Circadian Clocks: What are They Telling Us Besides Time?
Circadian clocks

- Endogenous
- Period ~24 hrs
- Entrainment
- T - compensation
Ticking Genes of the Circadian Clock

Sauman and Hashimi (1999)
Illuminating the Circadian Clock in Monarch Butterfly Migration
Monarch spectacular migration

Migrations & Breeding Ranges
In Spring & Summer

Fall Migrations & Wintering Areas
Over-wintering grounds in Mexico
Monarch navigation

Magnetic compass

Sun compass
Monarch butterflies (*Danaus plexippus* L.) use a magnetic compass for navigation

Jason A. Etheredge*, Sandra M. Perez†, Orley R. Taylor‡, and Rudolf Jander*

*Department of Entomology, University of Kansas, Lawrence, KS 66045; and †Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721

PNAS November 23, 1999 | vol. 96 | no. 24 | 13845–13846

---

**RETRACTION**

ECOLOGY. For the article “Monarch butterflies (*Danaus plexippus* L.) use a magnetic compass for navigation” by Jason A. Etheredge, Sandra M. Perez, Orley R. Taylor, and Rudolf Jander, which appeared in number 24, November 23, 1999, of Proc. Natl. Acad. Sci. USA (96, 13845–13846), the authors note the following: “The positive response to magnetic fields in two experiments cannot be repeated. Further experiments show the false positives in these tests result from a positive taxis by the butterflies to the light reflected off the clothing of the observers. We therefore retract our report. We regret the inconvenience that publication of this study may have caused.”

---

Retraction: Monarch Butterflies (*Danaus plexippus* L.) Use a Magnetic Compass for Navigation

Monarch navigation

Magnetic compass  Sun compass

?
Sun Compass

- Light receptors
- Time compensation
- Circadian clock?
Circadian clock  
Light requirements for navigation

[A diagram showing different light conditions and their effects on navigation, including plots and graphs demonstrating the number of events in different directions for various filters.]
Compass has its own eyes ... polarized!!!
Polarized UV light for Monarch navigation
Compass - Clock connection

Dorsal rim axonal projections
Clock-compass model
Collaborations

Steven M. Reppert
Amy Casselman
Haisun Zhu
Oren Froy
Anthony Gotter
Patrick Emery

Adriana D. Briscoe
Acknowledgments

David Doležel  Roman Neužil
Silvie Fexová  Hanka Sehadová
Alena Kobelková  Jan Stehlík
Jan Němec  Radka Závodská
Hassan Mir Mohamed Hashimi
Collaborations

Magda Hodková          Steven M. Reppert
Marek Jindra           Bambos Kyriacou
Vladimír Košťál        Makio Takeda
Helena Illnerová       Daniel Bopp
Alena Sumová           Jeff Hall
# Clock Gene Expression in Brain

<table>
<thead>
<tr>
<th>PER</th>
<th>Silkmoth</th>
<th>Drosophila</th>
</tr>
</thead>
<tbody>
<tr>
<td>cell #</td>
<td>8</td>
<td>&gt;&gt;8</td>
</tr>
<tr>
<td>nucleus</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>axons</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>rhythms</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>per mRNA rhythm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>phase PER/per</td>
<td>synchronous</td>
<td>phase delay</td>
</tr>
<tr>
<td>per antisense RNA</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**TIM**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>co-localize</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>nucleus</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>axons</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Periplaneta americana

ICC

in situ hybridization
Fruitflies
and you can not see the smell...
Locomotor activity

FOOD

WATER
Musca domestica – L:D 12:12, 25°C

Musca domestica – L:D 12:12, D:D; 25°C

Musca domestica – L:D 12:12, L:L; 25°C
UAS/GAL4 binary system

GAL4 fly (homozygote)  UAS fly (homozygote)

hsp70 promoter
actin promoter
The “GeneSwitch” system

GeneSwitch Driver

UAS-Transgene

G₀

F₁

no ligand present

RU486

transcriptional activation of transgene
RNAi

“Dominant-Negative”
Recombinant Sindbis Virus System
What is Sindbis virus?

- Enveloped, single stranded plus-sense RNA virus
- Transmitted between birds and mosquitoes
- Non-cytopathic, persistent infection in mosquitoes, broad host range
- A second viral promoter enables expression of any transgene in vivo
Two CRYs of the butterfly
Functional analysis of CRY1 and CRY2
Photolyase/cryptochrome gene family
Phylogenetic analysis of \textit{pho/cry} gene family
Rescue of *Drosophila cry* mutant by monarch *cry1* transgene
Three models of insect clockwork
Circadian clock models

fruitfly

monarch
History

- Genetic bases of circadian clocks: 1971
- Molecular dissection of circadian clock: 1984
- Premier species: the fruitfly, *Drosophila*
- Molecular mechanism: negative feedback loop
- Conserved features = conserved mechanism???
- Cloning of *per* homolog outside *Drosophila*: 10 years
Cloning of Clock Gene *period* from Silkmoth

Reppert, Tsai, Roca and Sauman
*Neuron* 1994, 13:1167-1176
Silkmoth *per* Expression in *Drosophila*

**ZT0**  
**ZT12**

- CBr
- Ey
- OL
Locomotor Activity Rhythm

transgenic fly

per⁰ sibling
Class Insecta

- Archaeognatha: *Lepismachilis y-signata*
  - Zygentoma
- Ephemera: *Siphlonurus armatus*
- Odonata: *Ischnura elegans*
- Orthoptera: *Locusta migratoria*
- Plecoptera: *Perla burmeissteriana*
  - Blattaria
- Hemiptera: *Notonecta glauca, Gerris pallidum*
  - Coleoptera: *Pachnoda marignata*
  - Lepidoptera
- Hymenoptera: *Apis mellifera*
- Trichoptera: *Hydropsyche contubemalis*
  - Diptera: *Neobellaria bullata, Phormia recina*

THYSANURA

PALAEOPTERA

POLYNEOPTERA

PARANEOPTERA

HOLOMETABOLA
PER localization

- Drosophila
- Lepidoptera
- Hymenoptera
- Heteroptera (Pyrrhocoris)
- Blattodea (Periplaneta)
- Thysamura
- Mus
- Homo
Musca domestica ICC

PER

TIM

CLK

CRY
RPA - timeless RNA
Chymomyza costata
(Drosophilidae)

Vladimir Kostal Lab
TIM Immunocytochemistry

A

1

2

wild strain

1

2

mutant strain

B

C
Conclusions & Future Prospectives

**Drosophila vs. Insects?**

- Nuclear Translocation
- Antisense RNA

**Missing Genetics!**

- Transgenic Studies
- RNA Interference
- Sindbis virus
The major aim

development of new methods that would allow functional genetic studies in insects other than *Drosophila*.

(P-element does not work outside *Drosophila*)

Reject intuitive assumptions on gene function based on its homology, get hardcore genetic data.
Tribolium castaneum - a novel genetic model

- Easy breeding
- Short generation time
- Sequenced genome
- Efficient transgenesis
- Larval/parental RNAi
Antheraea pernyi

Sauman and Reppert
Neuron 1996, 17:889-900
Sauman, Tsai, Roca and Reppert
Neuron 1996, 17:901-909
Sauman and Reppert
Neuron 1998, 20:741-748
Model of *period* regulation

Light requirements for circadian clock entrainment

No UV (>394 nm)
Location of a circadian clock in monarch brain

PER

CRY
CRY fibers

Dorsal rim axonal projections
Monarch cry2 - a homolog of mammalian cry !!!
CRY2 in monarch brain
CRY2 fibers

Central body
Cirkadiánní biologické hodiny

- Endogenní
- Perioda ~24 hodin
- Entrainment
- Teplotní kompenzace
piggyBac transformation of the housefly
piggyBac  Sindbis

Marek Jindra Lab