Genetic basis of circadian activity in the fly *Drosophila suzukii*

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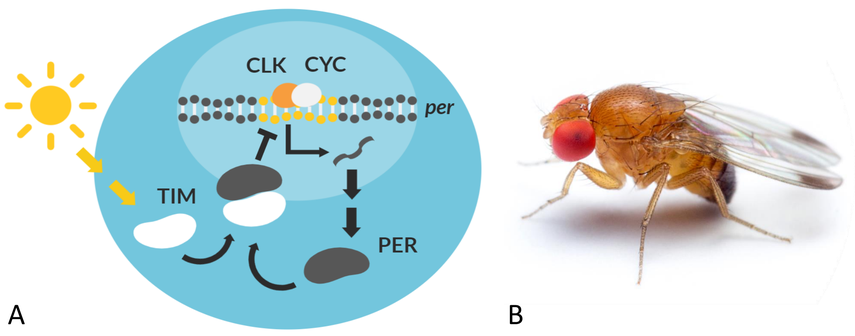
Introduction

- The fly *Drosophila suzukii* is an invasive pest that lays its eggs inside ripening fruits (Fig. 1A). As it shows strong seasonal phenotypes[1] and is closely related to the model *D. melanogaster*, it is an ideal species to investigate how the circadian clock interprets photoperiodic information to prepare for seasonal changes.
- The circadian clock is an internal system that adapts the behaviour of organisms to daily light changes. The oscillation in the levels of *period (per)* and *timeless (tim)* gene products are essential for the clock (Fig. 1B).[2]



A B

Fig. 1. A: Male *D. suzukii*. B. The fly master clock. *per* and *tim* regulate their own expression in response to light via a negative feedback loop with transcription factors (*cyc, clk*), resulting in daily clock protein oscillation.

1. We aimed to **disrupt the *tim* gene** and insert a visual marker using the CRISPR-Cas9 system to study the *D. suzukii* circadian clock. Specifically, this project focuses on the **generation of a donor plasmid** to be used for homology directed repair-mediated transgenesis (Fig. 2).

2. We also aimed to investigate the circadian behaviour of *D. suzukii* via **locomotor experiments**.

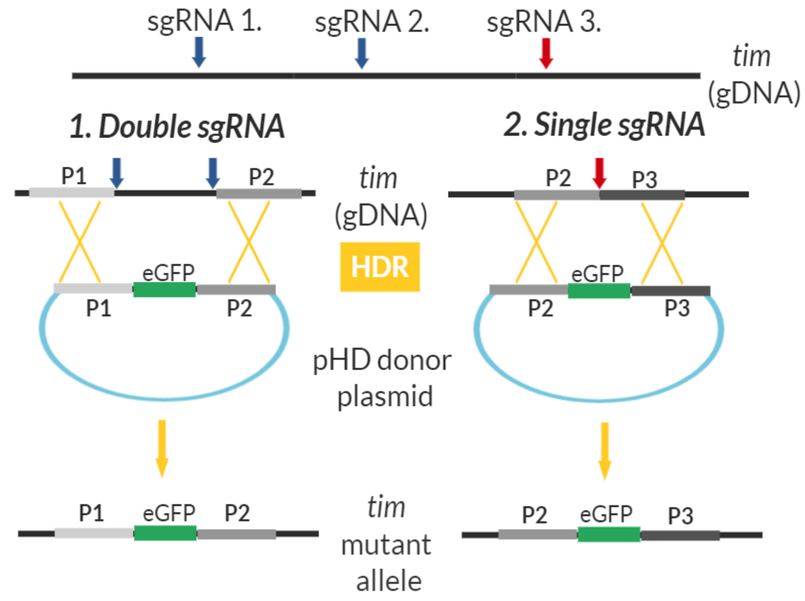


Fig. 2. Plan of the two approaches (single and double sgRNA) used to mutate the *tim* gene. Both employ two homology arms in the pHD donor vector to cause insertion of the marker gene (eGFP or RFP) into *tim* through recombination (HDR).

1. Methods

Donor plasmid generation[3]

1 Amplification of *tim* regions

D. Suzukii genomic DNA extraction

Amplification by PCR

3 ~1.5kb *tim* regions were PCR-amplified. Products were verified by gel electrophoresis and extracted from the gel.

2 Cloning into the pJET plasmid

Ligation into pJET

Transformation

Vectors were transformed into bacteria, plated, and incubated. Liquid cultures were set and plasmids were purified.

Identification of correct plasmids

Digestion with BglII. Sequencing.

3 Cloning P1 into donor plasmid

Amplification of homology arms

PCR to introduce AarI/SapI sites at the ends of the *tim* regions. Gel electrophoresis, extraction.

Digestion with SapI

Both P1 and plasmid digested.

Ligation into pHD-RFP/eGFP-attP plasmid

Transformation

Identification of correct plasmids

Digestion with BamHI. Sequencing.

4 Future steps

Cloning of P2 and P3 into the donor plasmid

The same step will be followed.

CRISPR transgenesis

By injection of the donor plasmid, Cas9 and sgRNA into *D. suzukii* embryos.

Identification of *tim* mutants

By RFP/eGFP marker driving in the eyes.

Results

The P1 homology arm was cloned into the donor plasmid (Fig. 3).

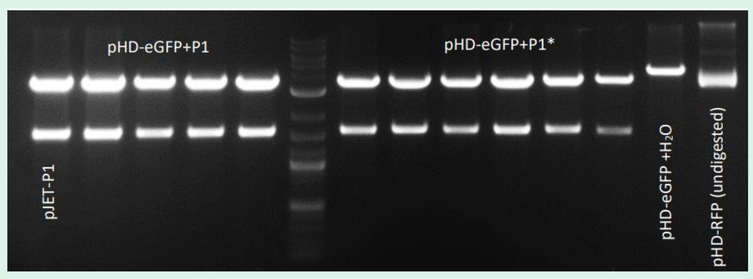


Fig. 3. Gel electrophoresis result of BamHI-digestion of the pHD-eGFP-P1-attP samples with controls. BamHI produced two bands (1.5kb, 3.5kb) if P1 was inserted successfully.

2. Methods

The activity of *D. suzukii* and *D. melanogaster* individual flies and pairs was recorded using activity monitors in constant darkness (DD) for ~10 days after entrainment to 12-12h light and darkness cycles (LD) (Fig. 4).

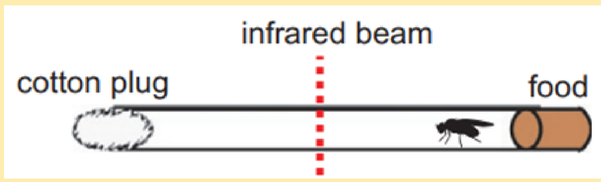


Fig. 4. An individual tube of the activity monitor used to locomotor assays

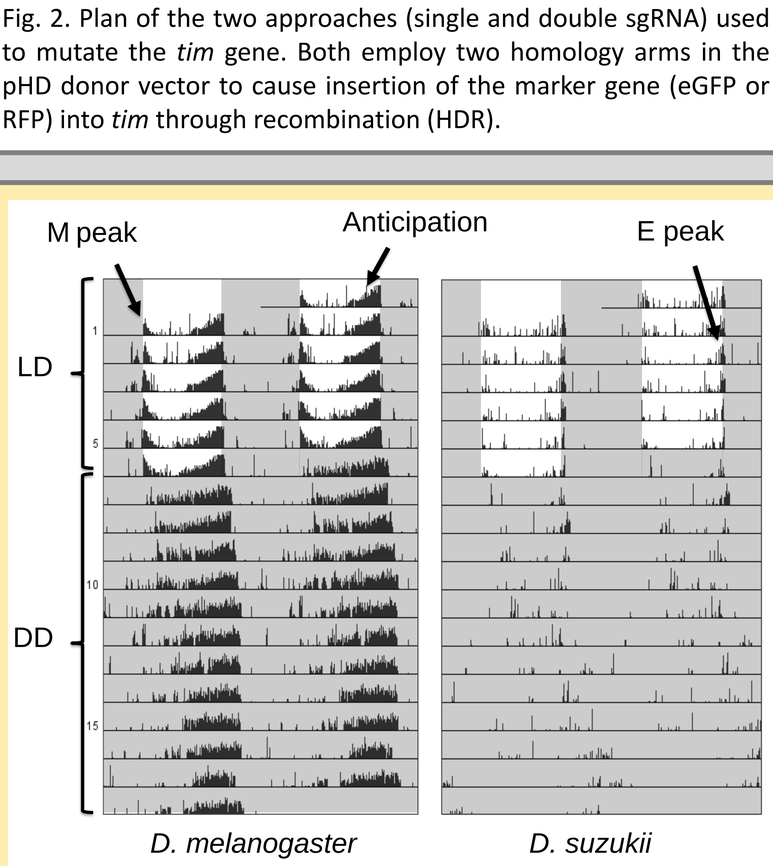


Fig. 5. Double plotted actograms representing the activity of a single rhythmic fly. (x: time; y: activity (crossings/min).

Results

Single fly assays:

D. suzukii flies were highly inactive compared to *D. melanogaster* (Fig. 5). As expected, *D. melanogaster* flies displayed crepuscular activity pattern – they were most active in the morning and the evening (M + E peaks). Most *D. suzukii* flies also displayed M and E peaks, but these were less pronounced. All *D. melanogaster* flies retained their rhythmicity under DD, while only 17% of *D. suzukii* flies became arrhythmic.

Pairwise assays:

Similar patterns, but *D. melanogaster* mixed sex pairs were active during the night as well as the day (Fig. 6). No such changed activity was observed in *D. suzukii*.

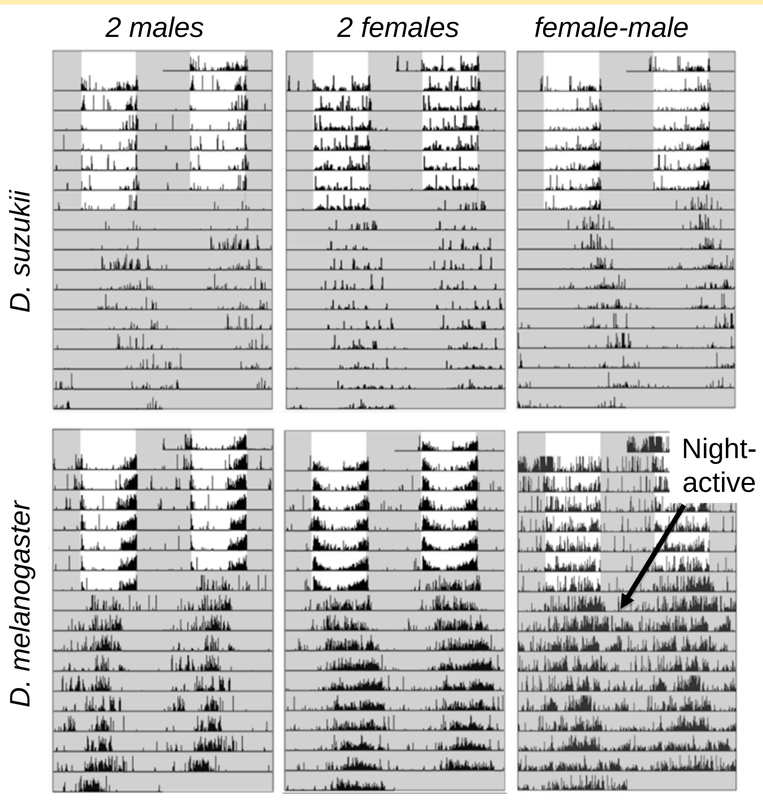


Fig. 6. Double plotted actograms representing a single pair.

Conclusions

1. **Cloning of the P1 homology** arm into the donor vector was achieved.

2.

- *D. suzukii* appears to interpret photoperiodic information less precisely than *D. melanogaster*.
- **The *D. suzukii* line used is primarily crepuscular.** Other strains were previously reported to be more diurnal, but the locomotor behaviour of *D. suzukii* lines is variable.[4]
- *D. suzukii* flies are **highly inactive** under laboratory conditions. This might be because they respond to stressful situations with inactivity, unlike *D. melanogaster.*
- Pairwise assays were performed to overcome the inactivity that might conceal the extent of circadian control in *D. suzukii*. They suggest that **the sex composition of pairs is an important determinant of circadian behaviour.** *D. melanogaster* mixed pairs display changed activity pattern due to circadian controlled courtship by males.[5] No such change was seen in *D. suzukii.*

Future steps

- After cloning the other homology arms into the donor plasmids, they will be used for transgenesis of *D. suzukii*. The visual marker will be strongly expressed in the eyes of mutant flies, making their identification possible. The use of two different markers might allow the generation of double clock mutants in future.
- *tim* knockout flies are expected to be arrhythmic in constant darkness (DD) due to the abolition of PER and TIM oscillation.
- Future behavioural experiments on *tim* mutant *D. suzukii* can further out understanding of how the circadian clock mediates seasonal phenotypic changes.

References

[1] Shearer, P. W. et al., 2016, *BMC ecology;* [2] Peschel, N. and Helfrich-Förster, C., 2011, *FEBS letters;* [3] Gratz, S. J. et al., 2015, *Current protocols in molecular biology.;* [4] Hansen, C. N. *et al.,* 2019, *Frontiers in physiology*.; [5] Fujii, S. et al., 2007, *Current Biology.*

Acknowledgments

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Further information: