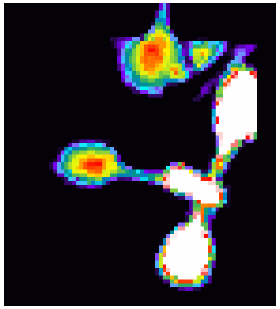
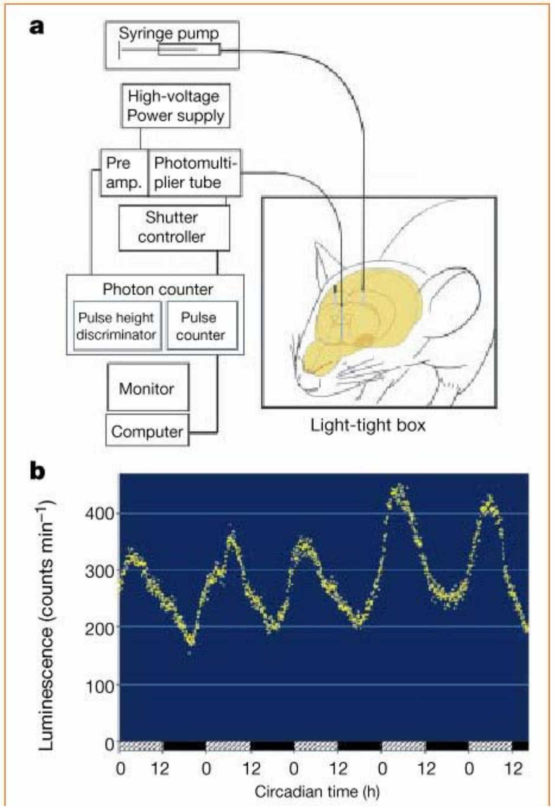
The circadian clock as a paradigm for Systems Biology

The requirements for systems biology are well-established in circadian research



- Dynamic, quantitative data *in vivo* and in cell cultures
- Crosses scales
  - Molecules to ecosystem or clinic
- Crosses taxonomic groups
- Major contributions from modelling since 1960's
- Worldwide research community



Yamaguchi *et al.*
Nature, 2001

Current:

Laszlo Kozma-Bognar
Kieron Edwards
Adrian Thomson
Vacancy !

Ozgur Akman
Paul Brown
James Locke
Treenut Saithong
Parantap Shukla
Vacancy !

Ruth Bastow (GARNet)

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Shigeru Hanano
Seth Davis
Mandy Dowson-Day
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Anthony Hall
Alex Morton
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Nickiesha Bromley
Victoria Hibberd
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Antony Dodd, Alex Webb, (Cambridge)
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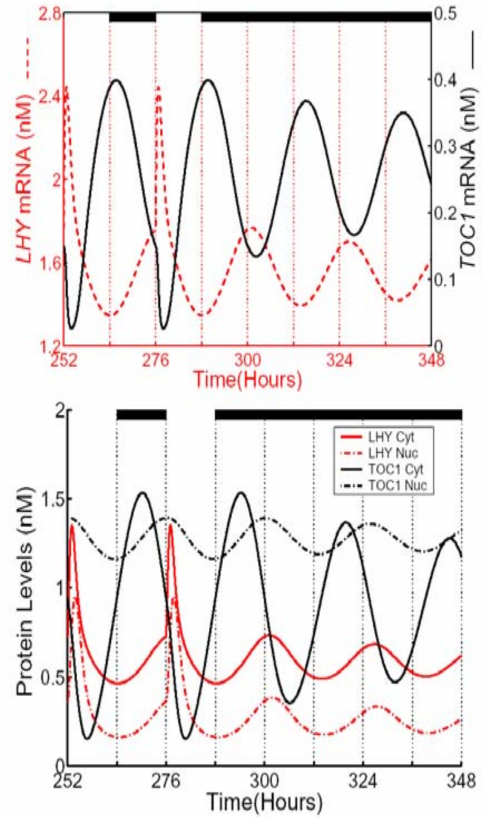
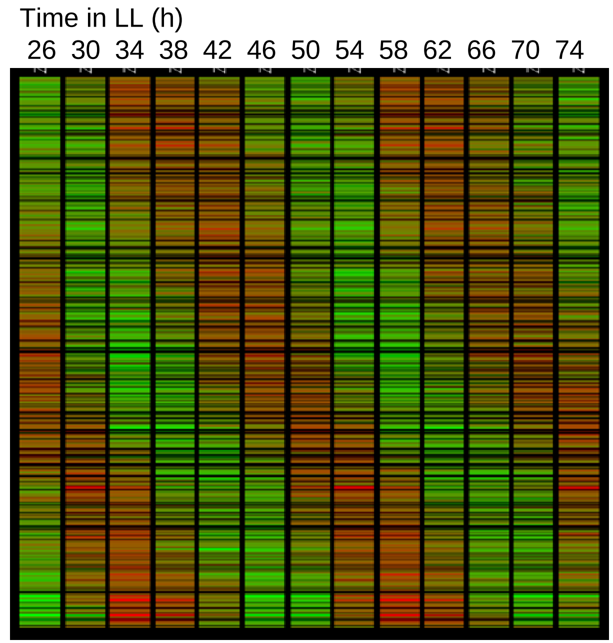
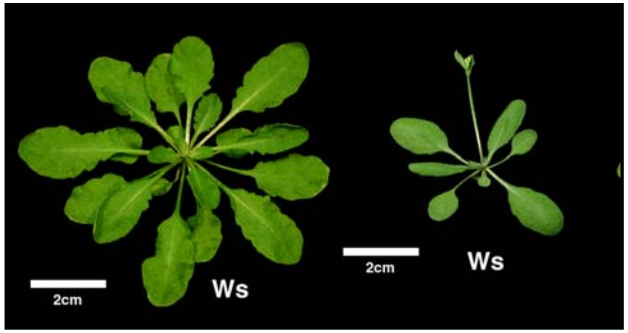
csbe

Funding: BBSRC, Gatsby, EPSRC, EU

Circadian clock in *Arabidopsis thaliana*



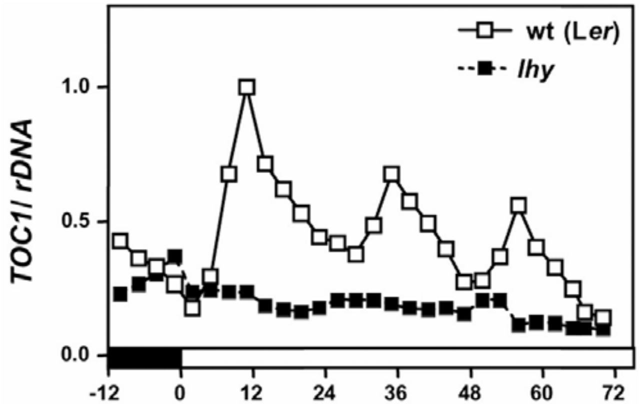




- Plant clock controls growth and flowering time
- Regulates >15% of RNA transcripts

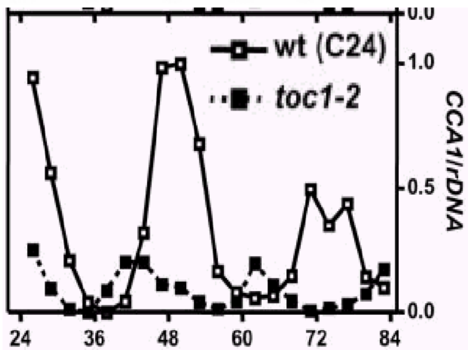
Edwards *et al.*
Plant Cell 2006

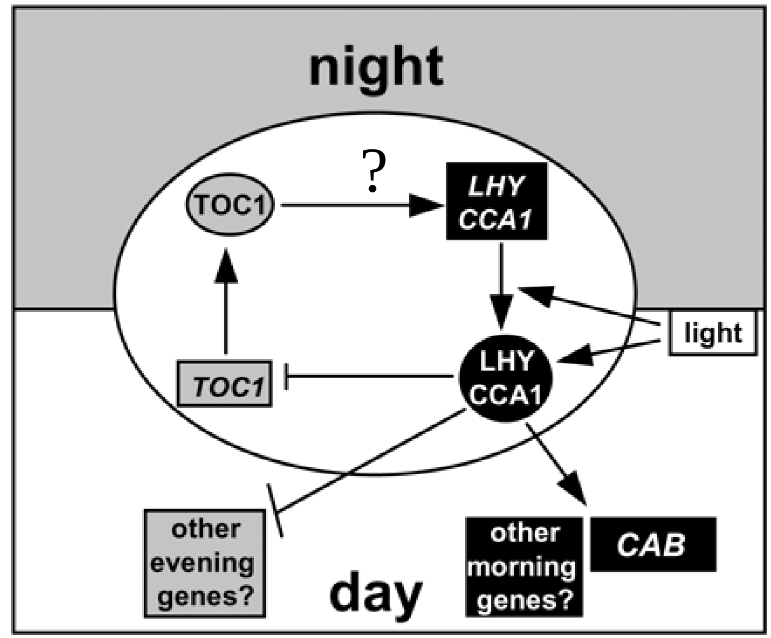
Mutant plants identify genes in the clockwork



WT = wild type (normal)

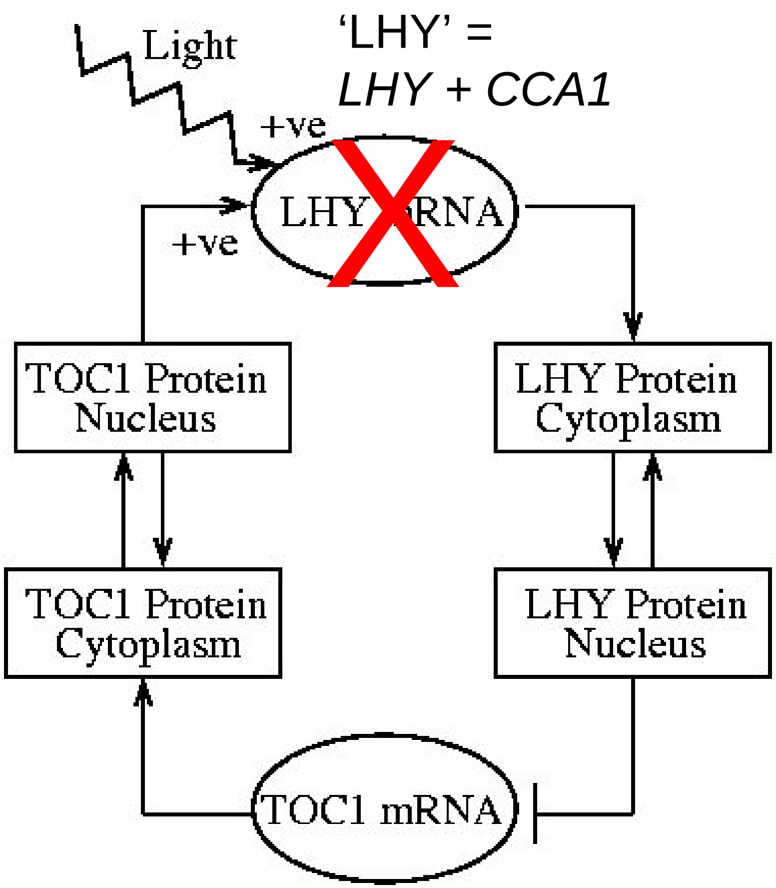
Alabadi *et al.*, 2001





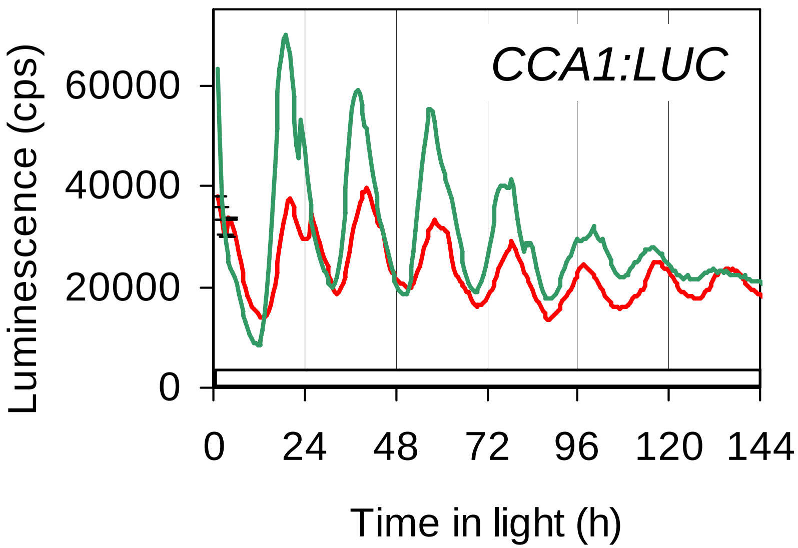
- Negative regulation during the day - *CCA1/LHY*
- Positive regulation at night – *TOC1* +++
- ODE model to test potential for regulation

Single loop is not sufficient

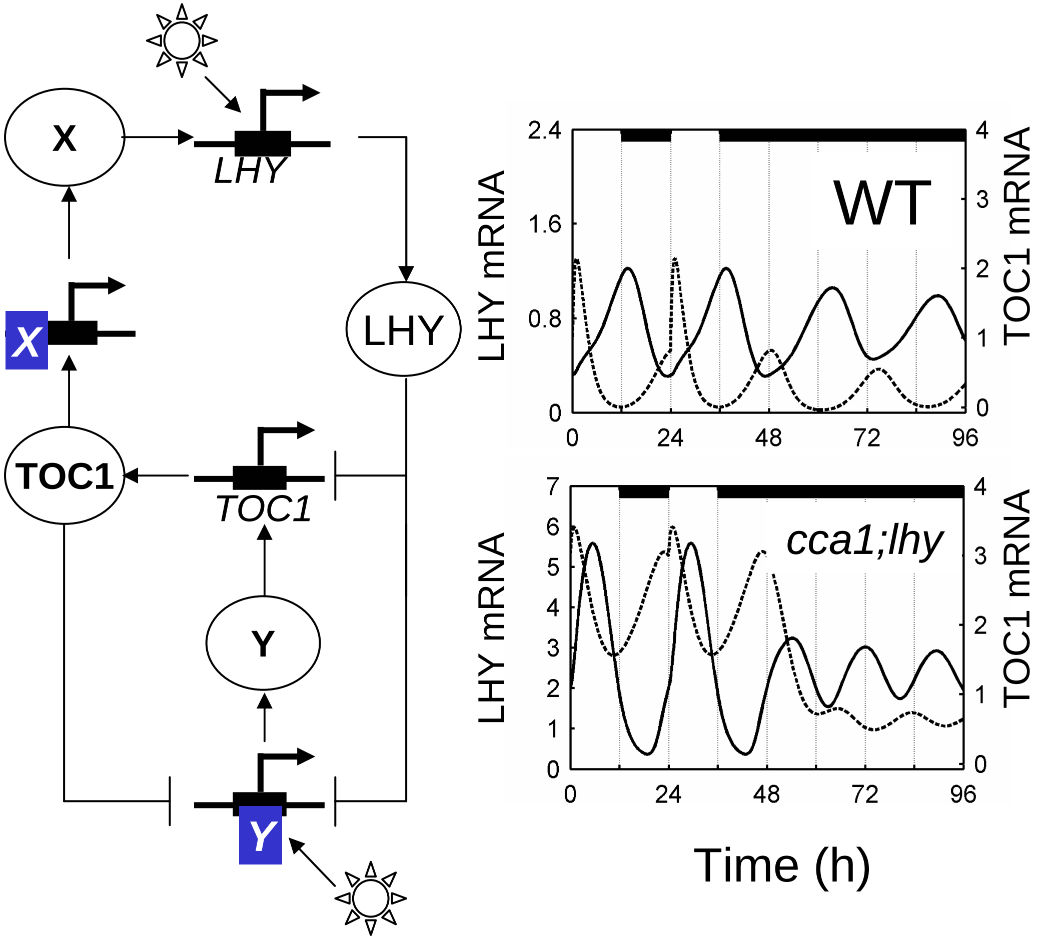


- Simulations contradict data
- Global parameter search finds no good solutions
  - *e.g. cca1;lhy* double mutants are rhythmic, ~17h period

⇒ another loop, without *CCA1* and *LHY*



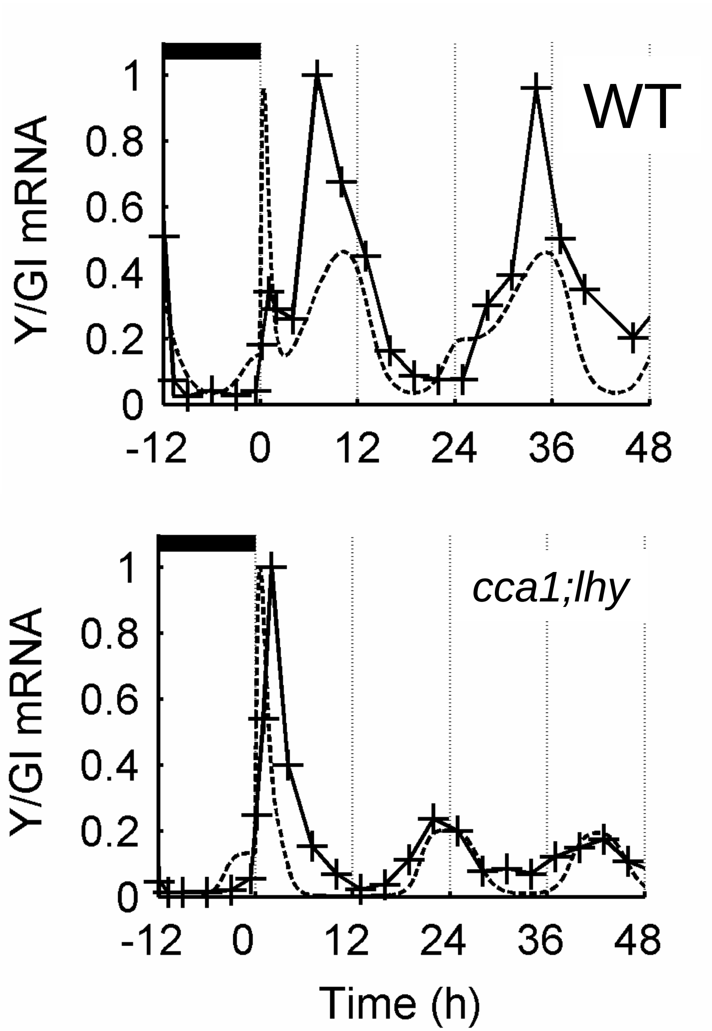
Interlocking loop model for Arabidopsis clock



- Model- J. Locke
- Hypothetical components *X*, *Y* added from inspection of data
- Fit model to WT and mutant data
- Predicts *X* and *Y* expression

Locke *et al.*, *Mol Syst Biol*, 2005: Data + interlocking loop model.

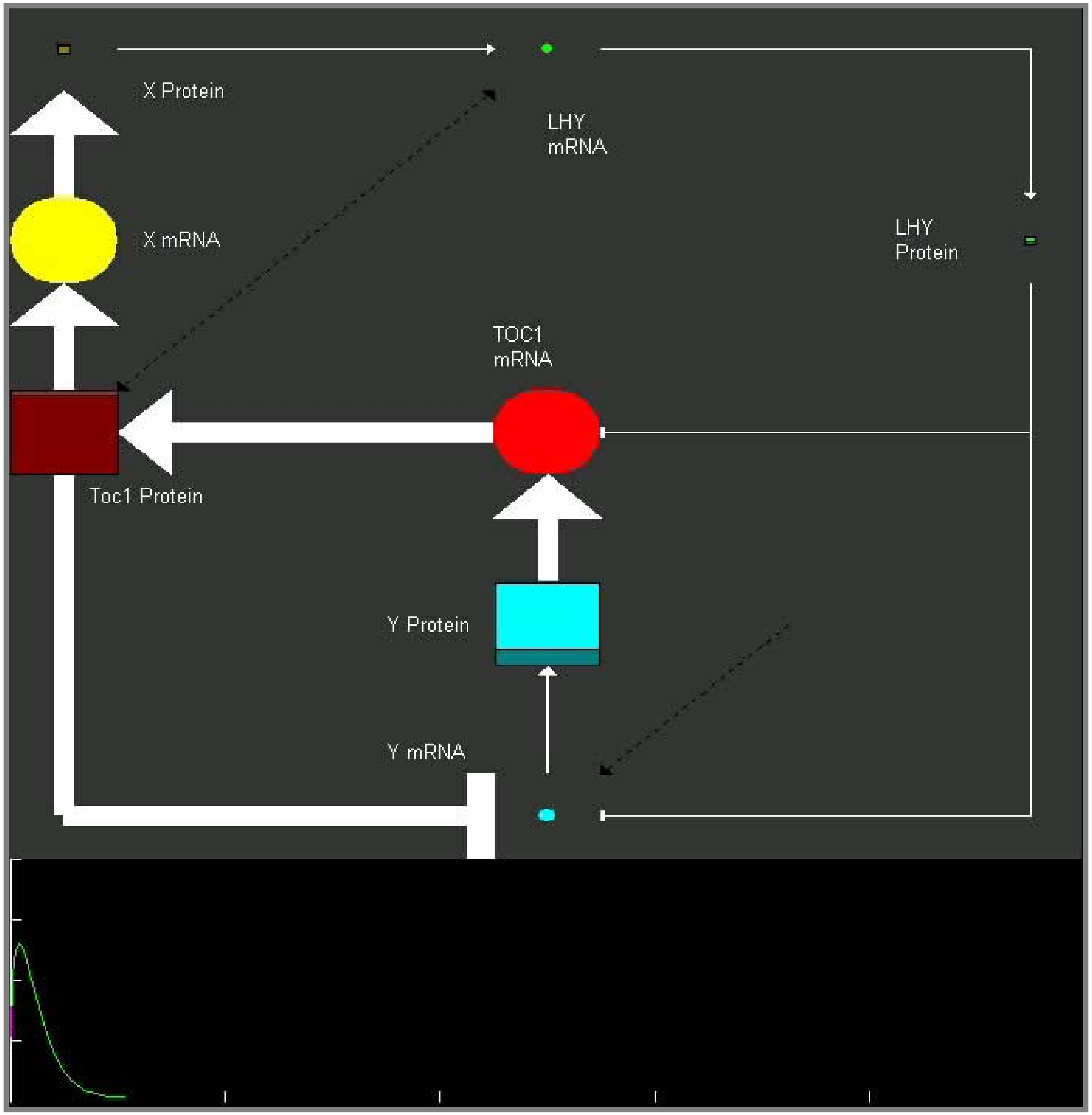
Experiments to identify *Y* turn up a good candidate



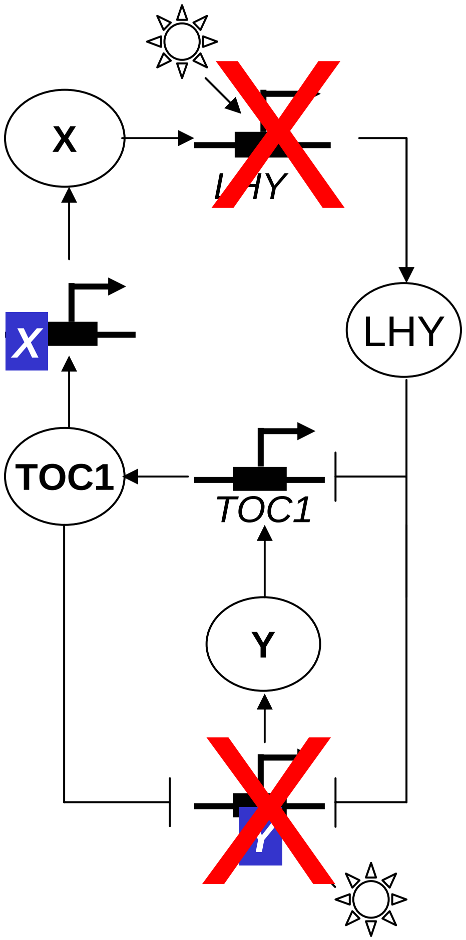
- Prediction = dashed line
- M. Southern tested candidate genes by qRT-PCR. Data = crosses
- Unexpected light response of *GIGANTEA* RNA matches prediction
- *GI* data in literature also matches predictions for *Y*

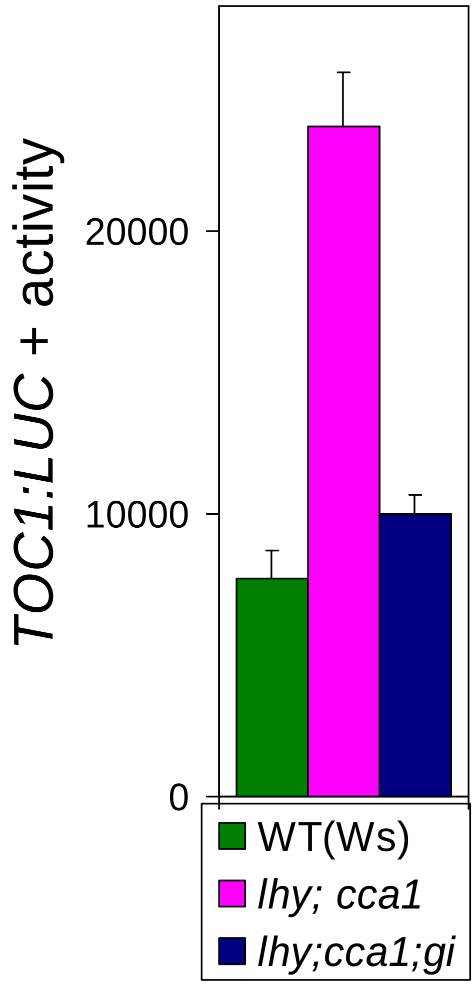
Simulation and visualisation software

- Paul Brown, software engineer
- Matlab animation
- Available at *www.amillar.org*



Validation 1, *TOC1* activation: *GI* is (part of) *Y*

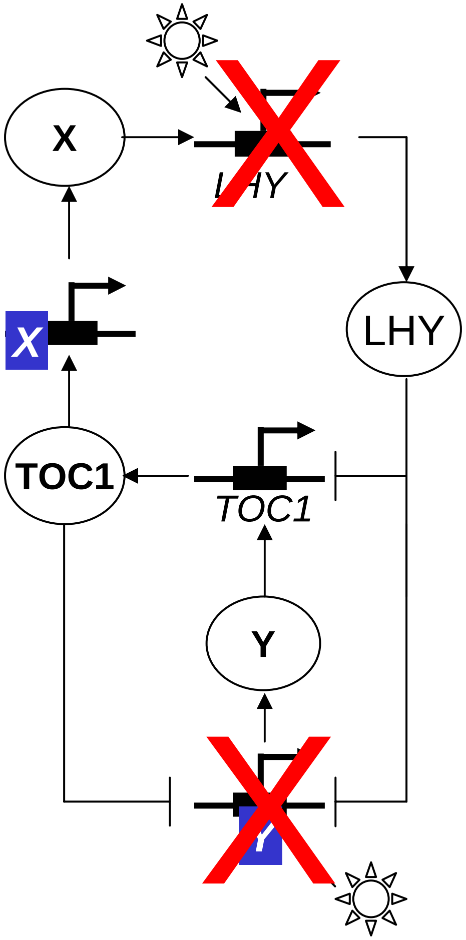


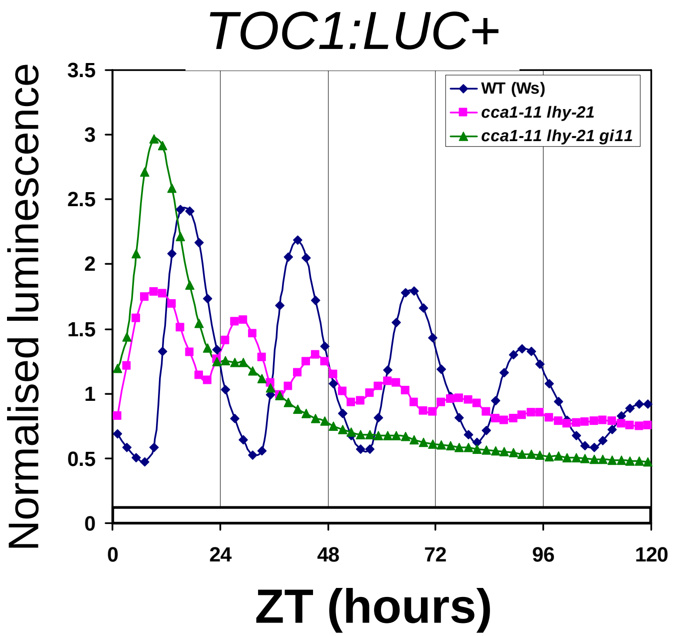


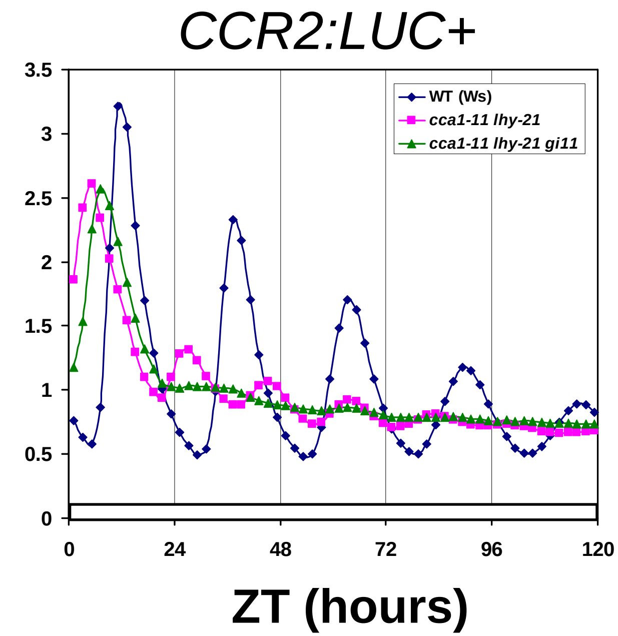
- *LUC* reporters crossed into mutant backgrounds
- *LHY/CCA1* inhibit *TOC1* expression
- *GI* activates *TOC1* expression

Locke *et al.*,
Mol. Syst. Biol. in press

Validation 2, rhythmicity: *GI* is (part of) *Y*





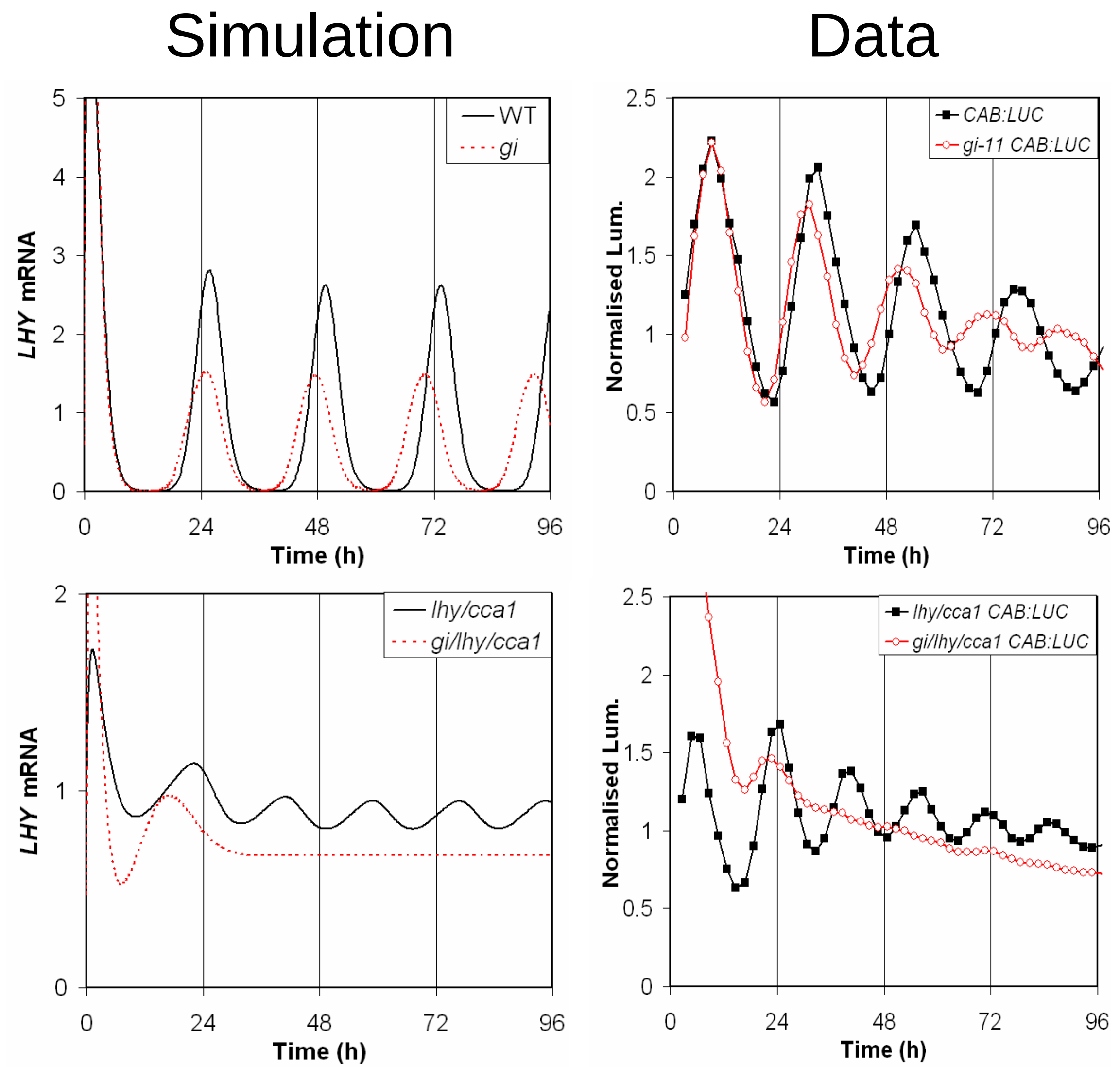


- *LUC* reporters crossed into mutant lines, tested in constant light
- *lhy;cca1* shows 18h rhythm
- *lhy;cca1;gi* does not, in light or darkness

Locke *et al.*,
Mol. Syst. Biol. in press

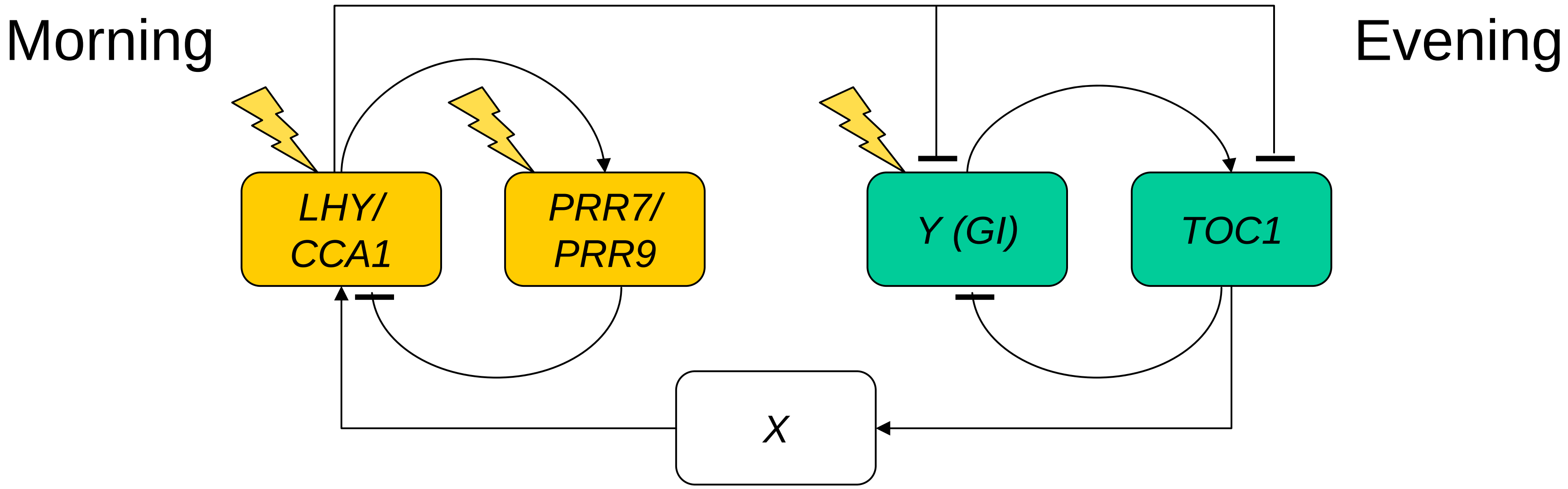
Validation 3, simulation: *GI* is (part of) *Y*

- *GI* is required for 18h rhythm
- Arrhythmia after one cycle in light
- Data closely match simulations using 3-loop model



Locke *et al.*,
Mol. Syst. Biol. in press

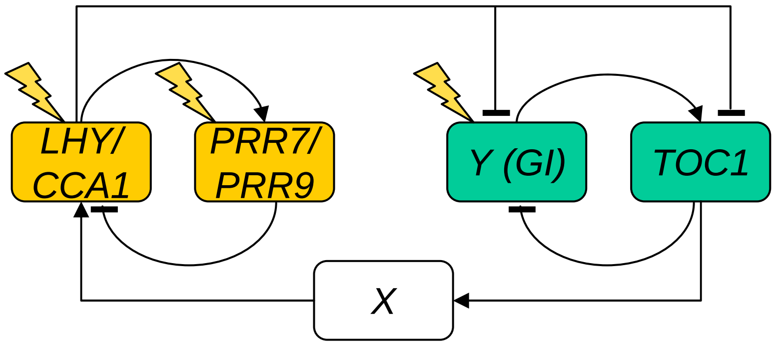
Three-loop model for the plant clock



- *PRR9, PRR7* genes are *TOC1 (=PRR1)* paralogues
- Form a feedback loop with *LHY/CCA1* (Farré *et al.* 2005)
- Add PRR9 and PRR7 (combined) to form 3-loop model

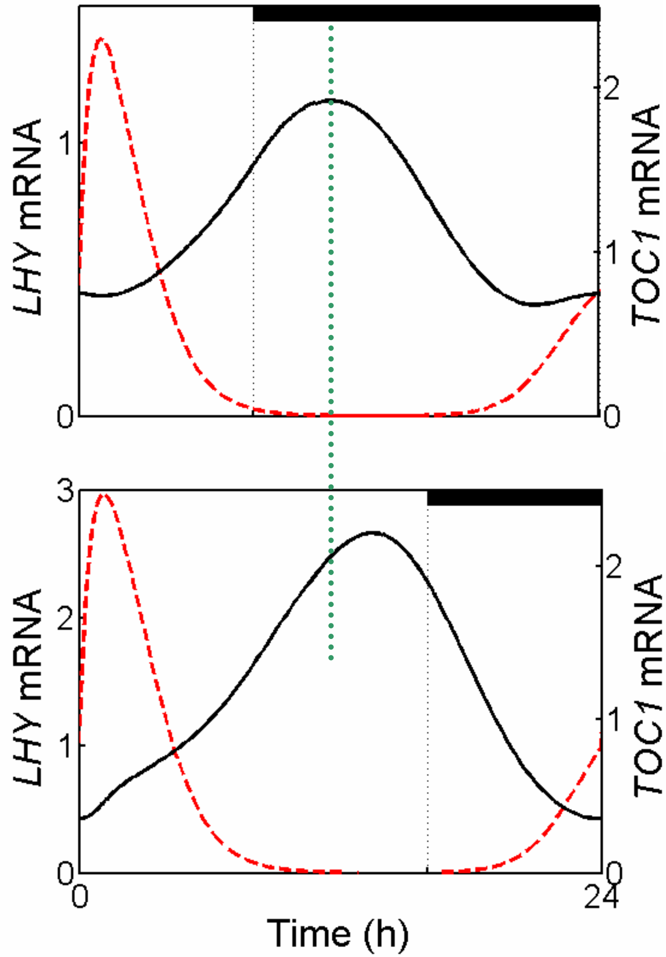
- Better match to *gi* and *toc1* mutant data, in addition to earlier data

Three-loop model: change relative peak times



- Compare simulations of long and short day length
- *LHY* peak constant relative to light-on
- *TOC1* peak also responds to light-off
- Observed in recent data

- Design similar to neural clocks in animals
  - Morning and evening cells
- <u>Intra</u>cellular in plants
  - no cell-cell coupling



Locke *et al.*,
Mol. Syst. Biol. in press

The Clock Shop

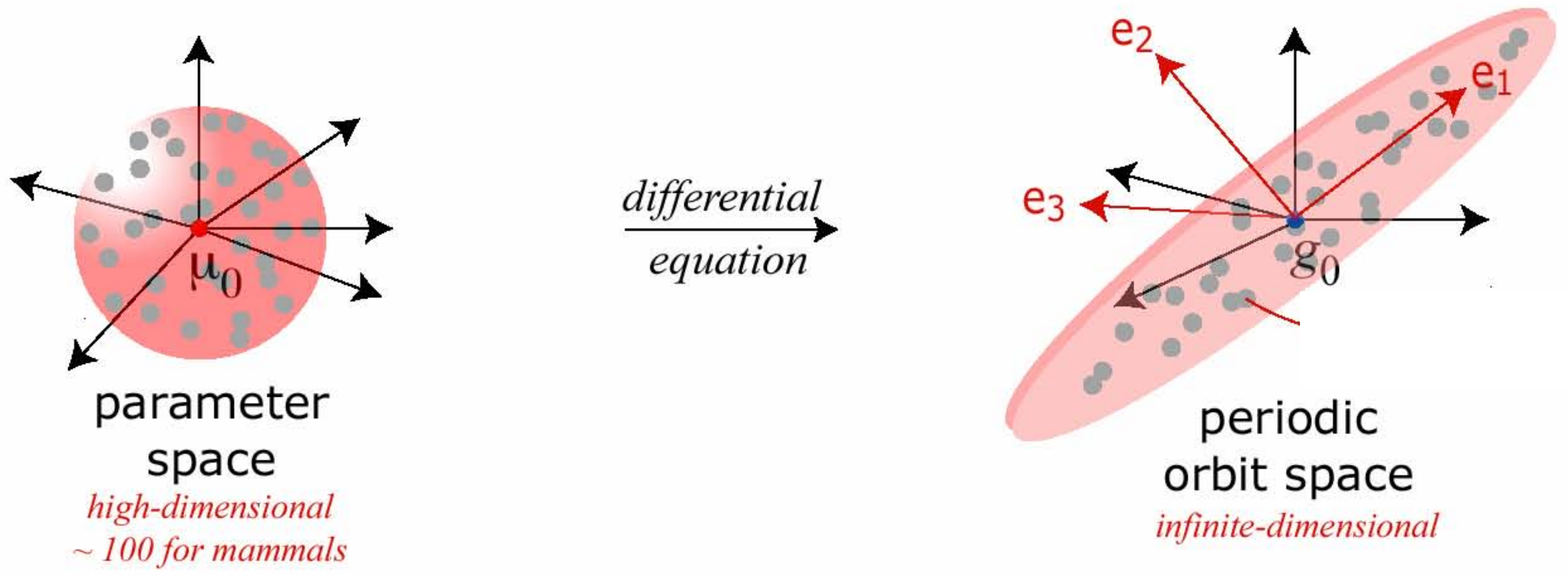
- Multiple genes
  LHY, CCA1
- Multiple loops
  >3 in plant clock
- Multiple clocks
  >1 per cell?

Why such complexity?

- Flexibility
  - Cycle to cycle
  - In evolution
- Robustness
  - Stochastic noise
  - Temperature compensation

Photo by Martin Lange

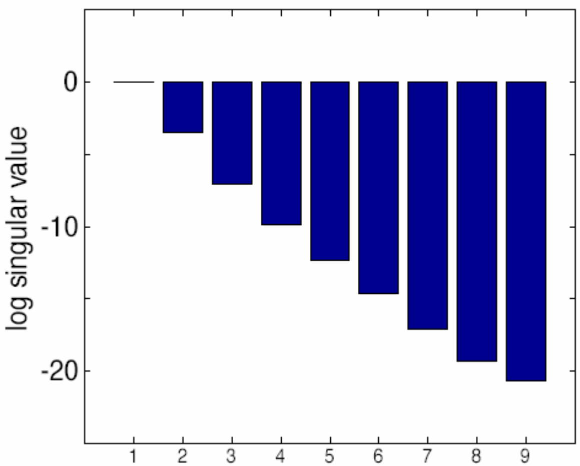
Flexibility: Principal Component Analysis or SVD



Leloup *et al.* fly model

- **10 variables**
- **41 parameters**
- ***1 dimension flexibility***
- **(phase or period)**

(from ≤1% μ perturbations under LD cycles)



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

Rand *et al.*, *Interface,* 2004

Flexibility in evolution: examples for clock models

Model	*Variables*	*Loops* (parts)	*Parameters*	*Flexibility* threshold 95% → 99.9%
Tyson *et al.* *Drosophila*	2	1	9	1, 1, 1, 1
Leloup *et al.* *Neurospora*	3	1	10	1, 2, 3, 4
Leloup *et al.* *Drosophila*	10	1 (2)	38	2, 3, 3, 6
Ueda *et al.* *Drosophila*	10	2 (3)	55	1, 1, 1, 2
Leloup *et al.* mouse	16	3 (4)	53	1, 2, 2, 5
Forger *et al.* mouse	73	5 (16)	36*	5, 7, 9,10

- Flexibility << Parameters
- Flexibility ~ Loop complexity

Rand *et al.*, *Interface,* 2004

Flexibility to evolve towards multiple targets

- Flexibility of single clock loops is low
  - evolution is constrained

- Design principle:
  Interlocking loops increase flexibility
  - biochemical mechanisms can vary, inter- or intracellular
  - deterministic

- Advantage for interlocking loops in evolution
  - if there is selective pressure on several, independent output properties (phases, temperature compensation, dawn/dusk tracking)

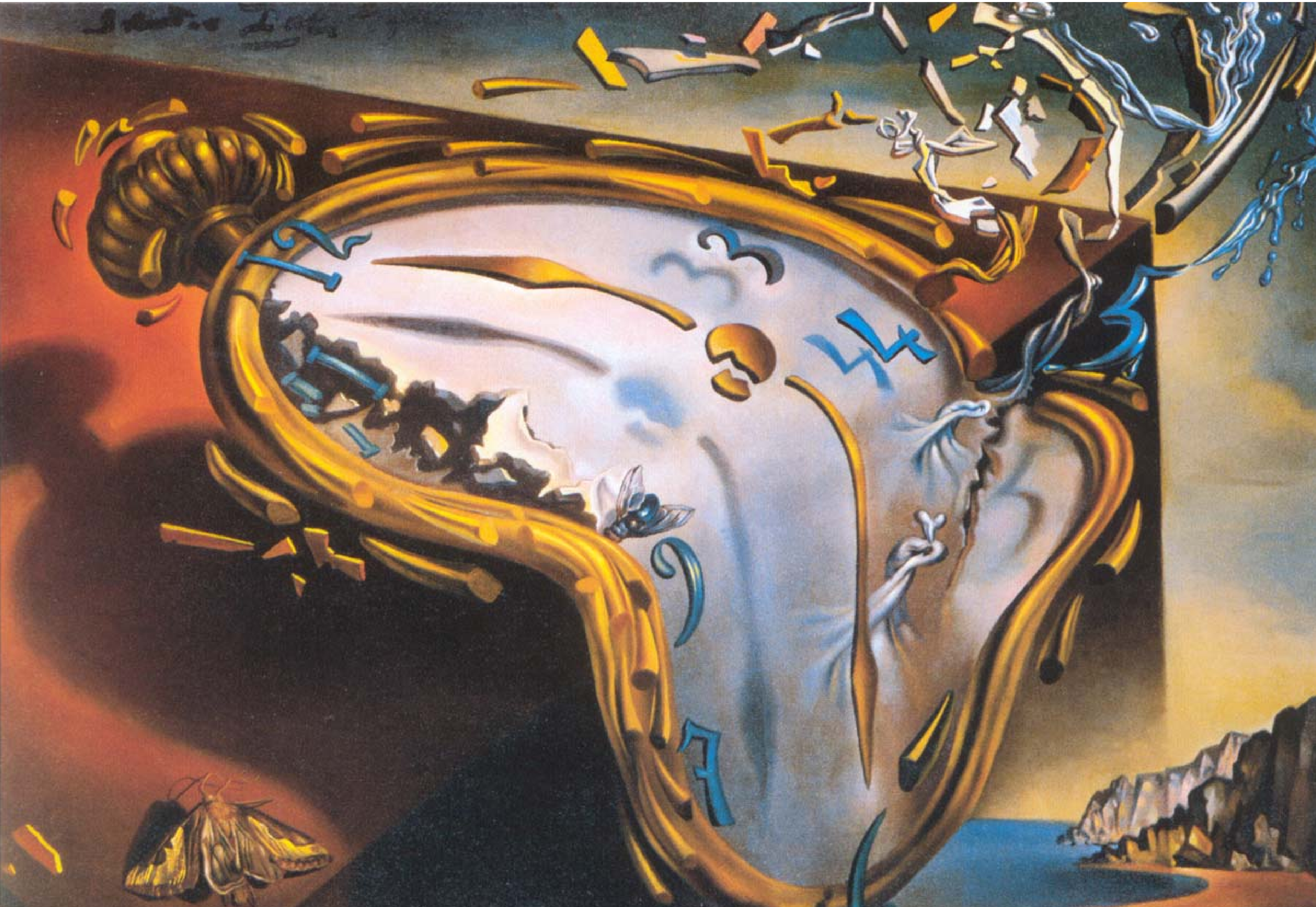
World Alliance for Clock Systems Biology

- Vision: Integrate clock systems research worldwide
  - Develop circadian system models for many species
- Aims
  - Shared informatics resources: data + models + tools
  - Stimulate data acquisition, computational tools
  - Enhance experimental – theoretical collaborations
  - Promote standards
  - Training, outreach: increase visibility
- Interim Steering Group

  Hiroki Ueda (RIKEN)

  John Hogenesch (U. Penn.)

  Andrew Millar (CSBE, Edinburgh)



Next steps

- Regional committees, cross subjects
  - Needed now, for fundraising
- Working groups, cross regions
  - Later, develop standards etc.
- Informal meeting Grand Sandestin lobby, 12:30
- Launch at ICSB Yokohama October 2006

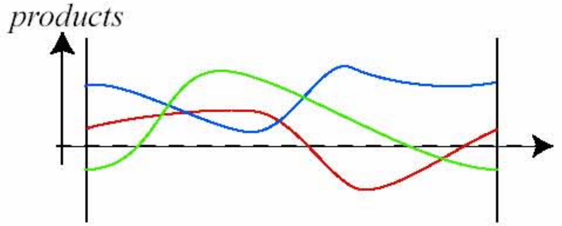
Flexibility

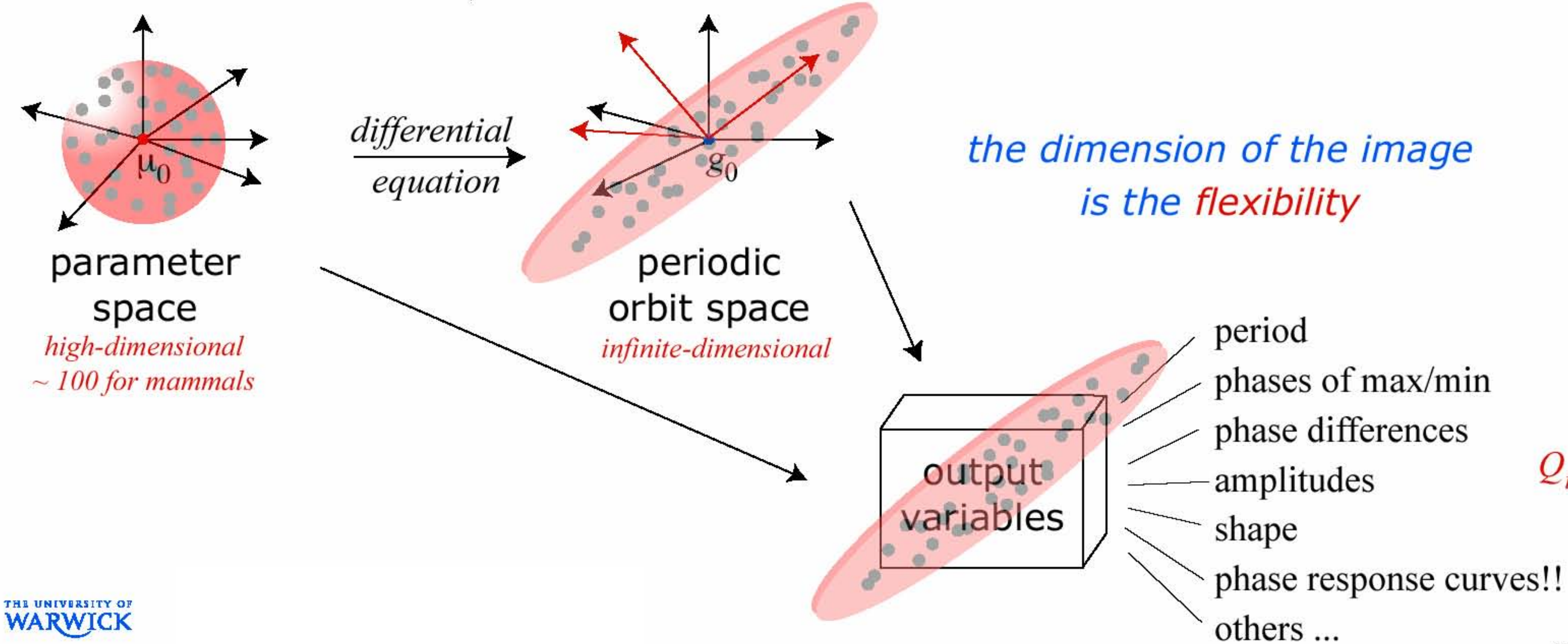
μ = (rates, couplings, ...)

μ_0 = base value

differential equation →

$g_\mu(t)$ = periodic orbit





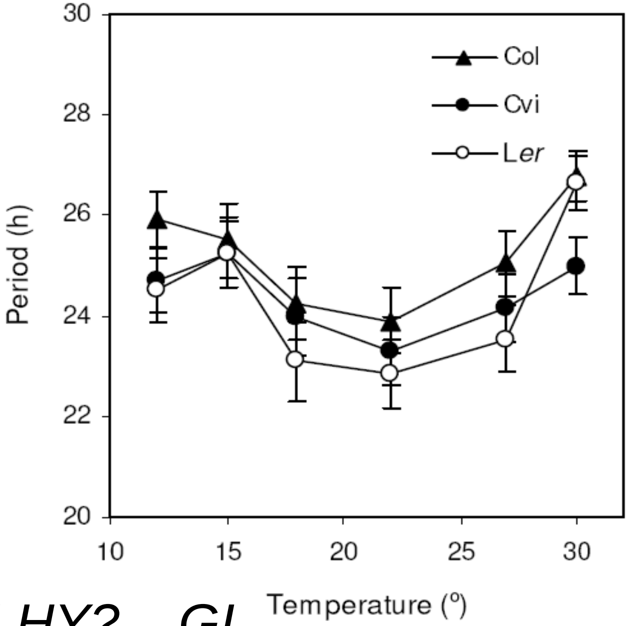
the dimension of the image is the flexibility

THE UNIVERSITY OF WARWICK

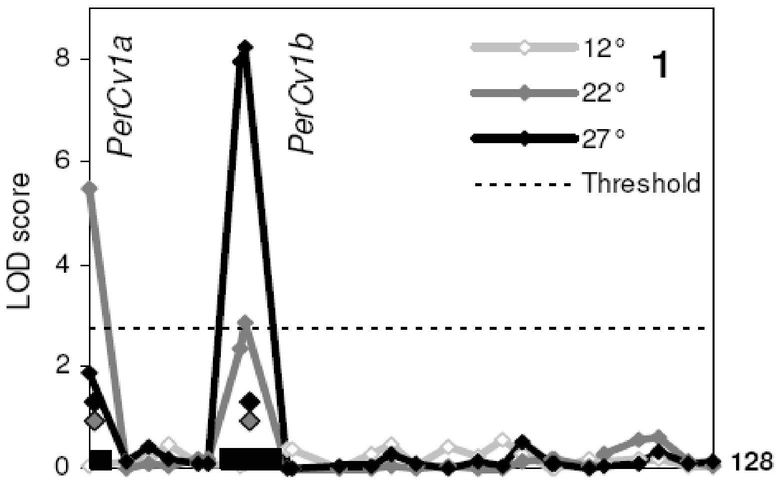
conclus

Clock complexity 2: temperature compensation

- Defining feature of circadian clocks
- Quantitative genetics (QTL) shows multiple genes are involved in Arabidopsis



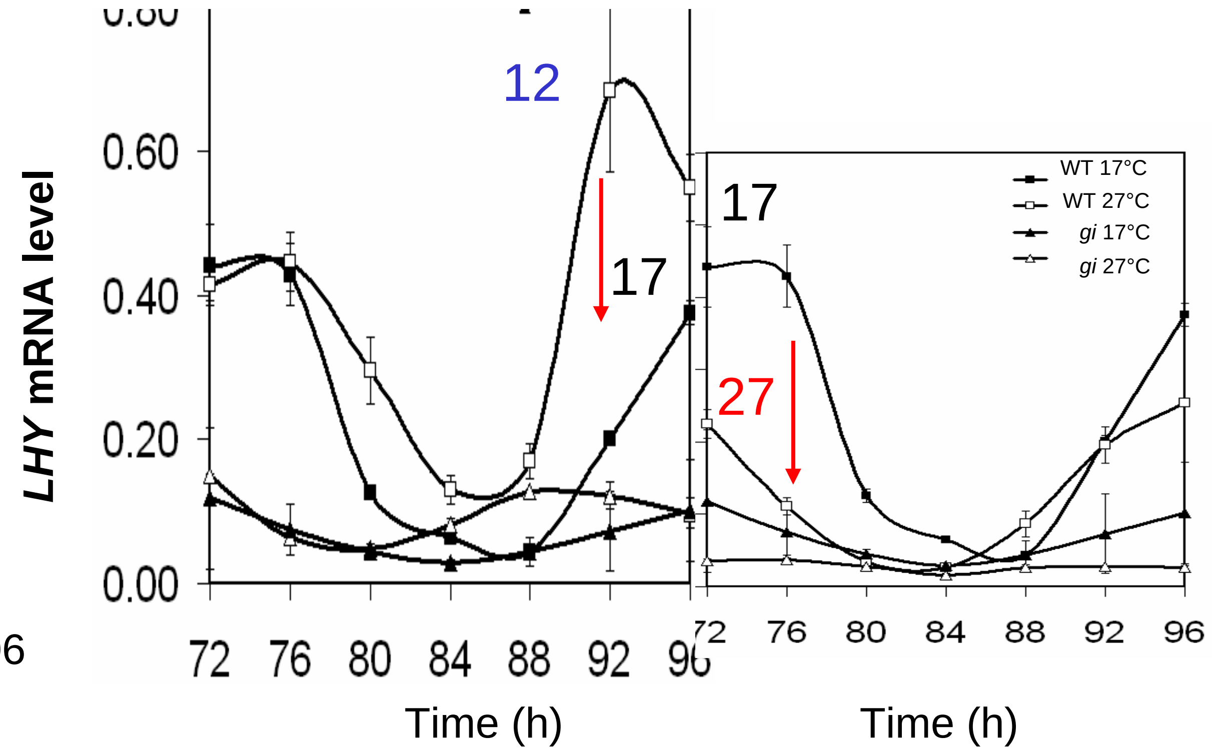
LHY? *GI*



Position along the chromosome

Edwards *et al*, Genetics 2005

Molecular mechanisms of temperature compensation



Gould *et al*,
Plant Cell 2006

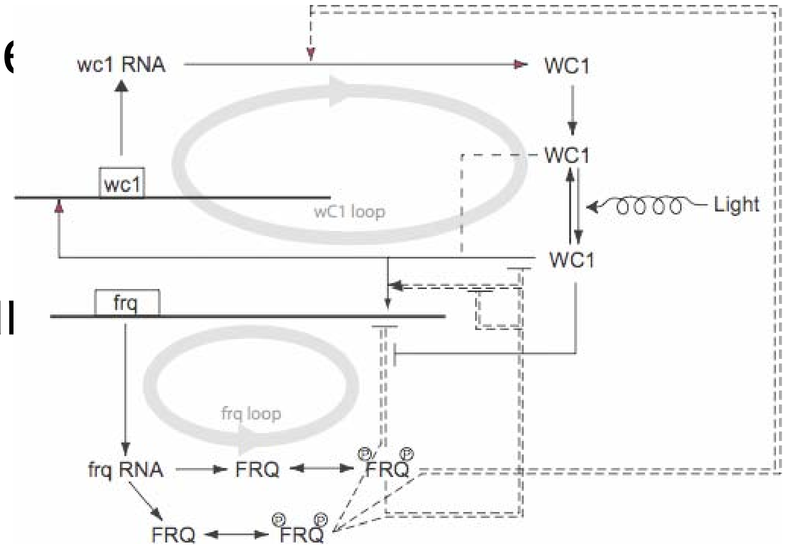
- *LHY* RNA levels fall with temp rise (*CCA1* rises)
- Mutant data confirms *LHY (*or *CCA1)* and *GI* are required
- Temperature-dependent substitution of homologous proteins?
  - To model, now split '*LHY'* to separate CCA1

Clock complexity 2: temperature compensation

- Homeostasis of period at a range of constant temperatures, unusual among biochemical oscillators
- Temperature affects many biochemical processes: parameters in the clock models
- ‘Antagonistic balance’ of period-lengthening and period-shortening effects
  - Gives local compensation at a particular temperature
  - Observed compensation covers wide temperature range
- Local constraints on parameters at many temperatures across the range? No, only TWO

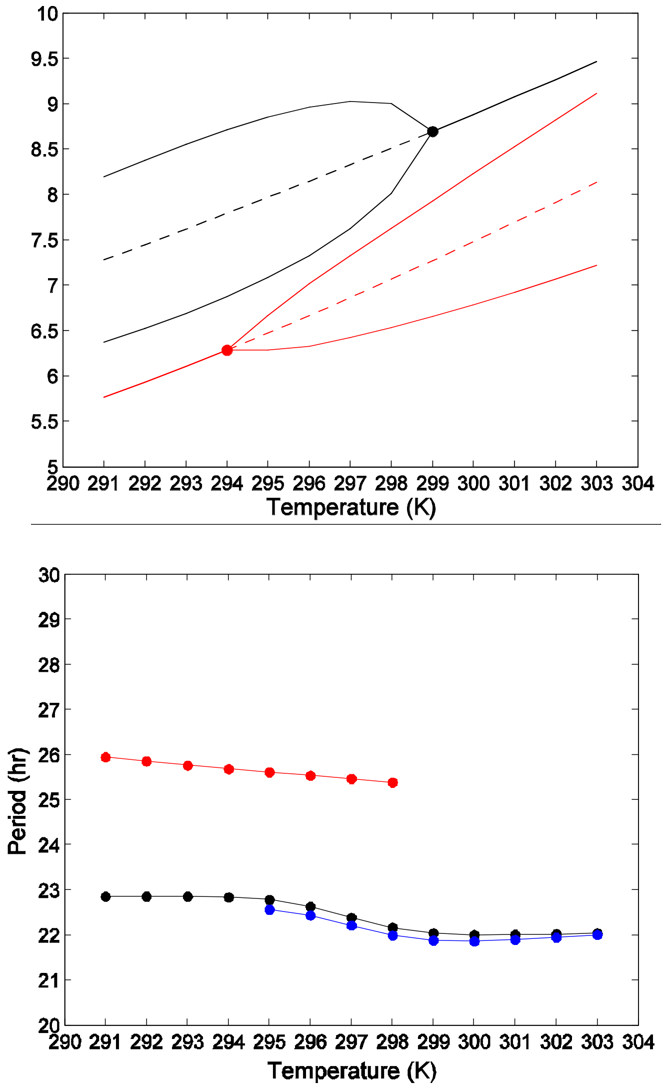
A switch for simple temperature compensation

- Alternative molecular speci
- Identical functions
- One dominates at low,
  the other at high temperatu

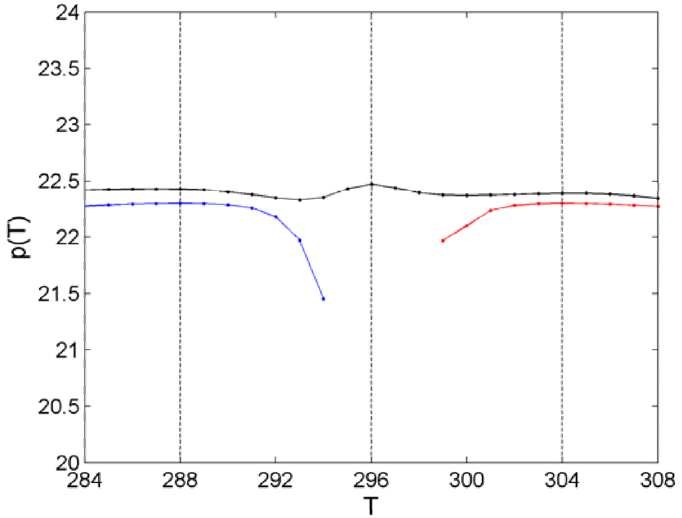


- Parameters affecting one species balance at one low temperature, the other at one high temperature
- Combining the two species gives compensation right across the temperature range

Switch between two molecular species



- Clock with only one species becomes arrhythmic at non-optimal temperature



- Rhythmicity and global temperature compensation with both species

Temperature compensation: summary

- Avoiding arrhythmia is a major challenge
  - If rhythmicity can be maintained across a temperature range, constant period is less surprising
- Design principle:
  Switch between two species allows tuning of some parameters for a high and a low temperature
  - biochemical mechanisms can vary, RNA or protein
  - deterministic
  - now to test experimentally…
- Global (temperature) compensation is readily achievable in evolution
- Long-standing questions…

Clock models are useful

- Inform experimental design
  - Simulation software for clock experiments (*www.amillar.org*)
  - Predict properties of undiscovered components (X and Y)
  - *GI* is part of *Y* function, another part also required
  - Analyse network properties, e.g. dawn/dusk tracking, temperature compensation
- Extend from clock to:
  - Molecular outputs: genome-wide rhythms at all phases
  - Whole organism: effect on growth via metabolism, morphology

Requirements for model building

- Multiple mutant data were key but will be limiting
  - ➢ Measure parameter values
  - ➢ Make more use of timeseries data to invalidate models
    - More and better timeseries, protein data
    - Environmental manipulation, e.g. light, nutrition
  - ➢ Modelling of species with hard genetics, e.g. crops, trees

- Large-scale informatics infrastructure
  - ➢ Organise diverse data, integrate with models, parameter search and analytical tools, High-Performance Computing
  - ➢ Coordination among centres should be possible

- Synthetic biological systems to validate models
  - ➢ Completely described, well-controlled
  - ➢ A biological "wind tunnel"