Genetic screens to identify loci affecting phase of entrainment

Summer Internship 2019

Under the Guidance of
Prof. Sheeba Vasu
Chronobiology and Behavioural Neurogenetic Laboratories
JNCASR, Bangalore

CHRONOBIOLOGY
&
BEHAVIOURAL
NEUROGENETICS
LABORATORIES

History behind Circadian Rhythms

- Most living organisms exhibit approximate 24 hour rhythms in behavioural and physiological processes.
- Period of (Circadian rhythms $\approx$ Daily environmental cycles).
- Led to a common conception (or rather misconception) that -

"Daily rhythms are mere passive responses to cyclic environmental changes."

History behind Circadian Rhythms

- Most living organisms exhibit approximate 24 hour rhythms in behavioural and physiological processes.
- Period of (Circadian rhythms $\approx$ Daily environmental cycles).
- Led to a common conception (or rather misconception) that -

"Daily rhythms are mere passive responses to cyclic environmental changes."

History behind Circadian Rhythms

- Most living organisms exhibit approximate 24 hour rhythms in behavioural and physiological processes.
- Period of (Circadian rhythms $\approx$ Daily environmental cycles).
- Led to a common conception (or rather misconception) that -

"Daily rhythms are mere passive responses to cyclic environmental changes."

Establishing that Circadian Rhythms are Innate

Journey from Daily rhythms to Biological Clocks

- Jean Jacques d'Ortous de Mairan (1729)
- Duhamel (1758)
- Erwin Bünning (1920s)
- Gustav Kramer (1950)
- Ronald J Konopka and Seymour Benzer (1971)
- Michael W. Young, Michael Rosbash, Jeffrey C. Hall (Noble Prize 2017)

Establishing that Circadian Rhythms are Innate

Journey from Daily rhythms to Biological Clocks

- Jean Jacques d'Ortous de Mairan (1729)
- Duhamel (1758)
- Erwin Bünning (1920s)
- Gustav Kramer (1950)
- Ronald J Konopka and Seymour Benzer (1971)
- Michael W. Young, Michael Rosbash, Jeffrey C. Hall (Noble Prize 2017)

Establishing that Circadian Rhythms are Innate

Journey from Daily rhythms to Biological Clocks

- Jean Jacques d'Ortous de Mairan (1729)
- Duhamel (1758)
- Erwin Bünning (1920s)
- Gustav Kramer (1950)
- Ronald J Konopka and Seymour Benzer (1971)
- Michael W. Young, Michael Rosbash, Jeffrey C. Hall (Noble Prize 2017)

Establishing that Circadian Rhythms are Innate

Journey from Daily rhythms to Biological Clocks

- Jean Jacques d'Ortous de Mairan (1729)
- Duhamel (1758)
- Erwin Bünning (1920s)
- Gustav Kramer (1950)
- Ronald J Konopka and Seymour Benzer (1971)
- Michael W. Young, Michael Rosbash, Jeffrey C. Hall (Noble Prize 2017)

Establishing that Circadian Rhythms are Innate

Journey from Daily rhythms to Biological Clocks

- Jean Jacques d'Ortous de Mairan (1729)
- Duhamel (1758)
- Erwin Bünning (1920s)
- Gustav Kramer (1950)
- Ronald J Konopka and Seymour Benzer (1971)
- Michael W. Young, Michael Rosbash, Jeffrey C. Hall (Noble Prize 2017)

Establishing that Circadian Rhythms are Innate

Journey from Daily rhythms to Biological Clocks

- Jean Jacques d'Ortous de Mairan (1729)
- Duhamel (1758)
- Erwin Bünning (1920s)
- Gustav Kramer (1950)
- Ronald J Konopka and Seymour Benzer (1971)
- Michael W. Young, Michael Rosbash, Jeffrey C. Hall (Noble Prize 2017)

Establishing that Circadian Rhythms are Innate

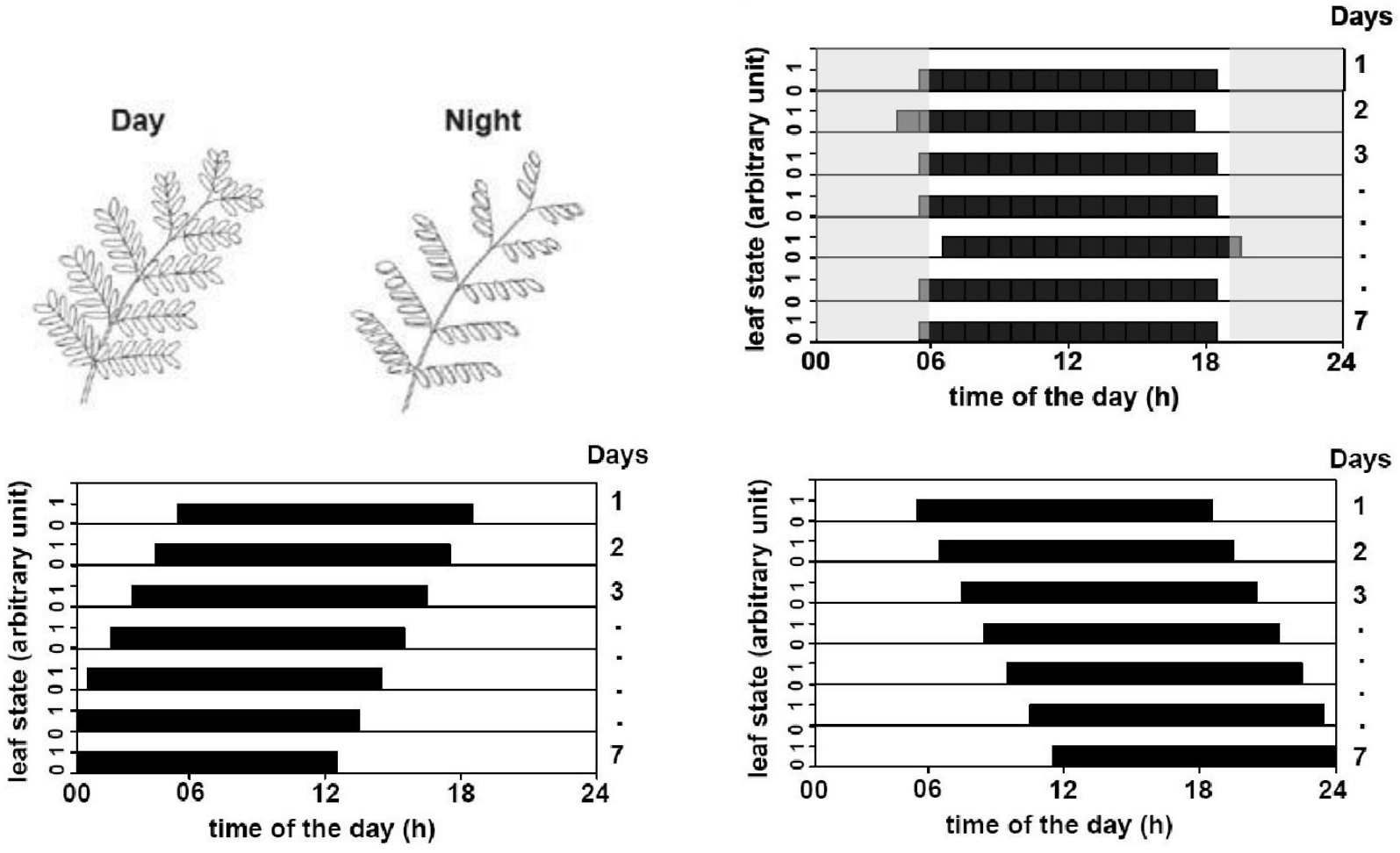


Figure 1: The first picture depicts the opening and closing of leaves in *Mimosa*. Open leaves are assigned the value of 1, while the closed leaves are assigned the values of 0. Graphs on the bottom are actograms taken under constant light (LL) conditions.

Properties of Circadian Rhythms

- Rhythms are innate, with a periodicity of $\approx$ 24 hours
- Temperature Compensated
- Entrainable to external environmental cues (Zeitgebers) i.e. maintain stable, reproducible Phase relationship with the Zeitgeber

Entrain (en·train) *verb*
(of a rhythm or something which varies rhythmically) cause (another) gradually to fall into synchrony with it.

Properties of Circadian Rhythms

- Rhythms are innate, with a periodicity of $\approx$ 24 hours
- Temperature Compensated
- Entrainable to external environmental cues (Zeitgebers) i.e. maintain stable, reproducible Phase relationship with the Zeitgeber

Entrain (en·train) *verb*
(of a rhythm or something which varies rhythmically) cause (another) gradually to fall into synchrony with it.

Properties of Circadian Rhythms

- Rhythms are innate, with a periodicity of $\approx$ 24 hours
- Temperature Compensated
- Entrainable to external environmental cues (Zeitgebers) i.e. maintain stable, reproducible Phase relationship with the Zeitgeber

Entrain (en·train) *verb*
(of a rhythm or something which varies rhythmically) cause (another) gradually to fall into synchrony with it.

Properties of Circadian Rhythms

- Rhythms are innate, with a periodicity of $\approx$ 24 hours
- Temperature Compensated
- Entrainable to external environmental cues (Zeitgebers) i.e. maintain stable, reproducible Phase relationship with the Zeitgeber

Entrain (en·train) *verb*
(of a rhythm or something which varies rhythmically) cause (another) gradually to fall into synchrony with it.

Aim

To find loci in the fly genome which are responsible for maintaining phase of entrainment in *Drosophila melanogaster*.

Approach

- In order to achieve precise gene identification, we have used multiple deletion lines.
- Deletions or deficiencies are alterations in the chromosomes of an organism i.e. parts of their genome are absent or deleted.
- Study overt rhythms such as Eclosion and Activity-Rest rhythms exhibited by these deletion lines.

Approach

- In order to achieve precise gene identification, we have used multiple deletion lines.
- Deletions or deficiencies are alterations in the chromosomes of an organism i.e. parts of their genome are absent or deleted.
- Study overt rhythms such as Eclosion and Activity-Rest rhythms exhibited by these deletion lines.

Approach

- In order to achieve precise gene identification, we have used multiple deletion lines.
- Deletions or deficiencies are alterations in the chromosomes of an organism i.e. parts of their genome are absent or deleted.
- Study overt rhythms such as Eclosion and Activity-Rest rhythms exhibited by these deletion lines.

DrosDel deficiency lines

Characteristic features of the *DrosDel* deletion lines are:

- Molecularly mapped deletions on an Isogenic background
- *DrosDel* deficiency lines comprise of both wide and narrow deletions
- Deletions with large average size have been chosen initially
- The wider deletions cover $\sim 75\%$ of the *D. melanogaster* genome
- Deletions on different chromosomes are maintained on different chromosome specific balancers such as FM7h, SM6a, CyO and TM6C.

Overt Rhythms

Studying multiple overt rhythms, allows us to identify circadian behaviour exhibited as a result of different genes and understand circadian organization at different scales.

Eclosion

- It is the emergence of adult flies from their pupal cases.
- Eclosion is a heavily gated behaviour and usually peaks at dawn.

Activity-Rest rhythms

- It is basically the measure of locomotor activity in *Drosophila melanogaster*.

Deletion line maintenance

Deletion lines were maintained in

- LD 12:12 cycle
- Luminous intensity of $10.25 \mu W/(cm^2 \cdot s)$
- Constant Temperature of $25 \pm 1°C$
- Constant Humidity of $70 \pm 5\%$

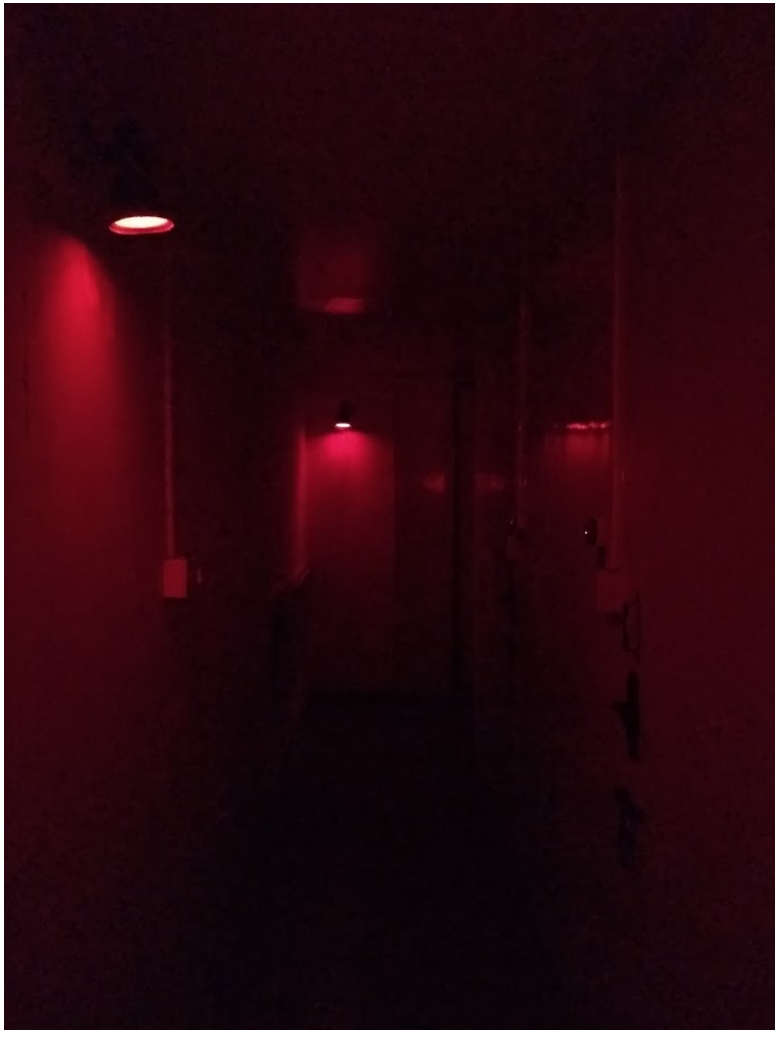


Figure 2: Dark cubicle in the Chronobiology Lab, JNCASR

Egg Collection and Blow up

- Flies are transferred to Plexiglas cages (13 $cm \times$ 16 $cm \times$ 21 cm).
- Eggs are collected from the cages by placing charcoal-corn-agar food plates covered with a generous dollop of yeast is placed.
- Eggs are collected after two days
- Lines for blowing up are provided food changes every 2-3 days after placing them inside vials.

Eclosion Assay

- About 100 eggs per vial and about 10 replicate vials for each deletion line is set up for the Eclosion assay.
- Assay conditions - LD 12:12, constant temperature (25±1°C), constant humidity (70±5%)
- Assay duration - 2 to 3 days
- Number of eclosed flies were counted every 2 hours :)

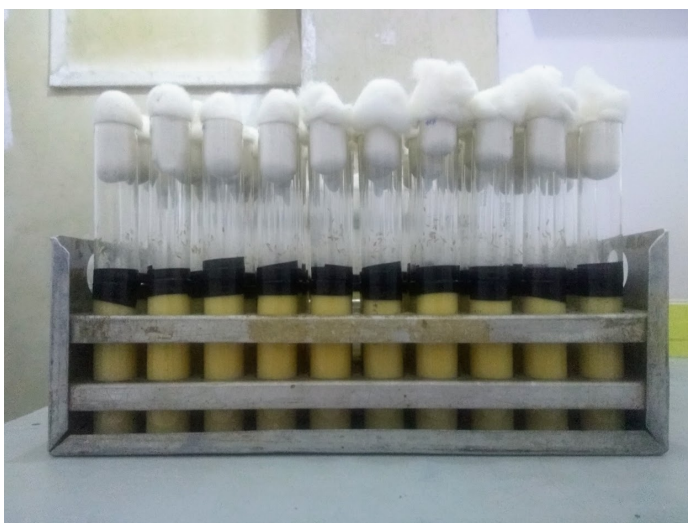


Figure 3: Eclosion vials - flies in developmental stages

Figure 4: Eclosion Experiment set up

Eclosion Assay

- About 100 eggs per vial and about 10 replicate vials for each deletion line is set up for the Eclosion assay.
- Assay conditions - LD 12:12, constant temperature (25±1°C), constant humidity (70±5%)
- Assay duration - 2 to 3 days
- Number of eclosed flies were counted every 2 hours :)

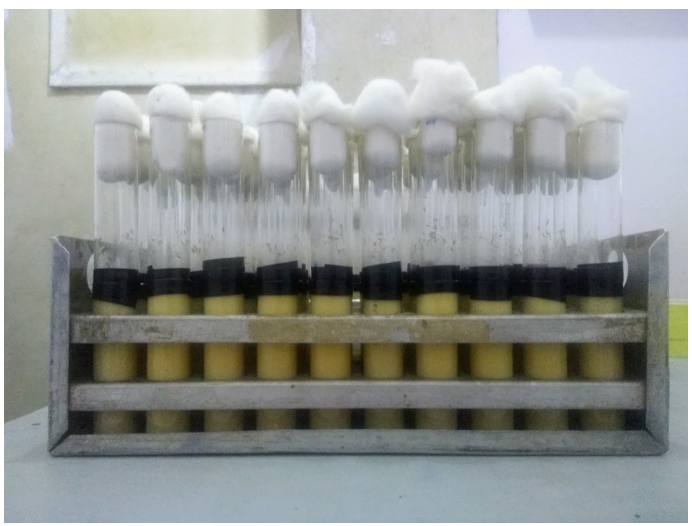


Figure 3: Eclosion vials - flies in developmental stages

Figure 4: Eclosion Experiment set up

Eclosion Assay

- About 100 eggs per vial and about 10 replicate vials for each deletion line is set up for the Eclosion assay.
- Assay conditions - LD 12:12, constant temperature (25±1°C), constant humidity (70±5%)
- Assay duration - 2 to 3 days
- Number of eclosed flies were counted every 2 hours :)

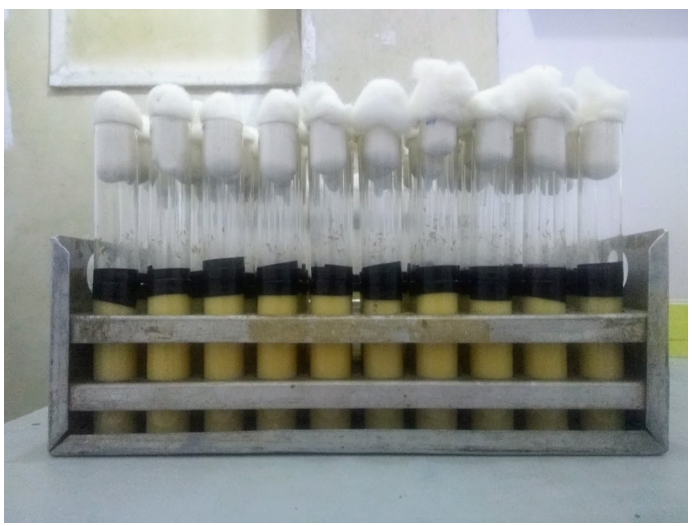


Figure 3: Eclosion vials - flies in developmental stages

Figure 4: Eclosion Experiment set up

Eclosion Assay

- About 100 eggs per vial and about 10 replicate vials for each deletion line is set up for the Eclosion assay.
- Assay conditions - LD 12:12, constant temperature ($25 \pm 1^{\circ}C$), constant humidity ($70 \pm 5\%$)
- Assay duration - 2 to 3 days
- Number of eclosed flies were counted every 2 hours :)

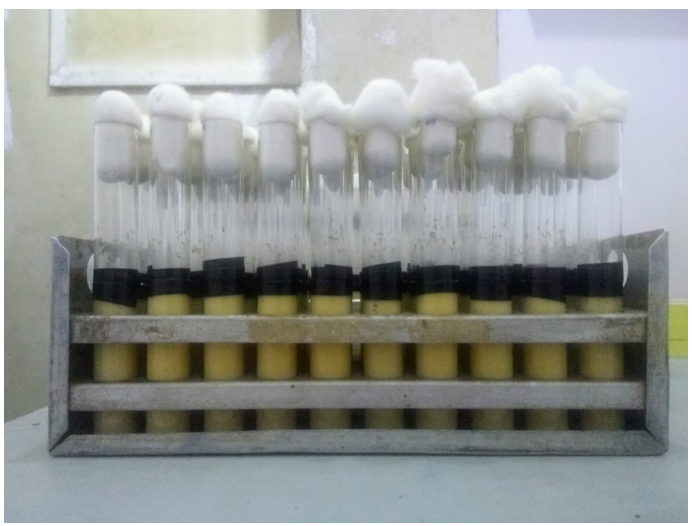


Figure 3: Eclosion vials - flies in developmental stages

Figure 4: Eclosion Experiment set up

Activity Rest Assay

- Flies needed for this assay were taken from the blown up stock.
- Flies were sexed i.e. males and females were segregated and virgin males were used for this assay.
- Assay conditions - constant temperature (25±1°C), constant humidity (70±5%)
- Assay duration - 6 days LD 12:12 and 4 days DD (total darkness)

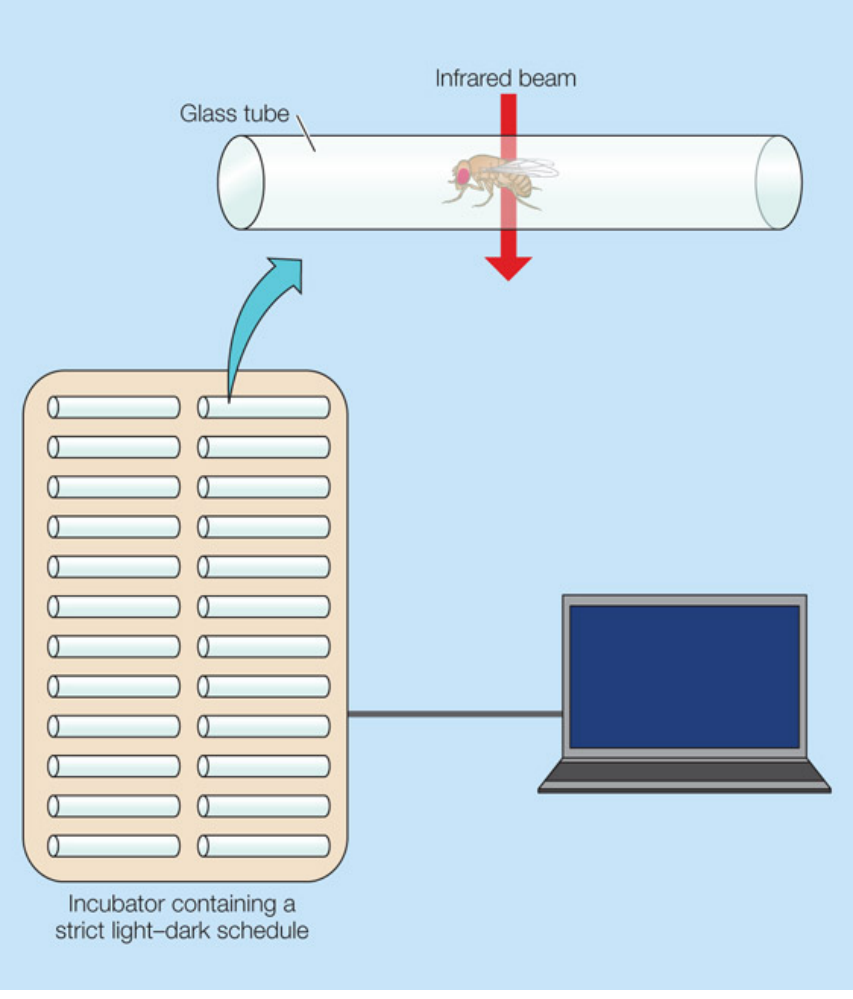


Figure 5: Activity-Rest assay set-up

Activity Rest Assay

- Flies needed for this assay were taken from the blown up stock.
- Flies were sexed i.e. males and females were segregated and virgin males were used for this assay.
- Assay conditions - constant temperature (25±1°C), constant humidity (70±5%)
- Assay duration - 6 days LD 12:12 and 4 days DD (total darkness)

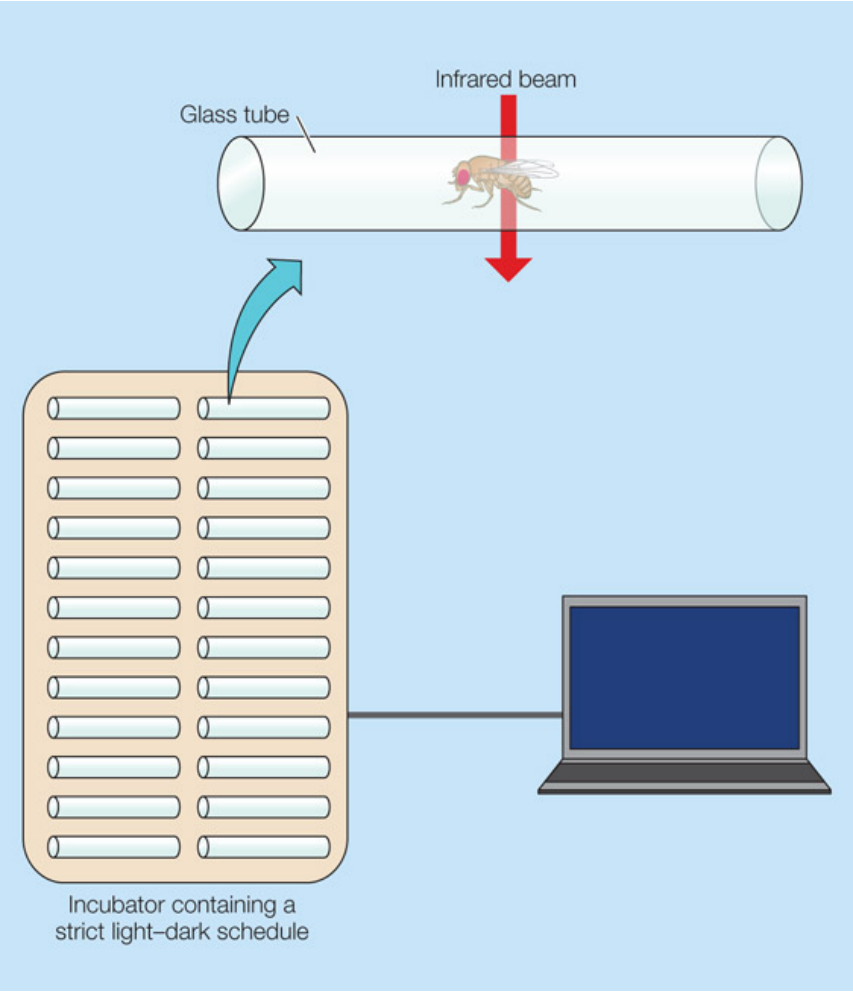


Figure 5: Activity-Rest assay set-up

Activity Rest Assay

- Flies needed for this assay were taken from the blown up stock.
- Flies were sexed i.e. males and females were segregated and virgin males were used for this assay.
- Assay conditions - constant temperature (25±1°C), constant humidity (70±5%)
- Assay duration - 6 days LD 12:12 and 4 days DD (total darkness)

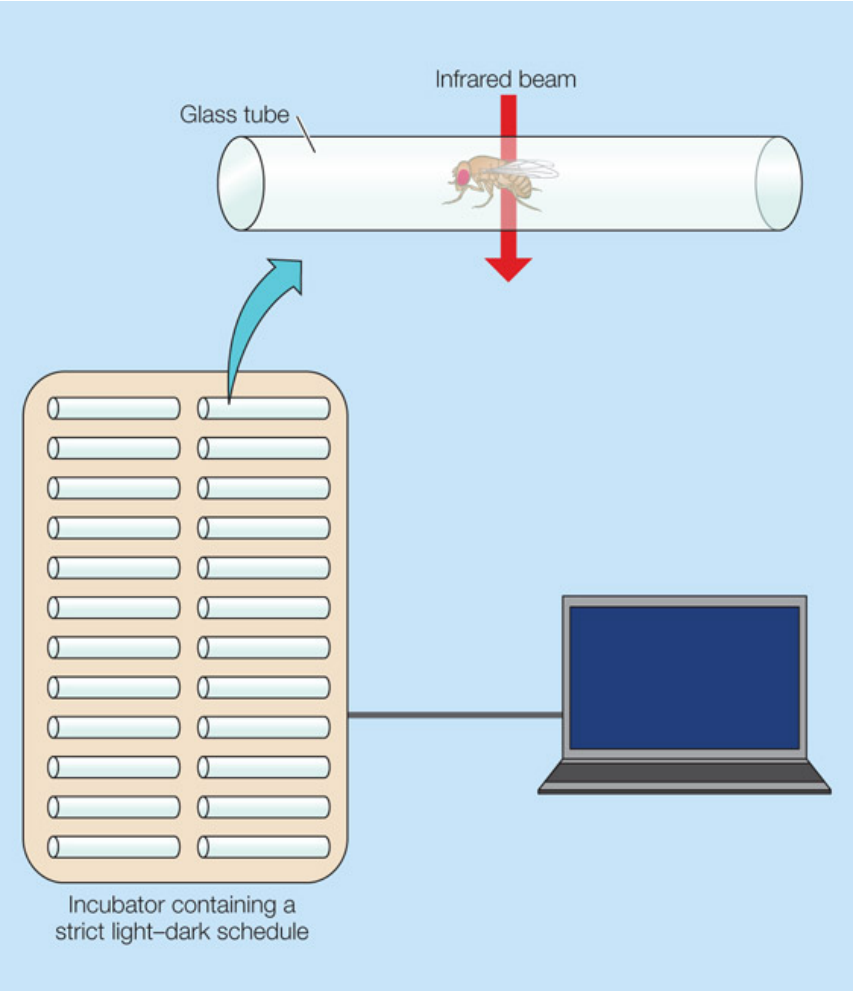


Figure 5: Activity-Rest assay set-up

Activity Rest Assay

- Flies needed for this assay were taken from the blown up stock.
- Flies were sexed i.e. males and females were segregated and virgin males were used for this assay.
- Assay conditions - constant temperature ($25 \pm 1°C$), constant humidity ($70 \pm 5\%$)
- Assay duration - 6 days LD 12:12 and 4 days DD (total darkness)

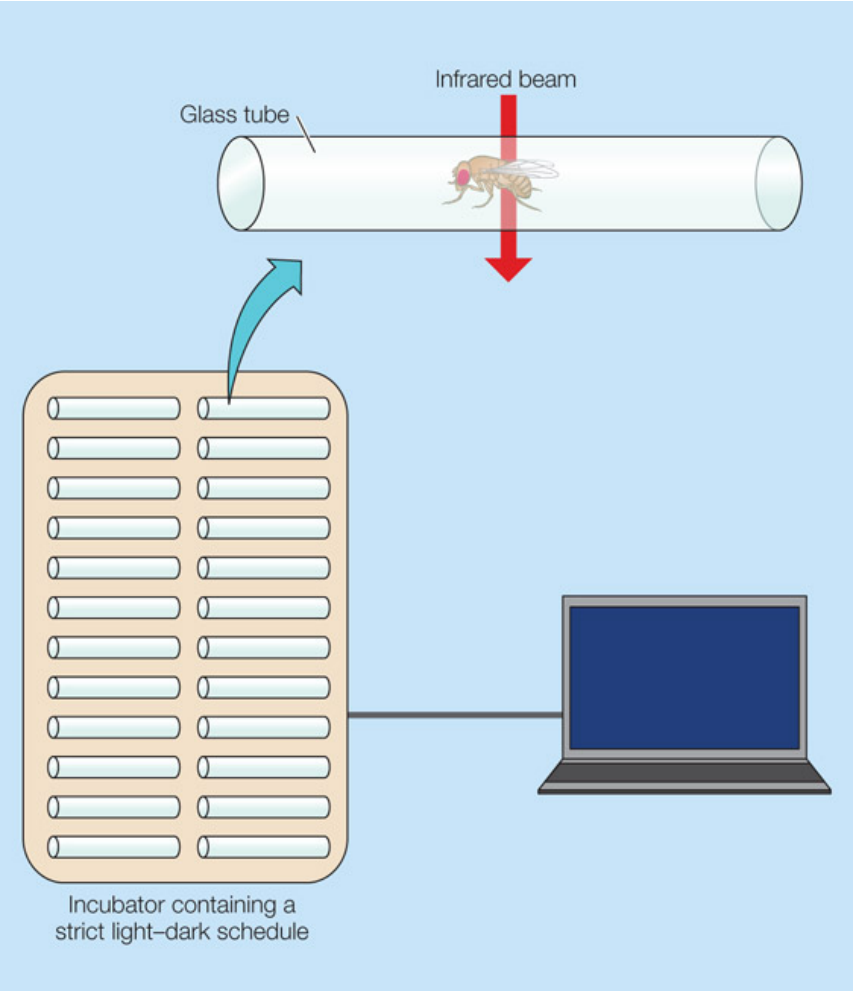


Figure 5: Activity-Rest assay set-up

Average emergence - 1

Note early emergence in line 4 and emergence before lights on in line 6.

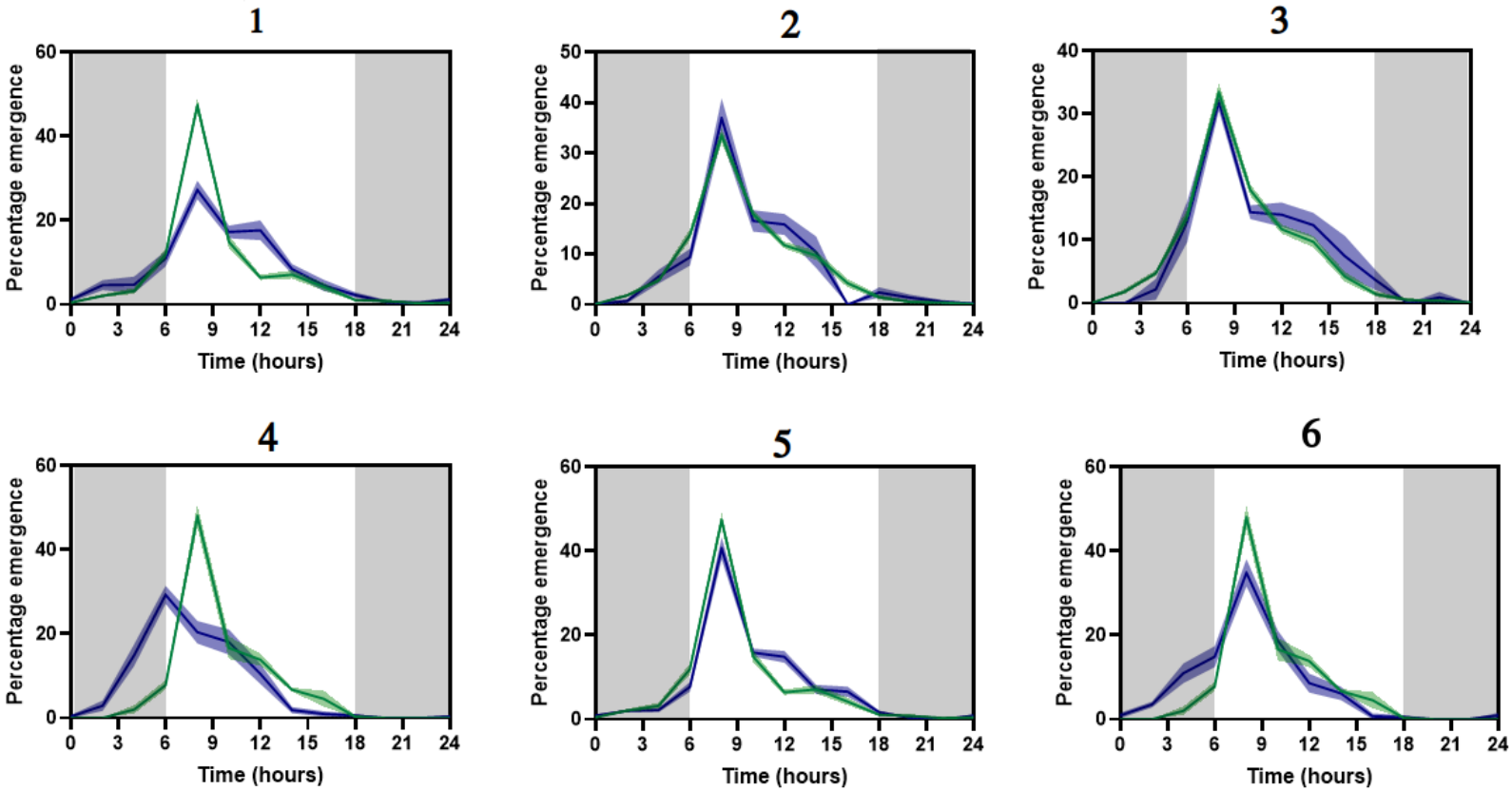
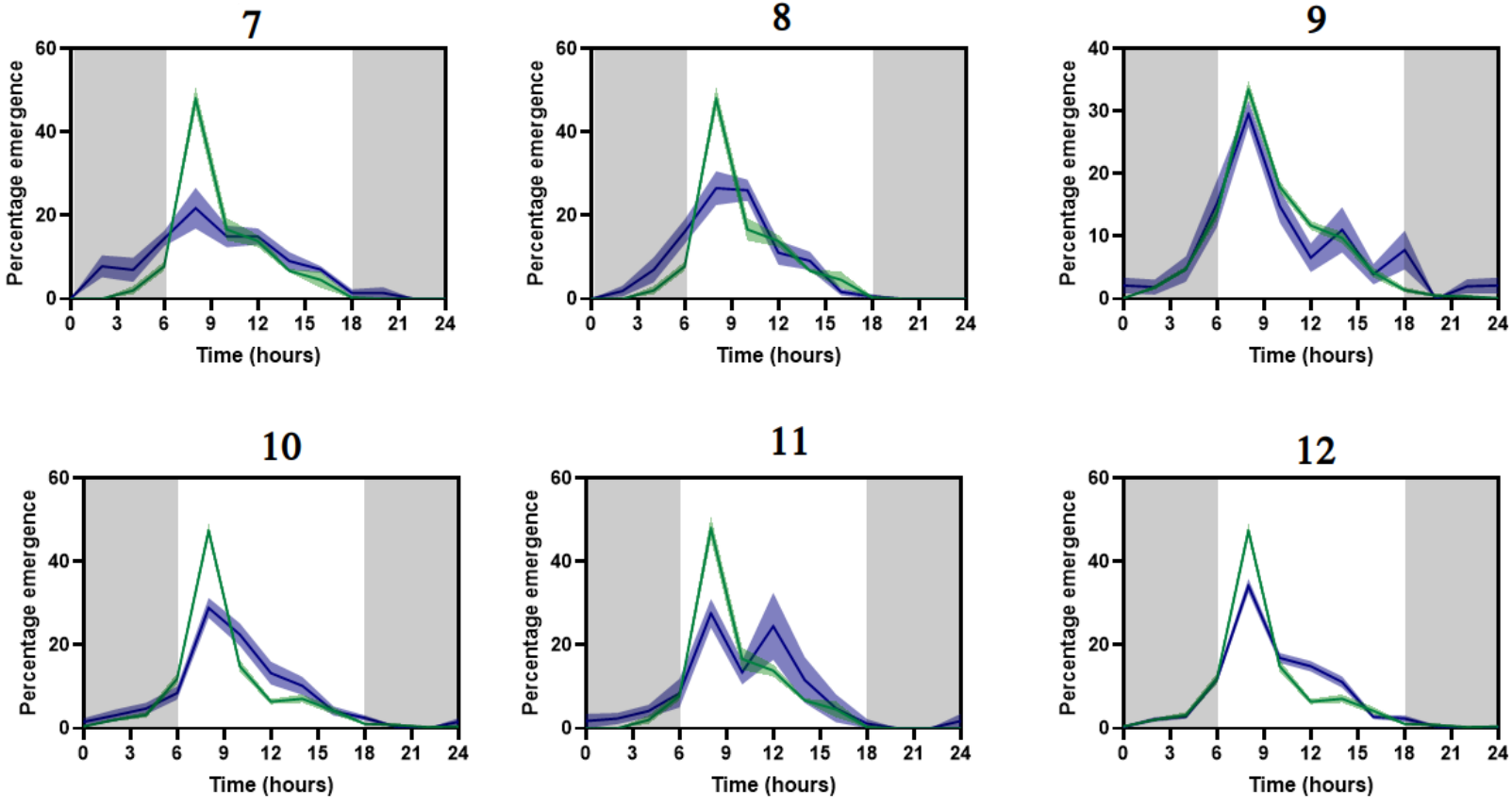


Figure 6-8: Blue solid lines represent the average emergence profile of the deletion lines. Green solid lines represent the average emergence profile of controls (line 534). Time 0 represents 4 AM.

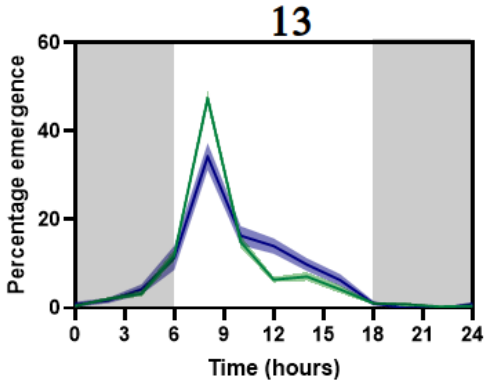
Average emergence - 2

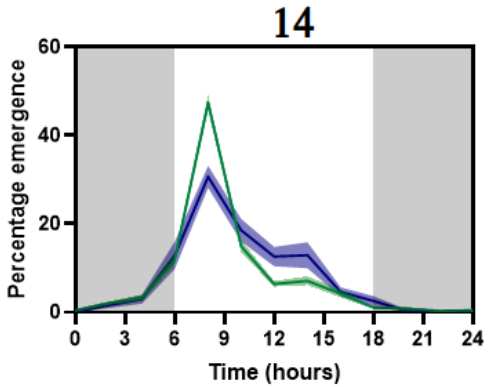
Note the late emergence in line 11

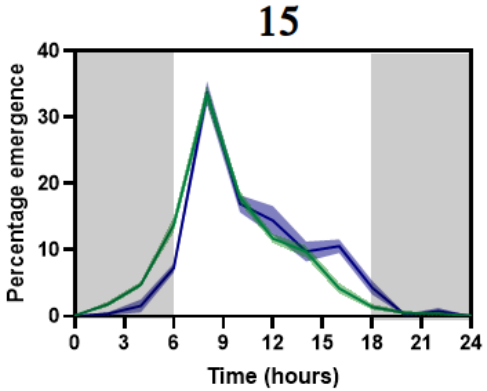


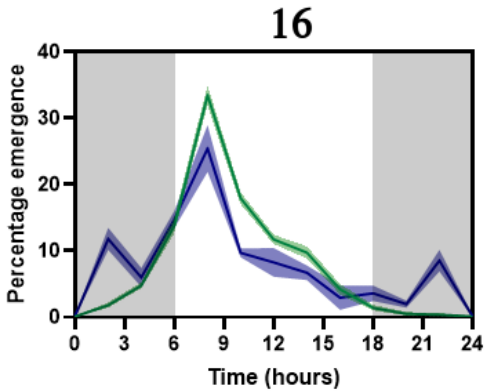
Average emergence - 3

Note the night/lights-off emergence in line 16









Polar plot - 1

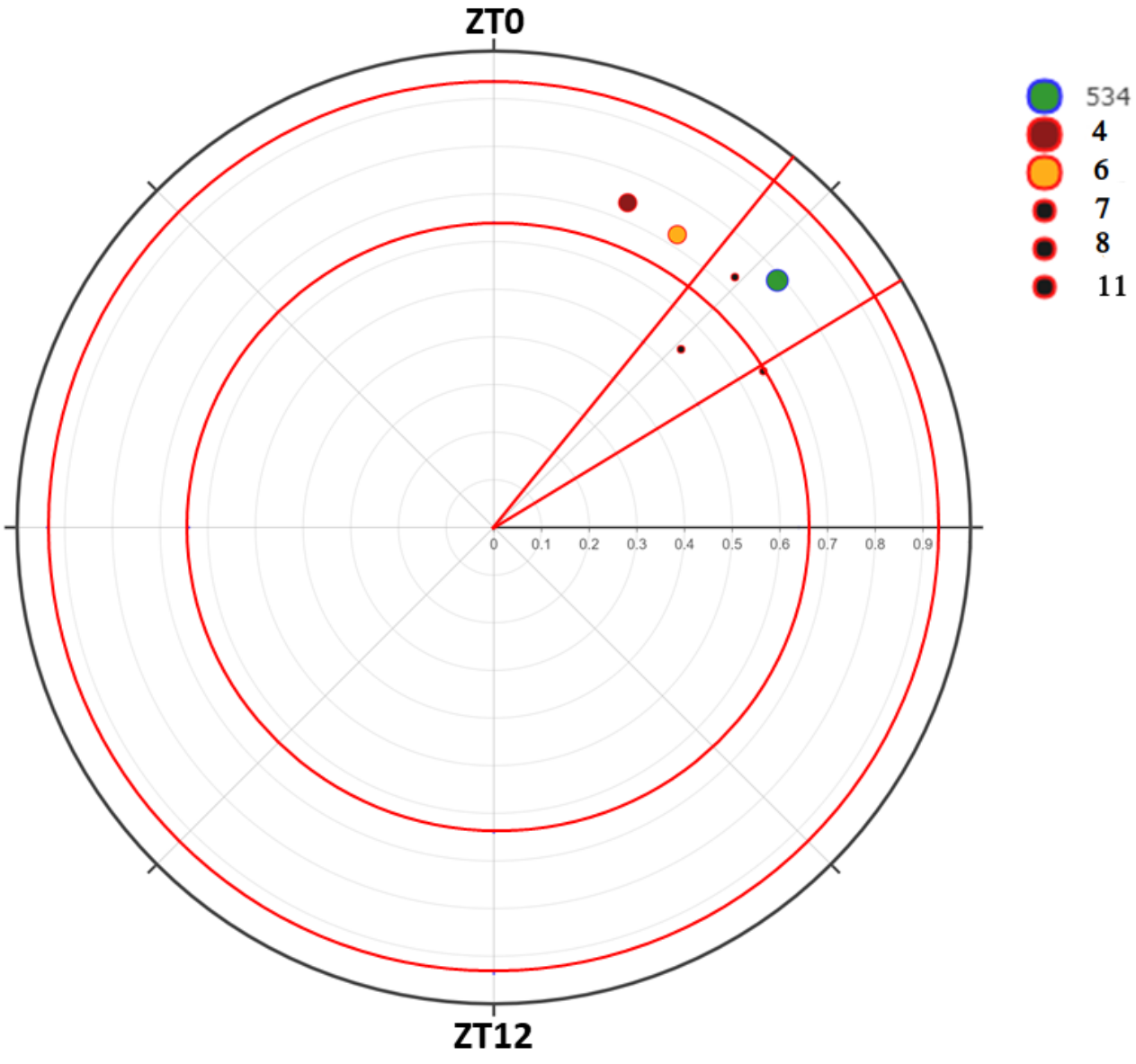
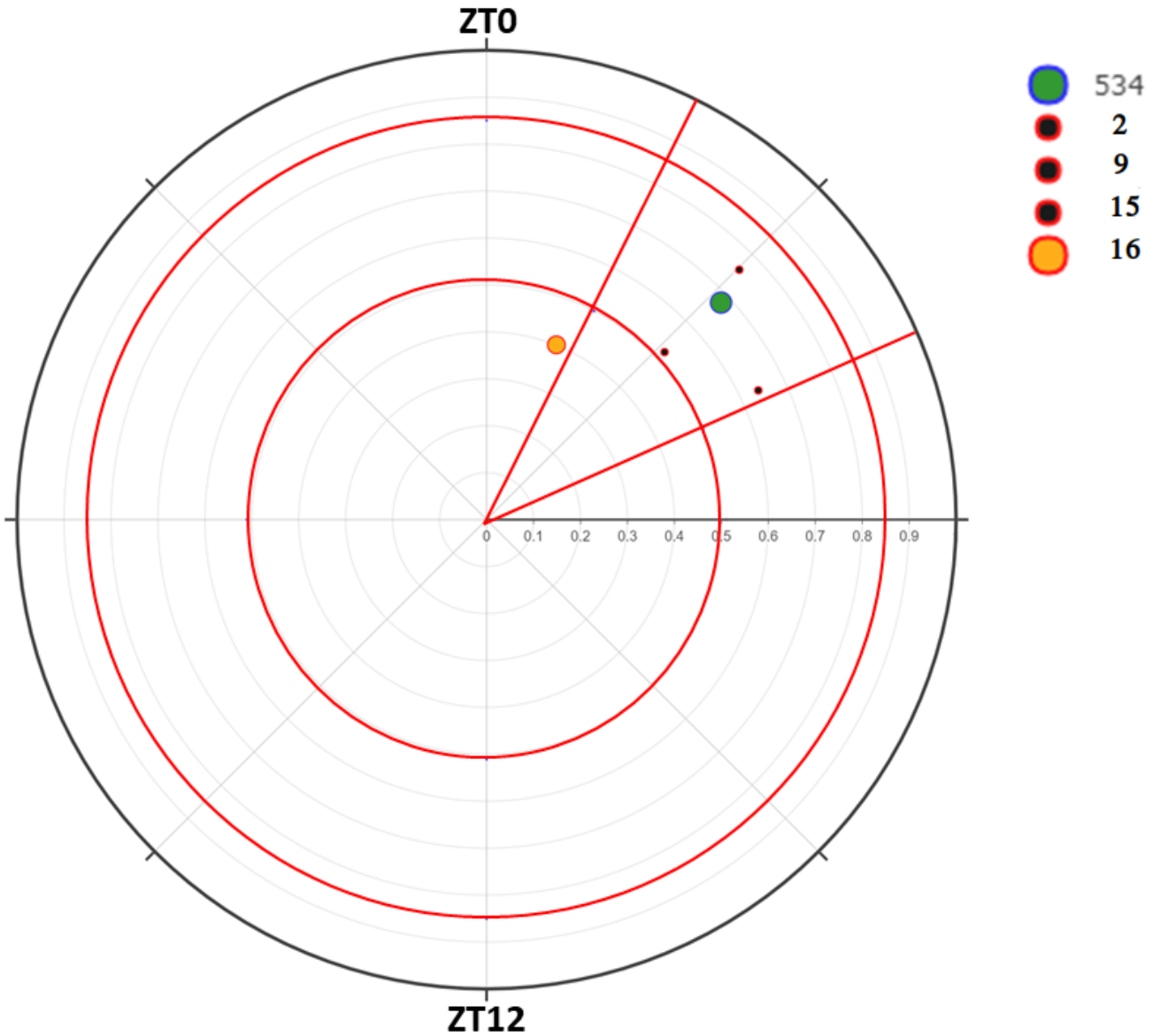
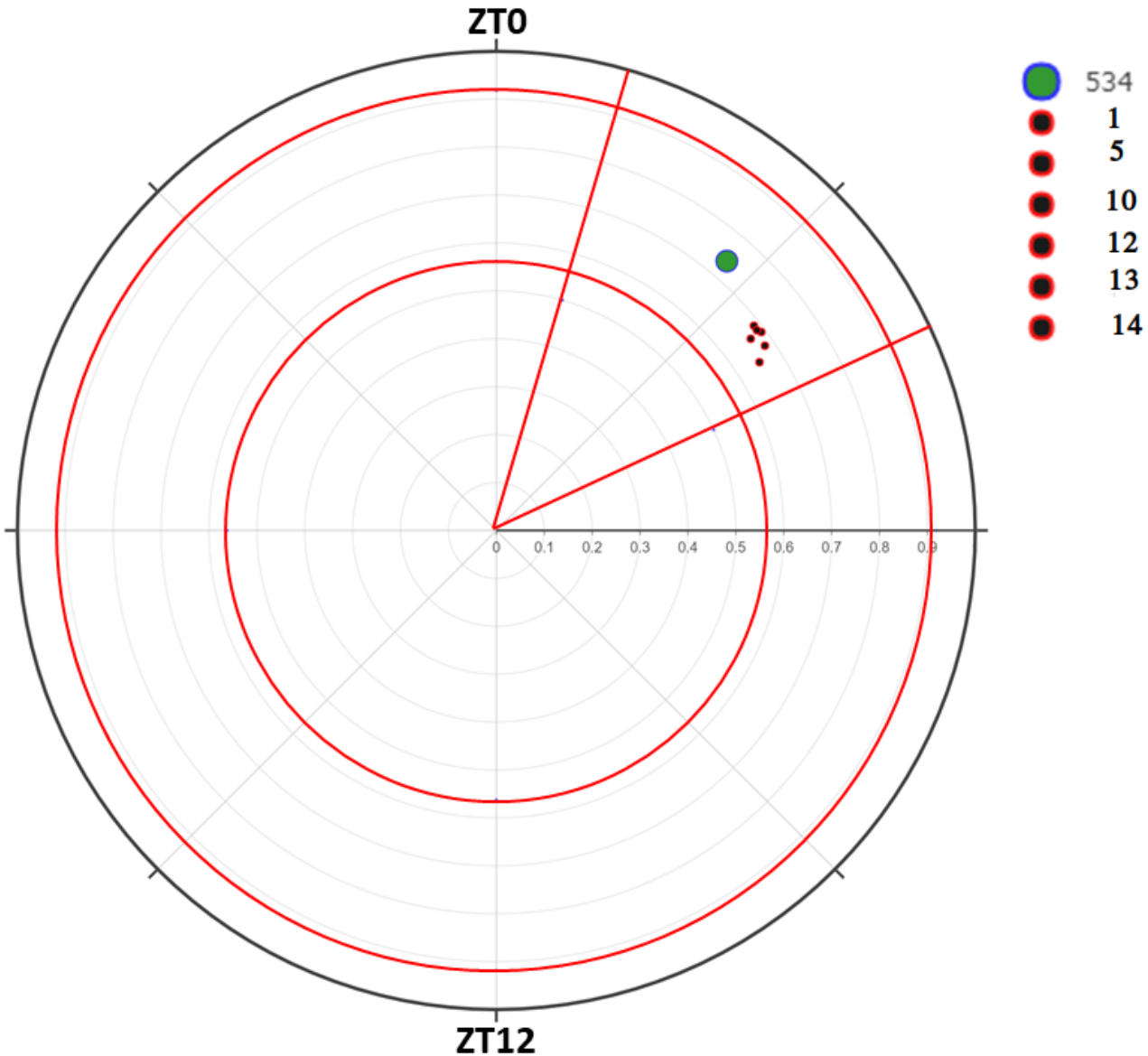


Figure 9-11: Mean phase and consolidation of emergence of the assayed deletion lines.

Polar plot - 2



Polar plot - 3



Eclosion assay - Hits

Deletion lines hits

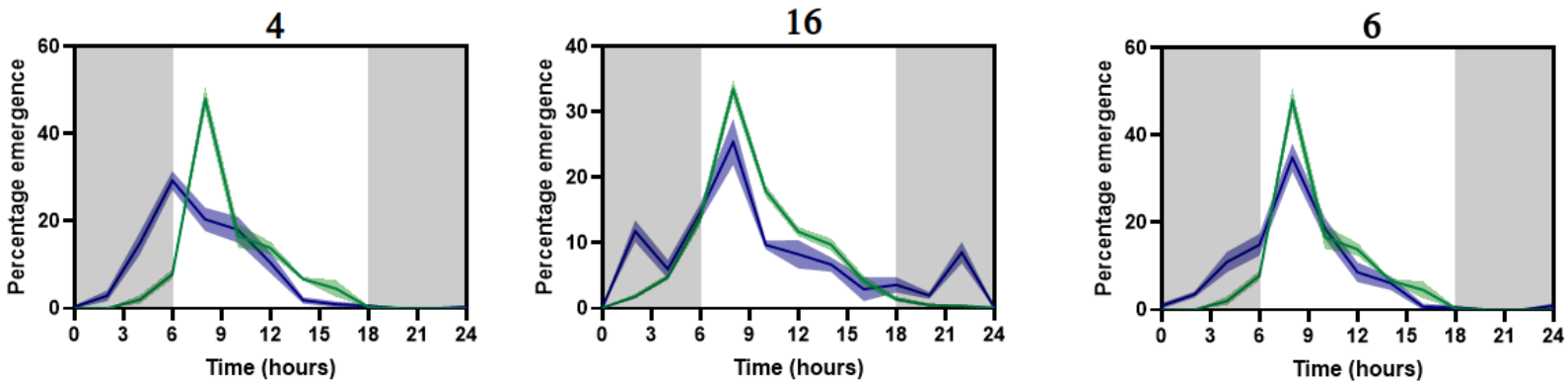


Figure 12: Average emergence profile of the three hits that we have gotten so far.

Actogram Data

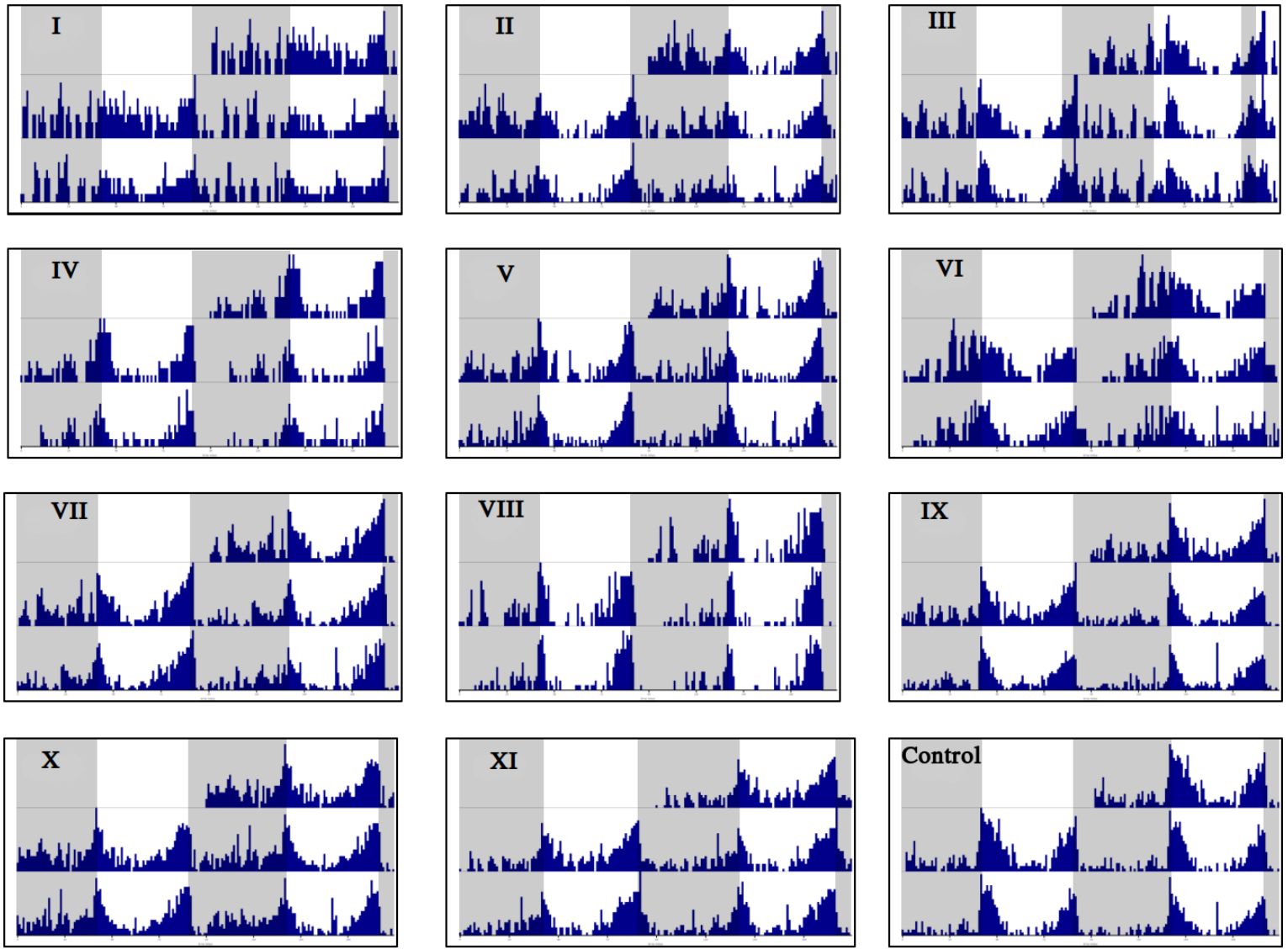


Figure 13: Actogram data

Work Advances and Applicability

Once all the wider deletions have been screened, we would start screening the narrower deletions and hopefully, we can identify genes that are essential for maintaining the same phase relation as their background.

This study can provide us with an insight into the molecular mechanisms underlying differential phase preference among individuals. Thereby, bringing us a step closer to understanding several of the prevalent sleep phase syndromes.

Thank You!


12
1
2
3
4
5
6
7
8
9
10
11
ES SCHLIESSEN SICH AM NACHMITTAG
ES OEFFNEN SICH AM VORMITTAG