# Modelling and Analysis of Cellular Regulatory Networks: Circadian Clocks and Flower Induction

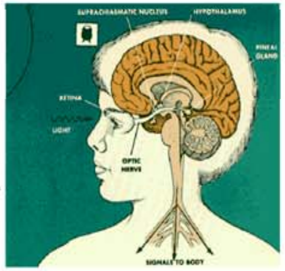
Domingo Salazar (Syngenta)

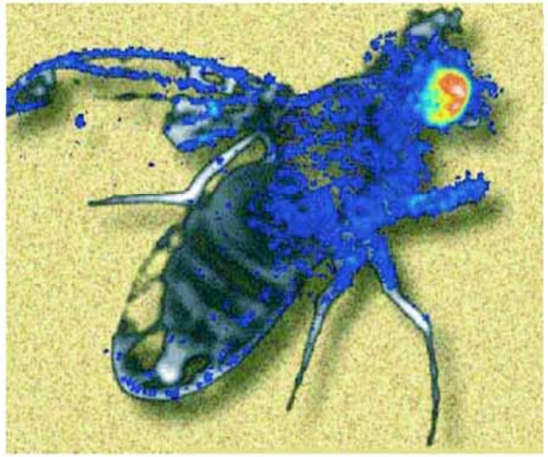
Main Collaborators:

- David Rand and Boris Shulgin (Warwick)
- Millar's Lab (Warwick, Edinburgh)
    - Andrew Millar
    - James Locke
    - Paul Brown
    - Saithong Treenut
    - Julia Foreman
    - Karen J. Halliday
- Isabelle Carrè (Warwick University)

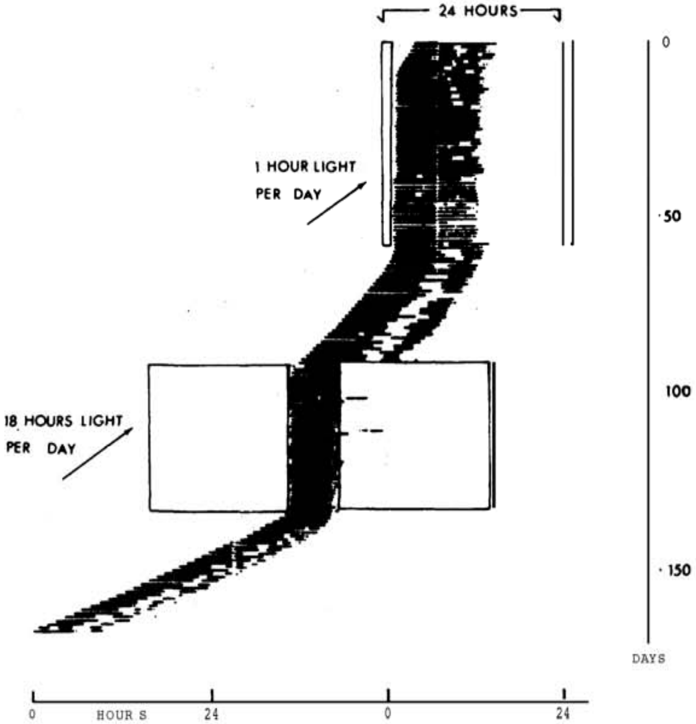
# Circadian rhythms

- **circadian rhythms,** endogenous cycles of behavior or biological activity with a period of about 24 hours



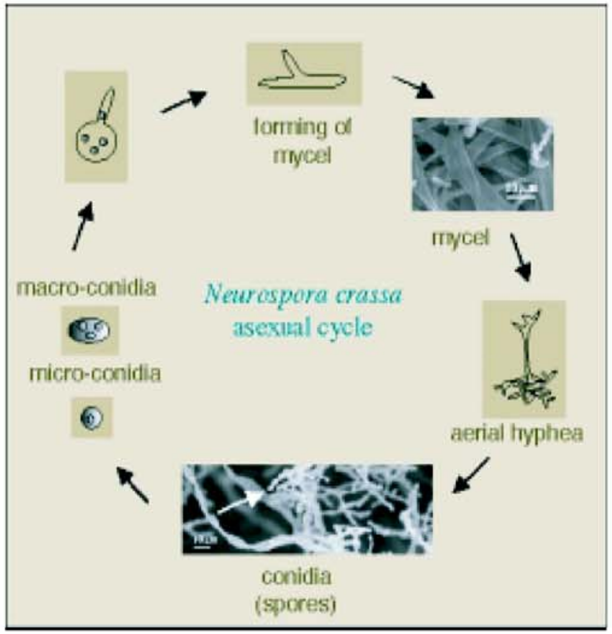


*Kay lab*




*Millar lab*

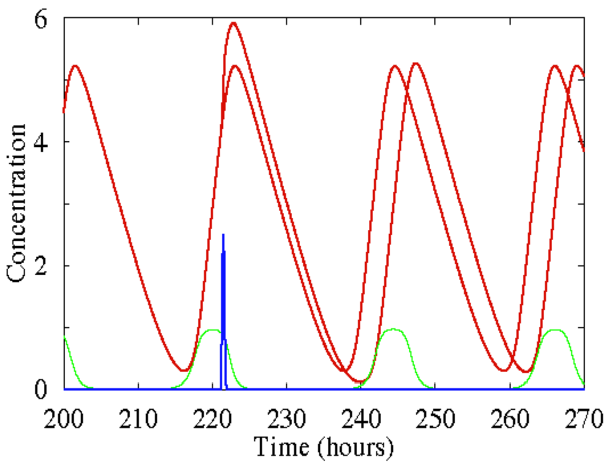
- in total darkness a **free running rhythm** that is independent of the local time
- environmental cycles (light-dark, temperature) **entrain** circadian rhythms

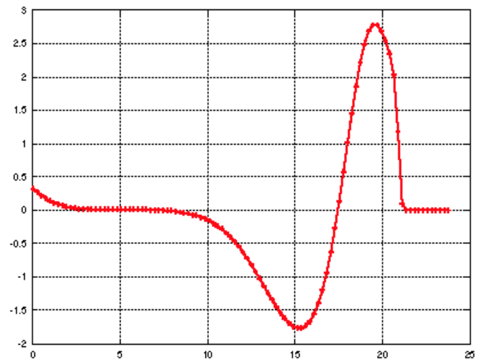



*van Gooch lab*

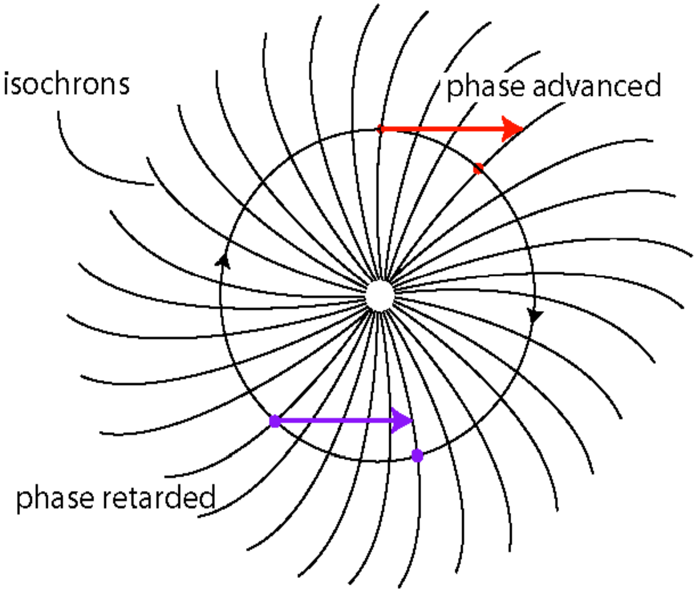
# Phase response curves (PRCs)

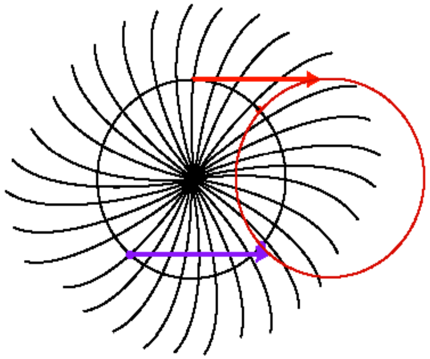
A **PRC** is a plot of phase-shifts as a function of circadian phase of a stimulus
- in our contex usually a light pulses, sometimes a temperature pulse.



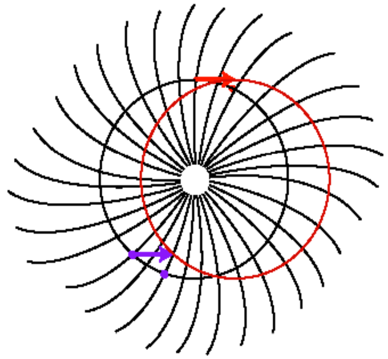



**Phase response curve**



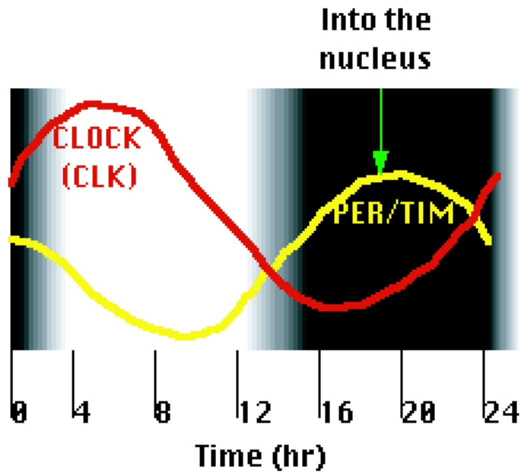



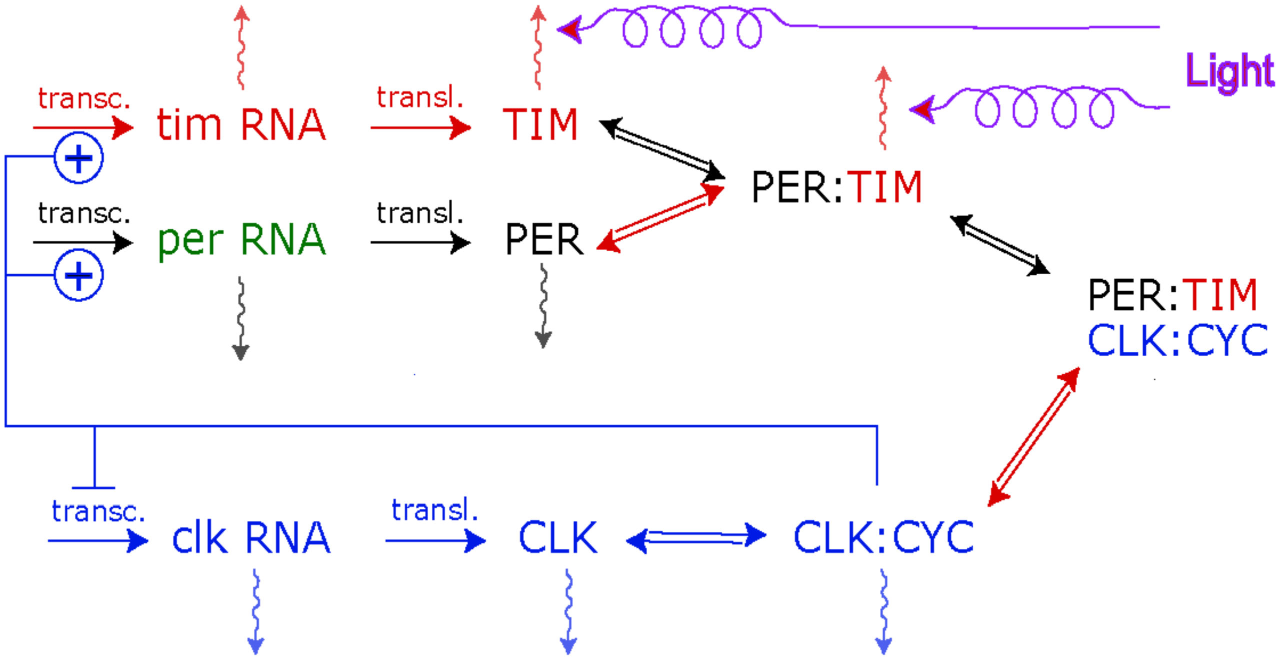
type 0



type 1

# Diagram for the *Drosophila* clock



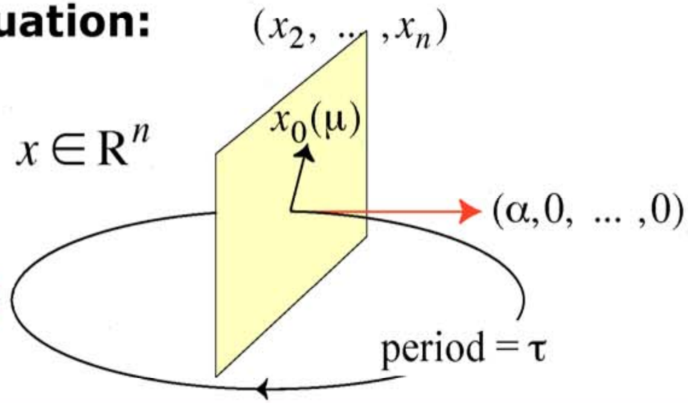



# Perturbation theory

**Differential equation:**

$$\frac{dx}{dt} = F(x,\mu) \quad x \in \mathbb{R}^n$$

$$g(t) = x(t, x_0, \mu_0)$$




**Variational equation:**

$$\frac{dX}{dt} = A(t) \cdot X \quad (*)$$

where

$$A(t) = \left( \frac{\partial F_i}{\partial x_j}(g(t), \mu_0) \right)$$

and

$X = X(t)$ an $n \times n$ matrix

$X(t)$ = fundamental matrix solution with $X(0) = I_n$

**Floquet theory:** can write $X(t) = Z(t)e^{Rt}$

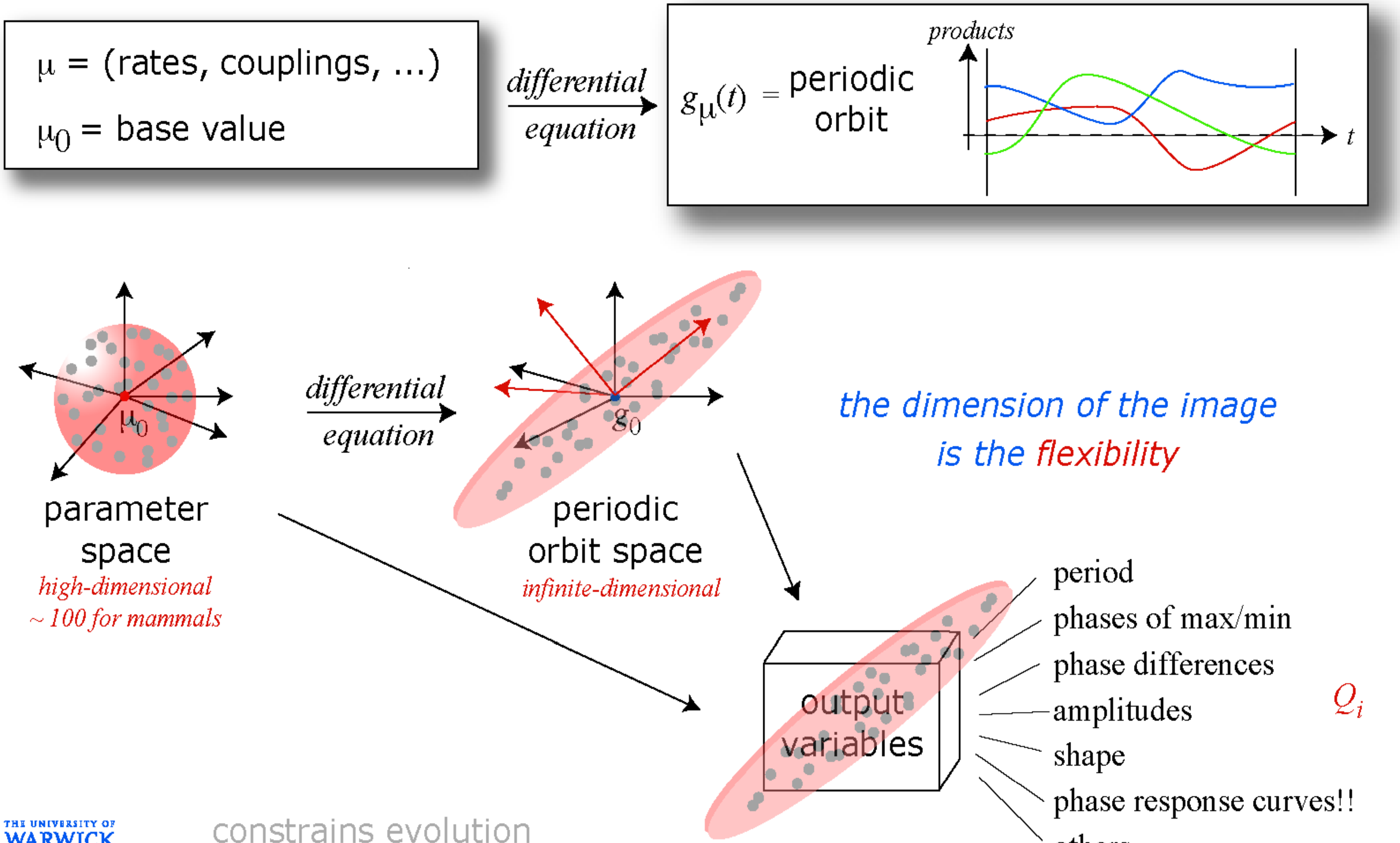
where $Z(t + \tau) = Z(t)$, $Z(0) = Identity$

eigenvalues $\lambda_1, \ldots, \lambda_n$ of $e^R$ are Floquet multipliers

$\lambda_j = e^{\chi_j}$ Floquet exponents

$$\frac{\partial}{\partial \mu_j}\left(p(\mu), x_0(\mu)\right)\Big|_{\mu=\mu_0} = -\left(X(\tau) - diag[\alpha, I_{n-1}]\right)^{-1} \cdot \int_0^\tau X(\tau)X(s)^{-1} \frac{\partial F_i}{\partial \mu_j}(g(t), \mu_0)\, ds$$

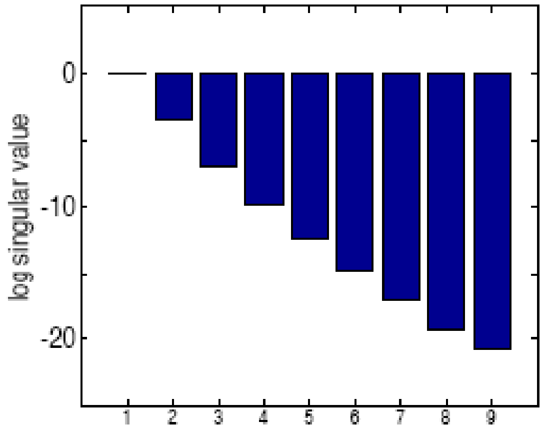
# Flexibility



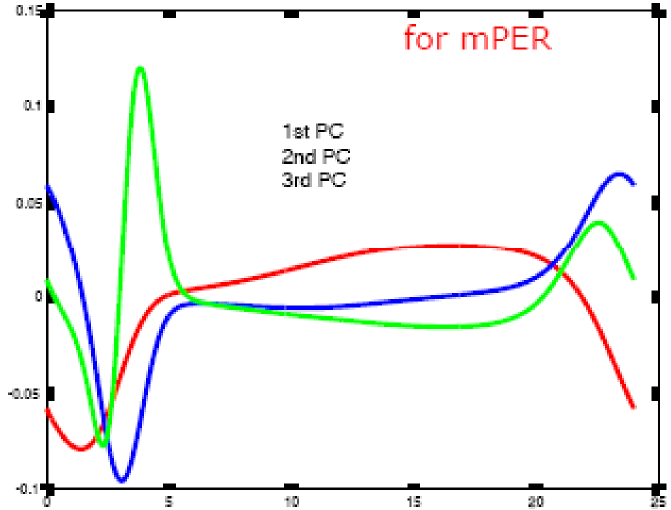

THE UNIVERSITY OF WARWICK

constrains evolution

# Two-loop forced Leloup-Gonze-Goldbeter model of the *Drosophila* clock



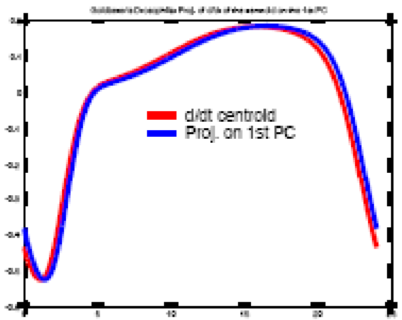

| Singular value | % of Variance |
|---|---|
| 1.041425287 | 97.023028951 |
| 0.031026676 | 2.890559794 |
| 0.000870290 | 0.081079393 |
| 0.000052507 | 0.004891725 |
| 0.000004254 | 0.000396276 |
| 0.000000428 | 0.000039912 |
| 0.000000036 | 0.000003352 |
| 0.000000004 | 0.000000418 |
| 0.000000001 | 0.000000066 |
| 0.000000000 | 0.000000027 |

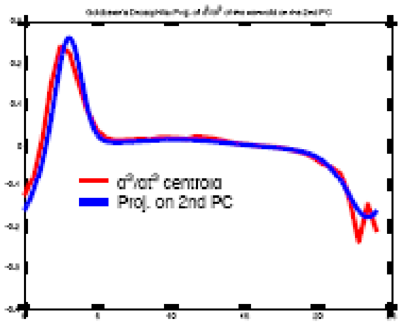



**Number of variables** in the model ... 10
Variable used as reference (mPER) ... 1
**Number of parameters** in the model ... 41
Number of perturbed parameters in this analysis ... 39
Size of the perturbations ... <= 1.00%

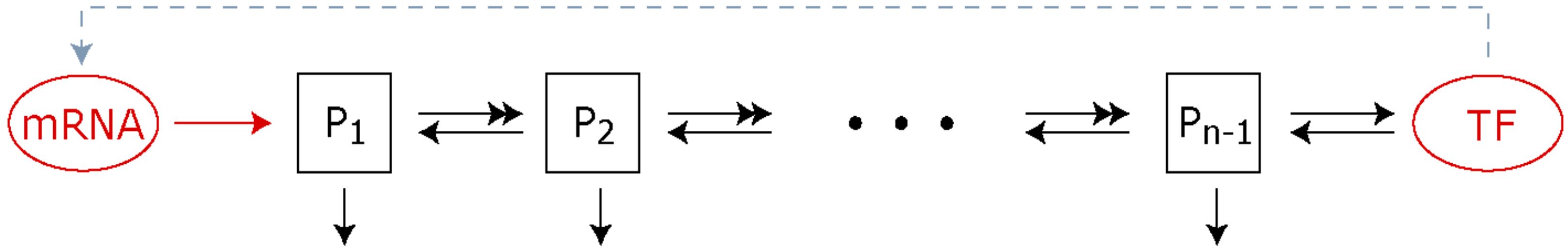
Projection coefficient of dt on 1st PC ... 6.6325
Projection coefficient of dt on 2nd PC ... -0.830736

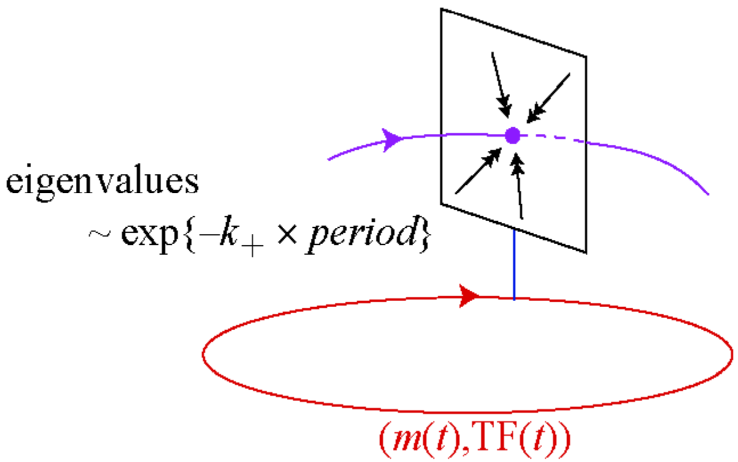
Projection coefficient of dt^2 on 1st PC ... -0.38426
Projection coefficient of dt^2 on 2nd PC ... -2.73112
Projection coefficient of dt^2 on 3rd PC ... -0.581293



# Loops



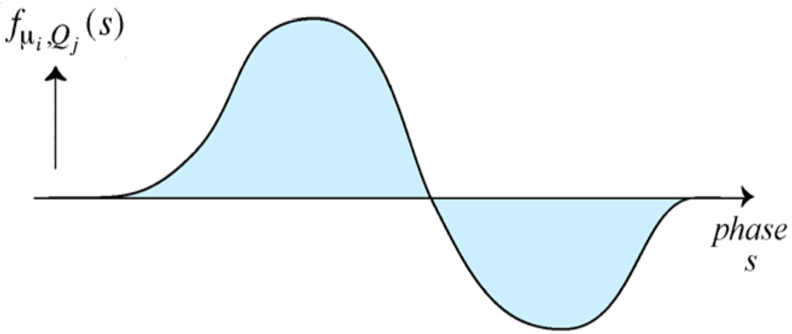

dominant mode is phase $\phi$ and perhaps $d\phi/dt$.

$\Rightarrow$ image of parameters $\rightarrow$ period orbit is low-dimension (typ. 2 in this case)

extra slow modes obtained by adding more genes (TF v mRNA), coupling etc

## IRCs: Infinitesimal Response Curves

for given parameter $\mu_i$ and output variable $Q_j$



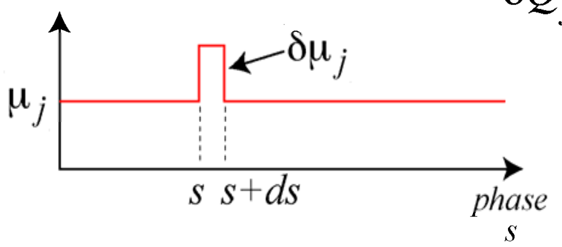

has the following properties:

- for sustained parameter change of $\delta\mu_j$:

$$\delta Q_j = \delta\mu_i \cdot \int_0^\tau f_{\mu_i, Q_j}(s)ds + O(\delta\mu_i^2)$$

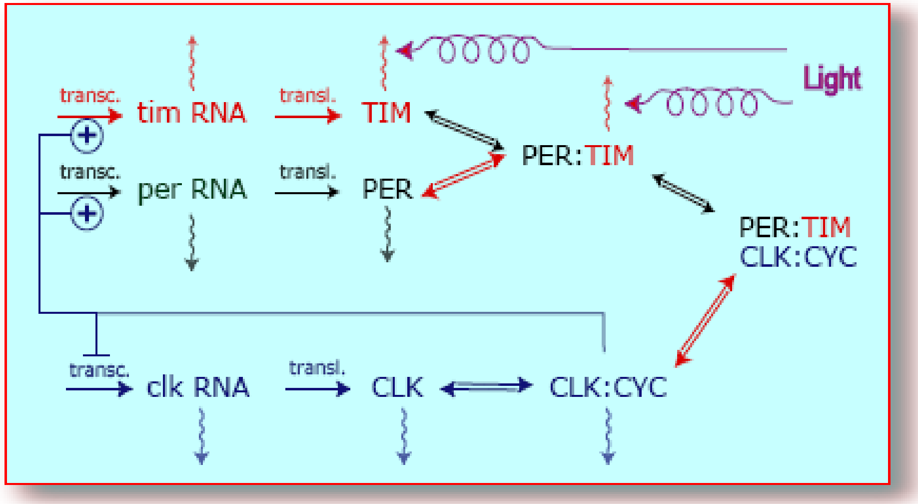
- for phase-restricted parameter change of $\delta\mu_j$ between $s$ and $s+ds$

$$\delta Q_j = \delta\mu_i \cdot f_{\mu_i, Q_j}(s)ds + O(\delta\mu_i^2)$$

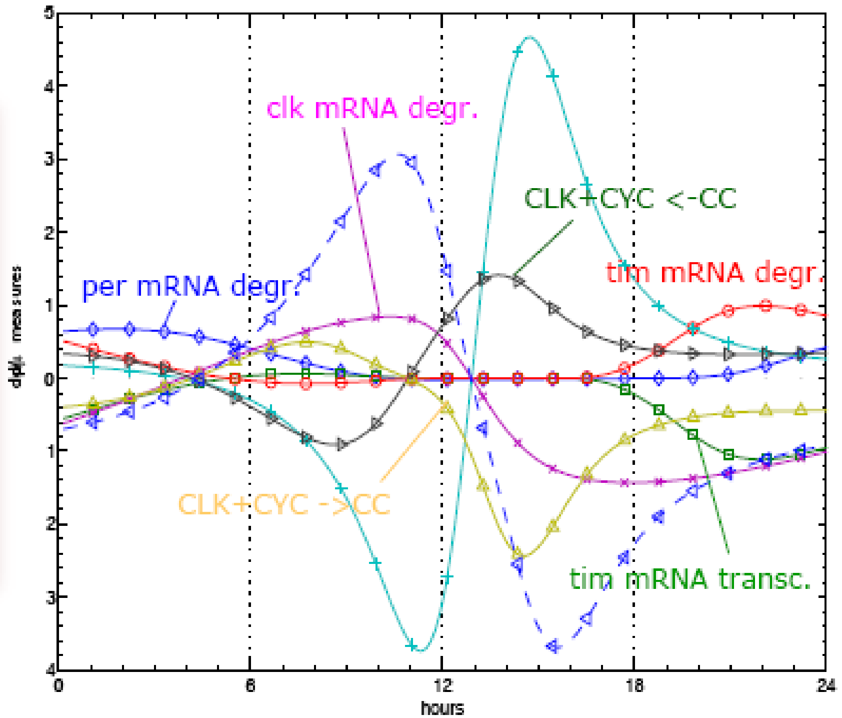


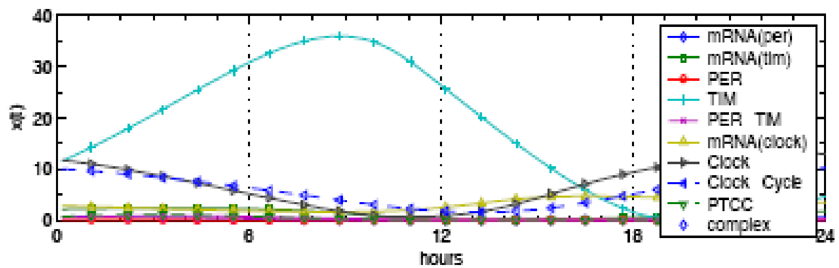

WHY

# *Drosophila* clock response analysis




| value | scaled deriv |
|---|---|
| 0.7 | 6.52 |
| 0.76 | 8.54 |
| 0.7 | 7.6 |
| 1 | 6.55 |
| 0.1 | 11.8 |
| 0.1 | 12.5 |
| 0.1 | 6.5 |
| 2 | 12.5 |



# Temperature Compensation

base temperature $T_0$,

**Parameters as function of temperature:** $\mu_j = \mu_j(T)$

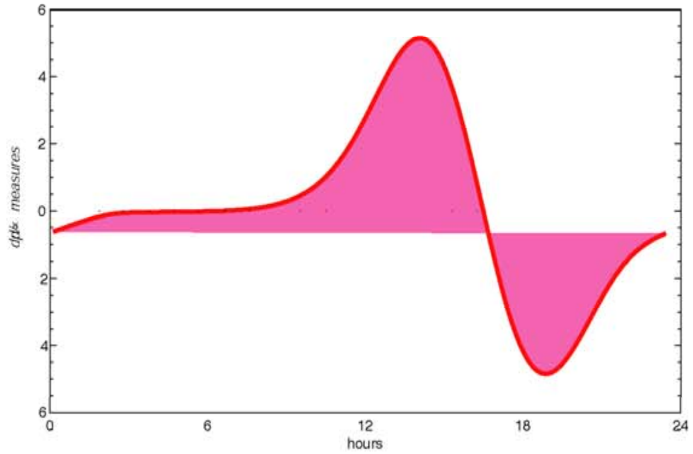
$$T_0 \rightarrow T_0 + \delta T \quad \text{causes change} \quad \mu_j(T_0) \rightarrow \mu_j(T_0) + \mu_j'(T_0)\cdot \delta T + O(\delta T^2)$$

**Temperature IRC:** $f_{T,period}(\varphi) \;=\; \sum_j \mu_j'(T_0)\cdot f_{\mu_j,period}(\varphi)$

**Temperature compensation**

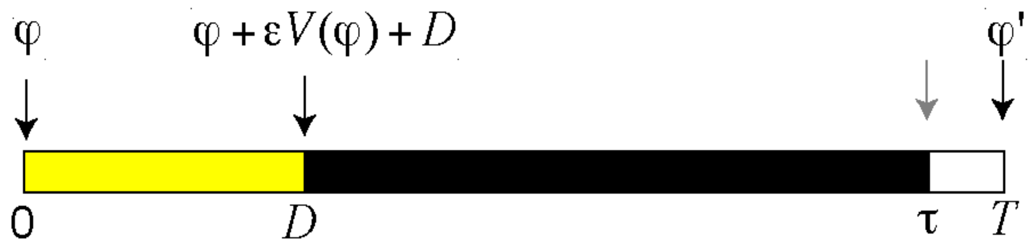
$$\Updownarrow$$

$$\int_0^{\tau} f_{T,period}(\varphi)\,d\varphi = 0$$




THE UNIVERSITY OF WARWICK

# Phase response maps

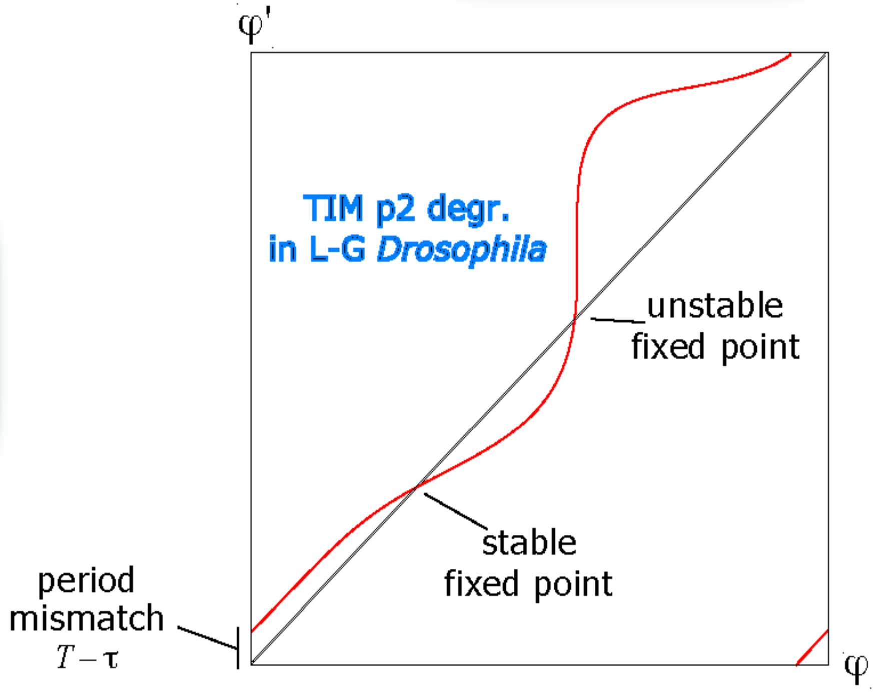


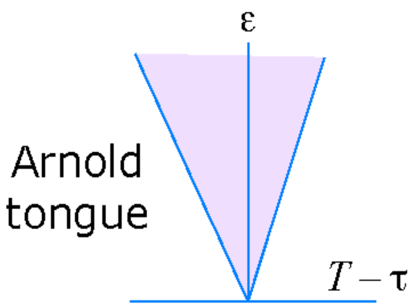

$$V \approx -f_{\mu_i, period}$$

$\tau$ = period of free oscillator

$T$ = forcing period (e.g. 24hrs)

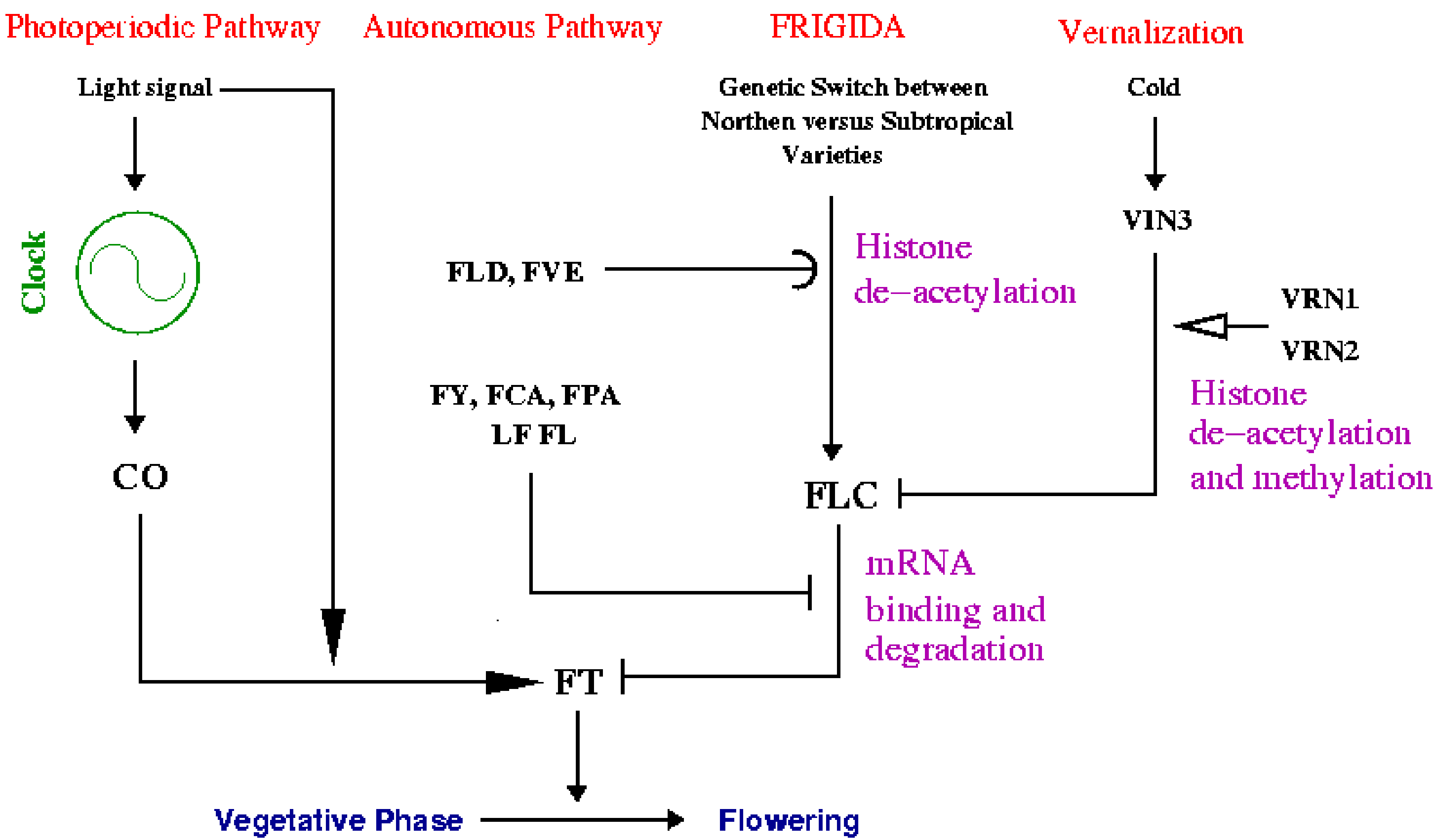
$$\varphi' = \varphi + (T - \tau) + \varepsilon V(\varphi)$$



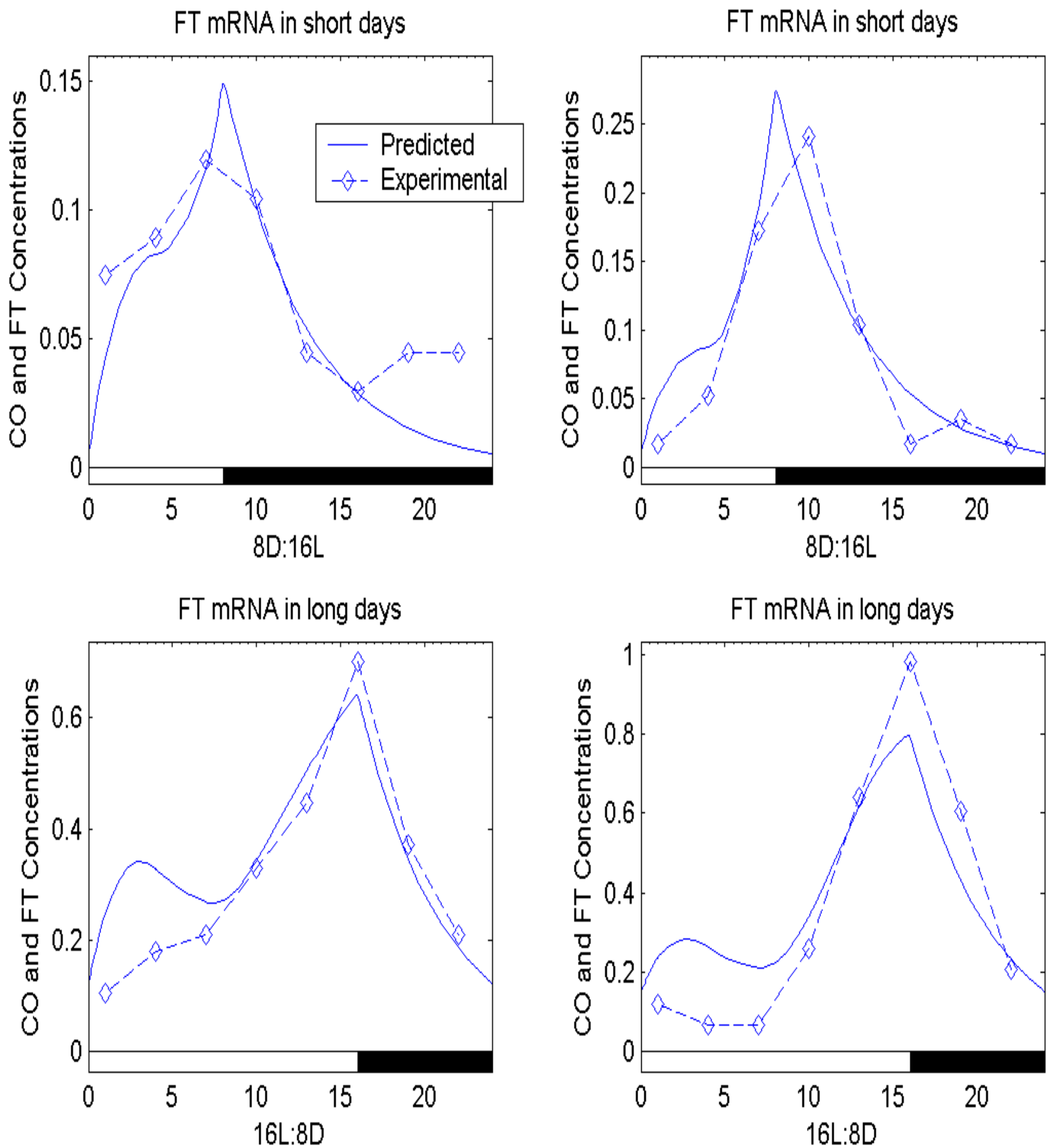

$\Rightarrow$ $V = -f_{\mu_i, period}$ **must have significant amplitude**

# Flowering Induction Network



# Parametrisation

- Two different short day (SD) and long day (LD) experiments were used as training data sets.
- The validation data sets included mRNA waveforms from both wild-type plants and some mutant types.
- The fit of the model to the relevant data set(s) was measured using a weighted mean square cost function (SDs vs. LDs).
- Initial parameter search was performed using simulated annealing (Sobol sequences). The Nelder-Mead unconstrained simplex optimization method (Matlab) was used to improved the fitting.
- In each case, the solution to the ODE was allowed to relax to the limit cycle (entrainment) and then the limit cycle was computed using MatLab ODE boundary value solver. The resulting solution was therefore guaranteed to be a true, attracting limit cycle.
- After optimisation, the difference in cost values of the 20 best solutions tended to be small, although some parameter values could be widely spread in parameter space.




The model shows a missing inhibitor gene in the morning.

We deduced from here its expression pattern and that has given us appropriate candidates.

Help in experiment design:

- Microchips
- Micro RNAs

# Conclusions

- New Molecular Biology techniques are giving us unprecedented details about the inner workings of living organisms.

- It is possible to adopt an interactive approach where existing knowledge is modelled, computer simulated and analysed mathematically; this in turn helps with the design of new experiments.

- Despite the great complexity of biological systems, it is possible to discern fundamental principles that are achieved through convergent evolution.

- New mathematical theories and techniques will emerge from the study of these systems, which may be fundamentally different from the more physically inspired theories that we have now.