no behavior because LHY is only inhibitory. This may suggest that not every light node is necessary to construct a control kernel² for this model.

The model also showed a good ability to regulate sporadic and uneven light inputs into a regulated output. This is behavior we would expect from a circadian system, designed to produce regulated output. The fact that the model produces long period behavior isn't even particularly concerning because it still is pseudo-periodic on a shorter 24hr period.

These results went against our initial expectations for this project. We had assumed the model would've taken varied, synchronous photoperiods better than the asynchronous options (including varied, asynchronous photoperiods), but this turned out to be not the case. The underlying reason for this is still unknown and would require a deeper understanding of Boolean network theory than we currently possess.

5 Conclusions

Ultimately this paper leaves a lot of room for further exploration of circadian Boolean network models. Because the size of the

model made fixing any of the shortcomings we identified unrealistic, the construction of a new more robust model may be in order. Akman also works briefly with a 3-loop *Arabidopsis* model, which may give the model better behavior near the edges of the parameter space. Also, understanding Boolean chaos could help illuminate if the potential chaotic nature of the model means anything for our biological understanding or is simply an artifact of a new modeling method. The success of the model in asynchronous light situations may at first appear simply to be an interesting mathematical exercise; however, there is research to suggest that the light nodes modeled here don't reflect a full spectrum of light and instead reflect certain wavelengths of light³. If this were the case, this model could prove useful in understanding the operation of circadian systems in environments with light conditions different than a full spectrum (sunrise, deep ocean, etc.)

6 Acknowledgements

The authors thank Dmitry Kondrashov for his guidance and advice throughout this project.

References

- 1 O. E. Akman, *Journal of The Royal Society*, 2012.
- 2 J. Kim, *Scientific Reports*, 2013.
- 3 D. E. Somers, *Science*, 1998.