Circadian clock and photoperiodism in the pea aphid *Acyrthosiphon pisum*

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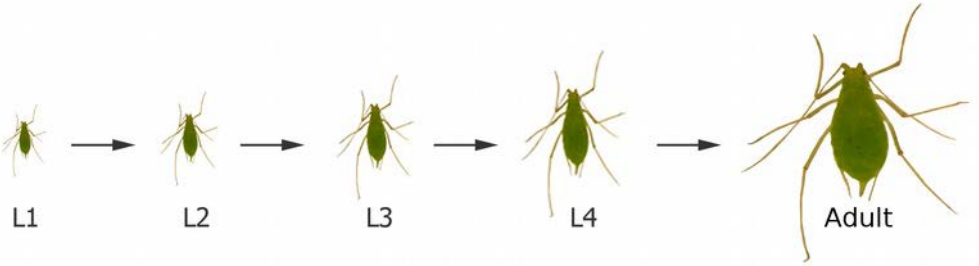
Aphids

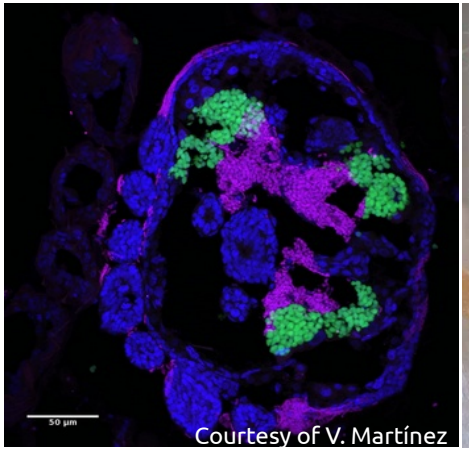
- Ovoid shaped, soft body small insects
- Stylet-like mouthparts
- 4 nymphal stages
- 4700 species, some crop pests
- Symbiosis
- Complex life cycles
- Several polyphenisms


Acyrthosiphon pisum


A. pisum




Megoura viciae

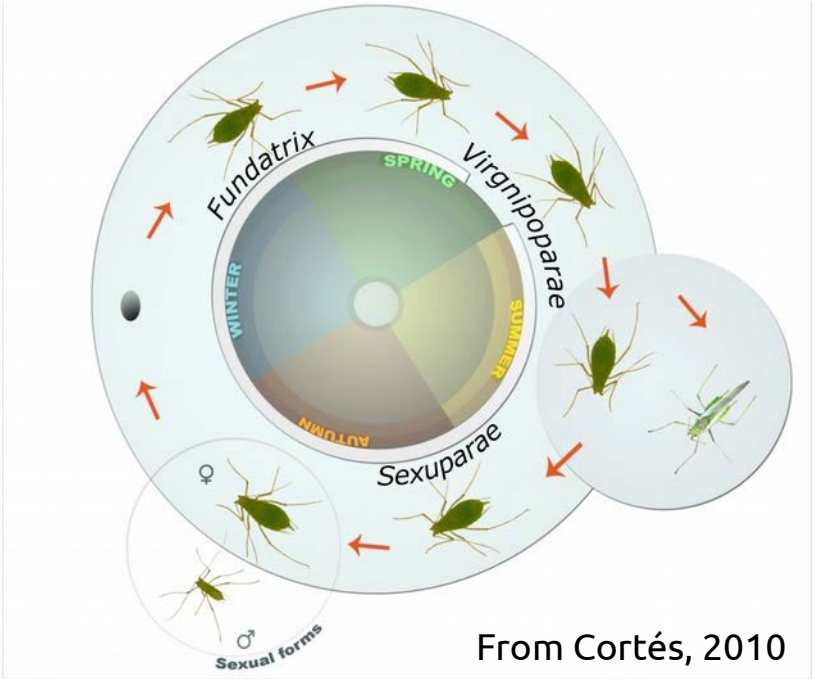

Courtesy of V. Martínez


Geoica pellucida
Courtesy of A. Salazar


Aphis sp

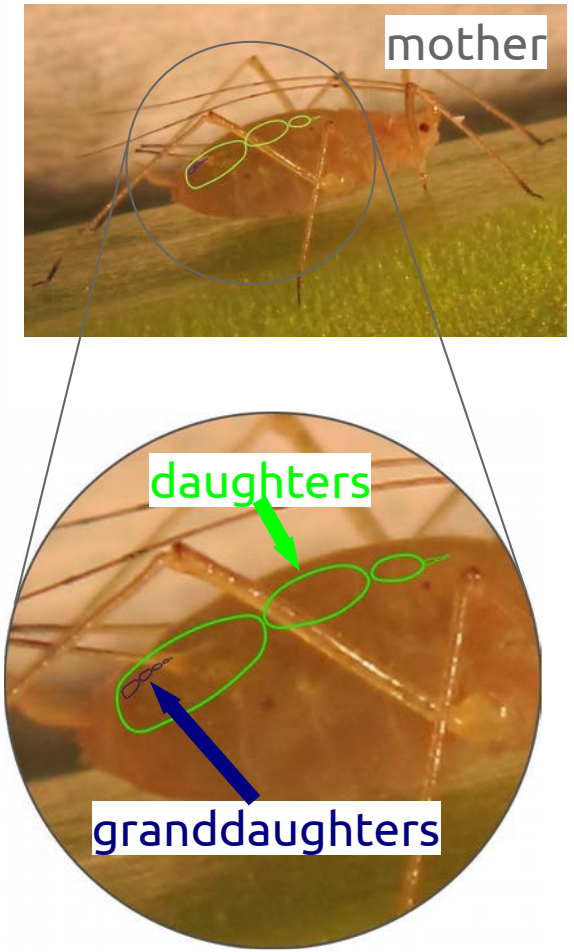
Cyclical parthenogenesis

Cyclical parthenogenesis



From Cortés, 2010

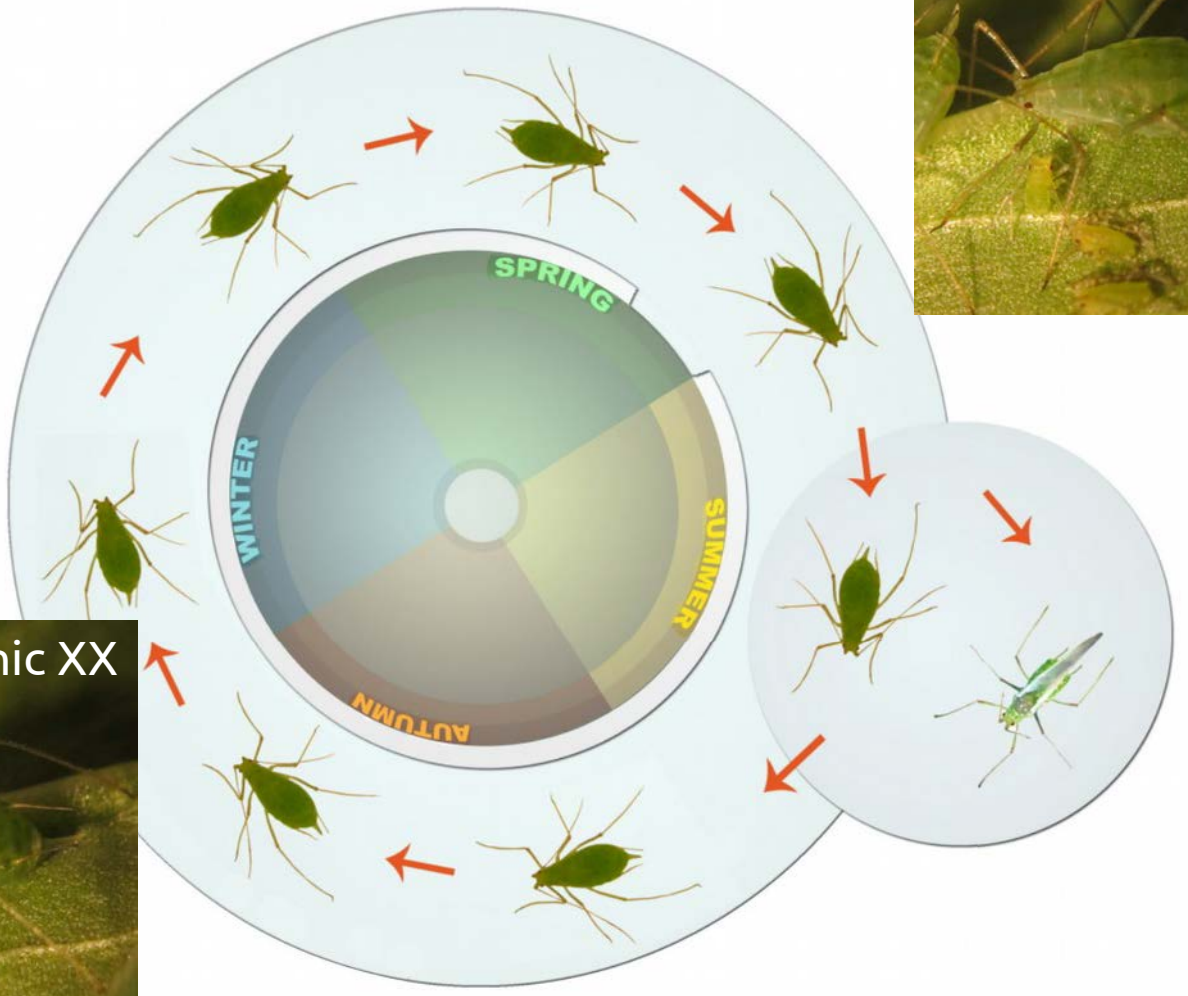
Telescoping of generations



Apomixis: asexual reproduction in which females produce diploid (2n) eggs by a modified meiosis with a single division.

Aphid life cycle

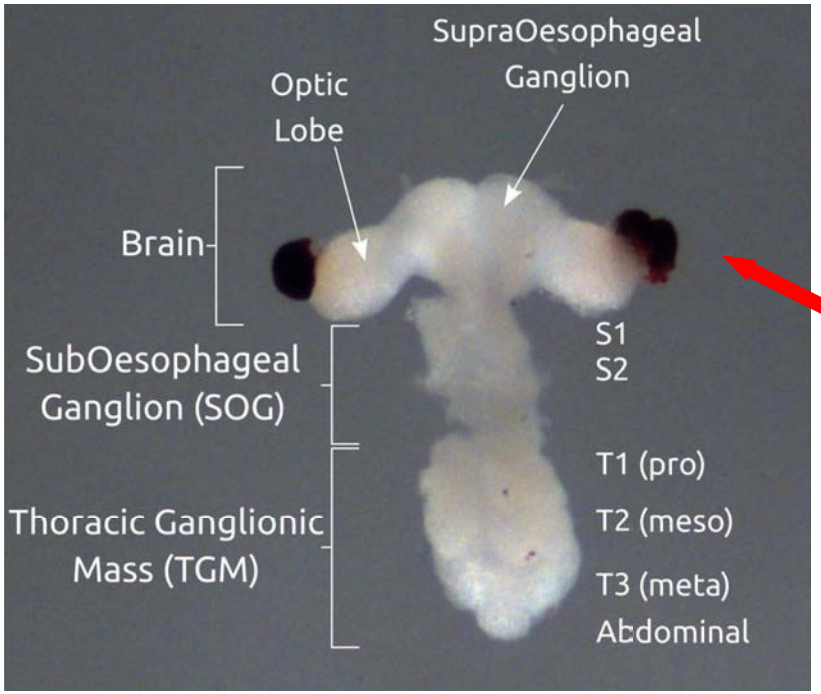
ANHOLOCYCLIC APHIDS

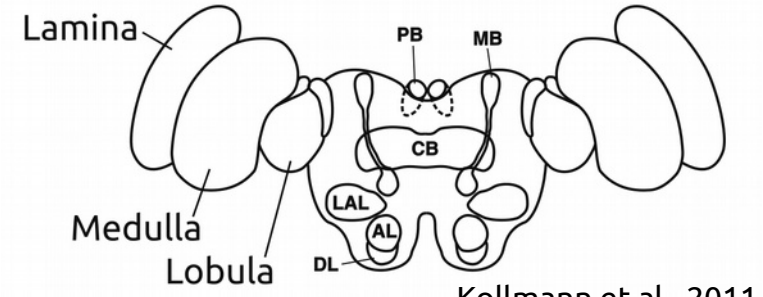


Modified from Cortés, 2010

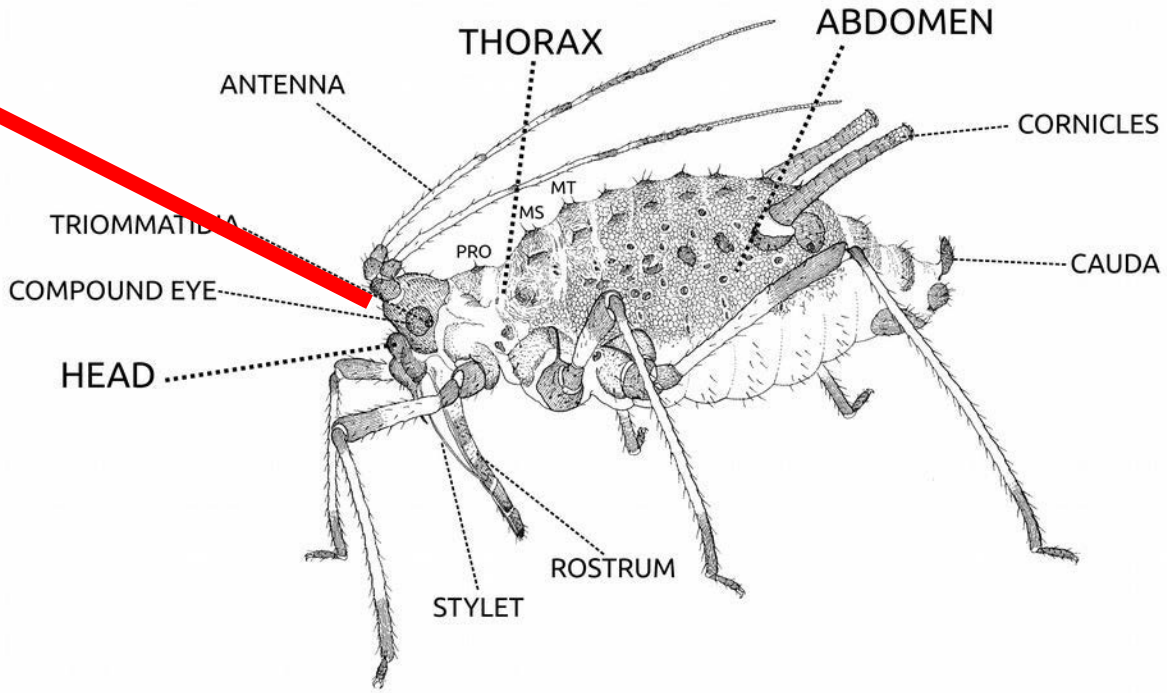
Aphid central nervous system

CENTRAL NERVOUS SYSTEM



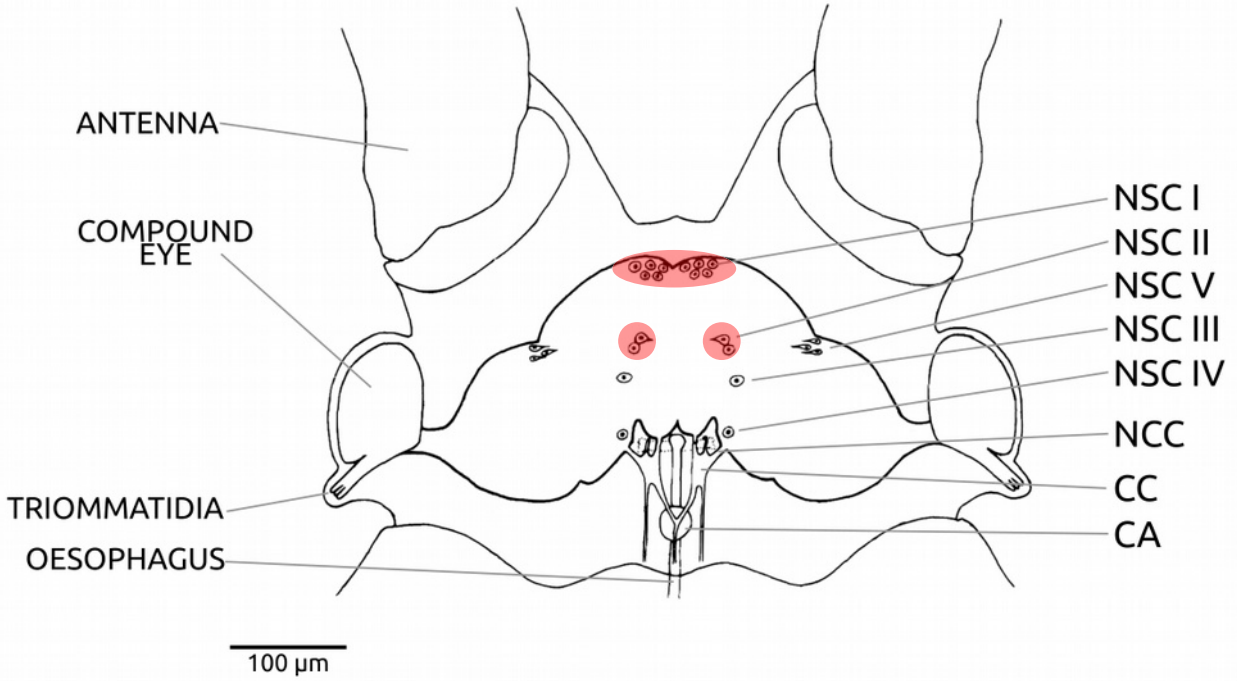


Kollmann et al., 2011

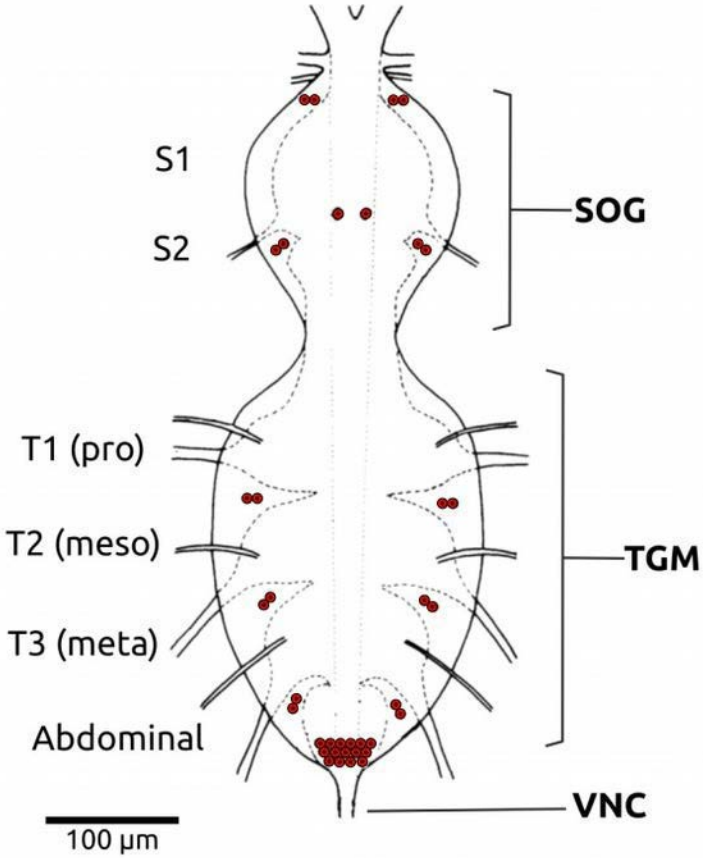


Aphid neurosecretory system

Modified from Steel, 1977

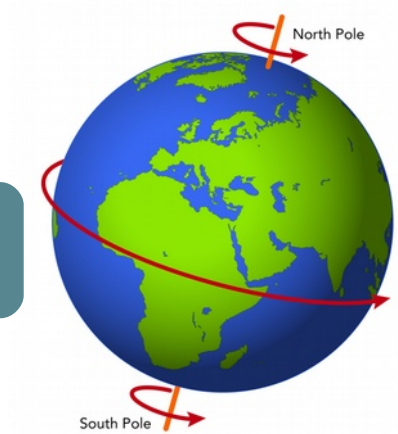


NSC = NeuroSecretory Cells



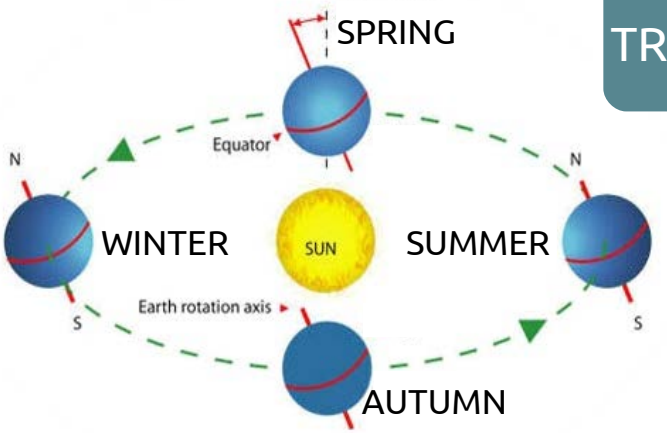
Biological rhythms

ROTATION

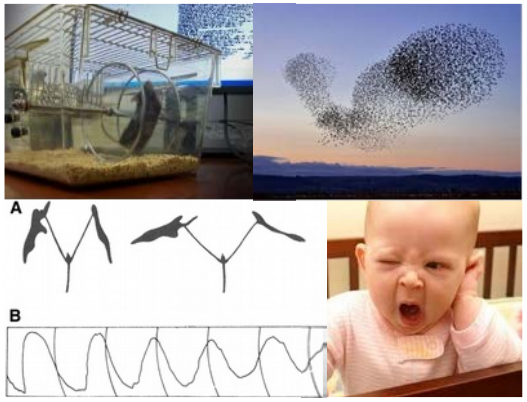


LIGHT/DARK cycles

TRANSLATION



SEASONS



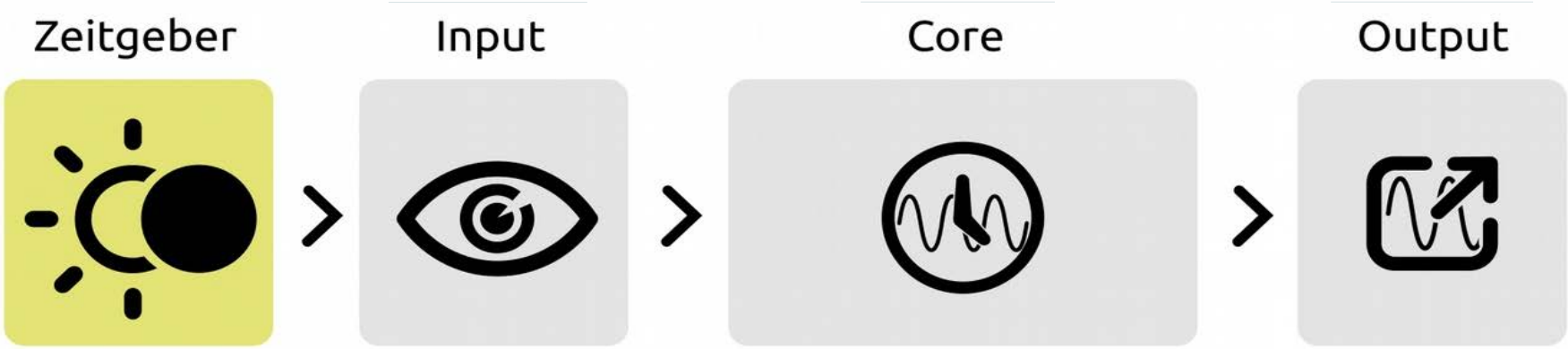
CIRCADIAN RHYTHMS
(24h period)

Zeitgeber → LIGHT

SEASONAL RHYTHMS
(1 year period)

Zeitgeber → PHOTOPERIOD

Circadian clock



Light
Temperature

Protein receptors
(Cryptochromes, Opsins)

Sense
External cues

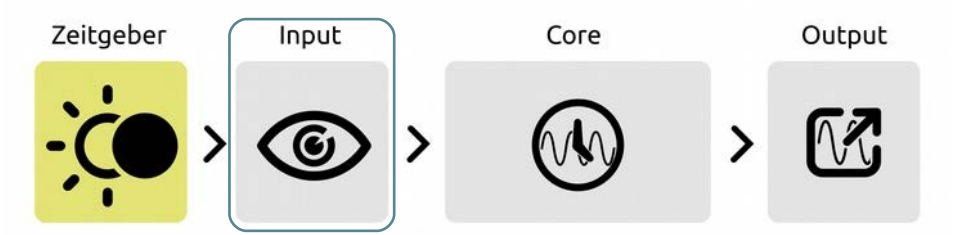
Transcription factors
Kinases & Phosphatases

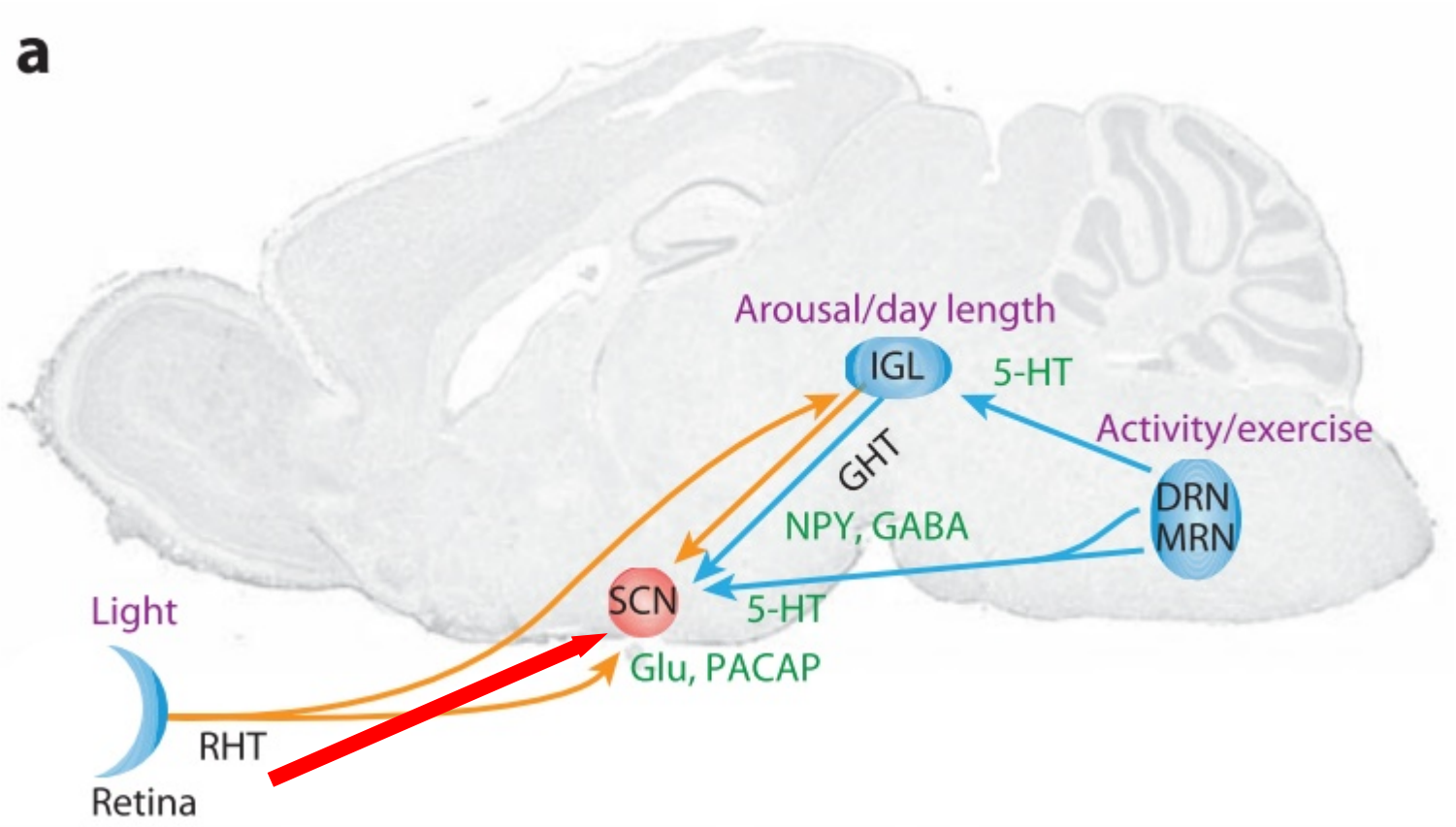
Autonomous
Oscillator

Neuropeptides
Hormones

Convey rhythmic
information
through the organism

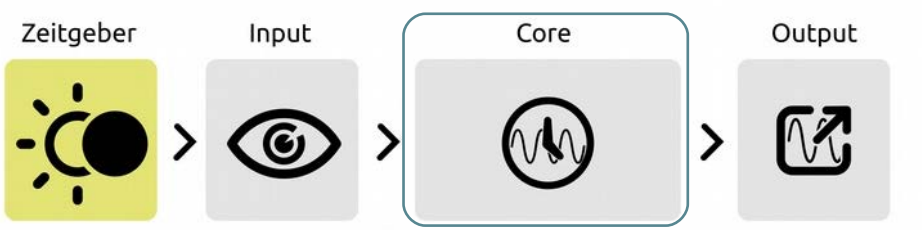
Circadian clock: input



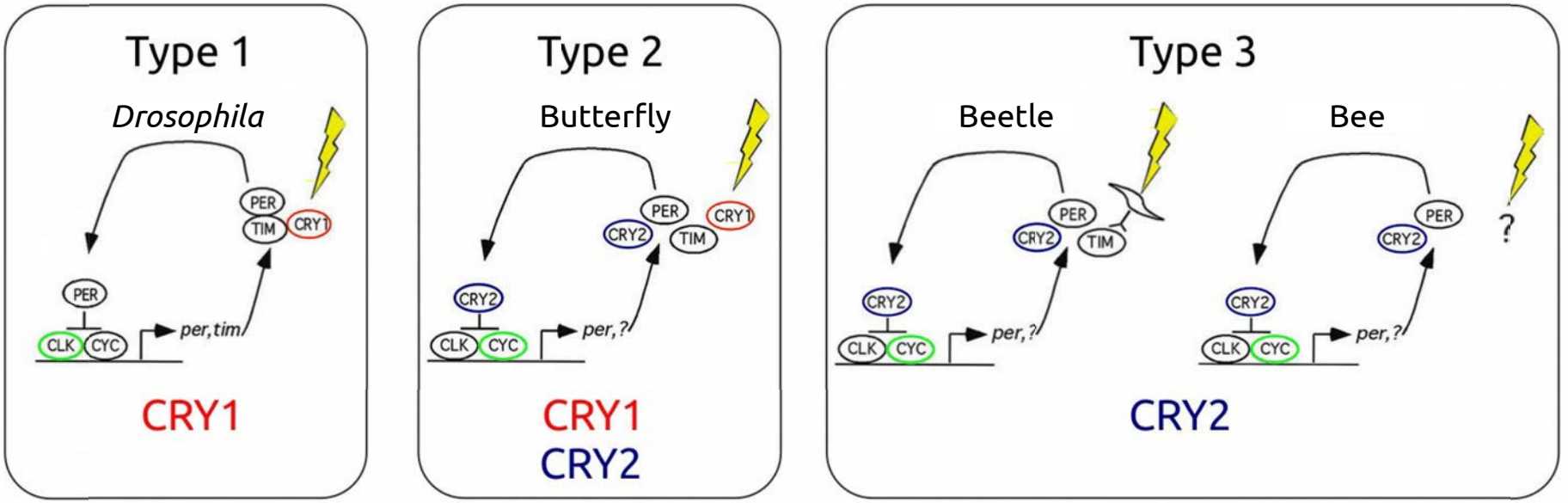


Dibner et al., 2010

Circadian clock: core

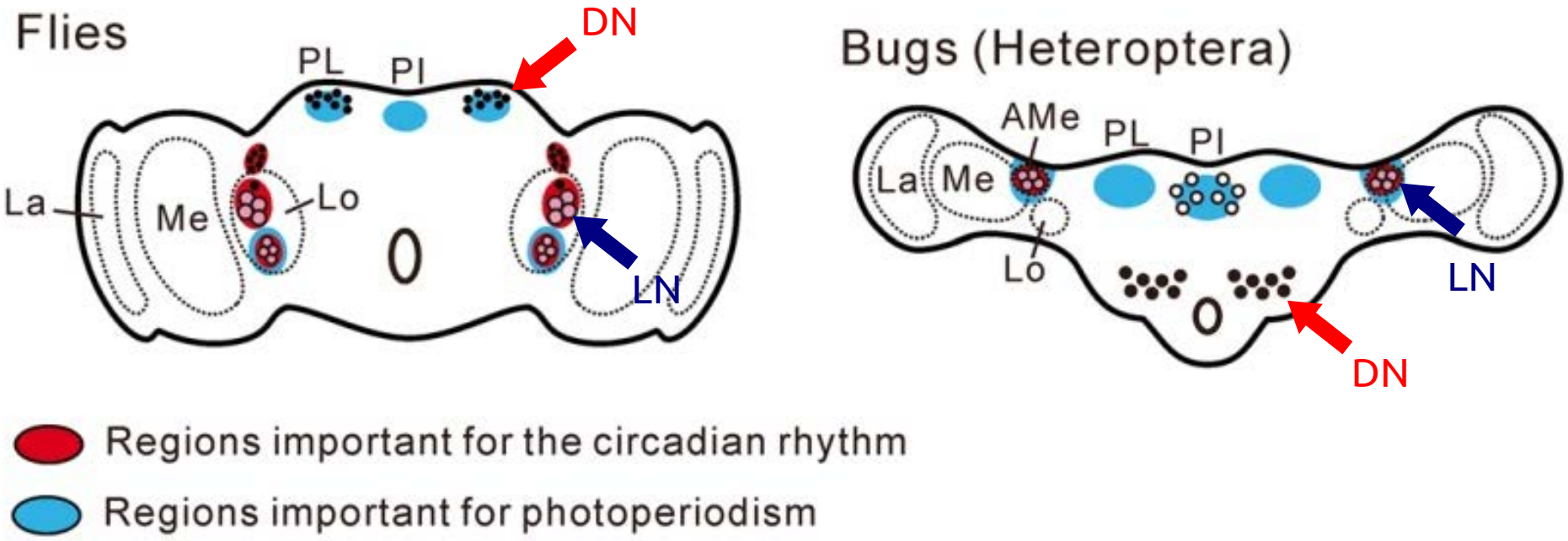


ANCESTRAL



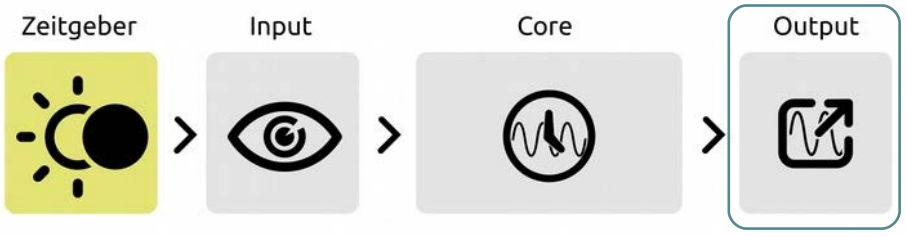
Modified from Yuan et al., 2007

Localization of circadian clock

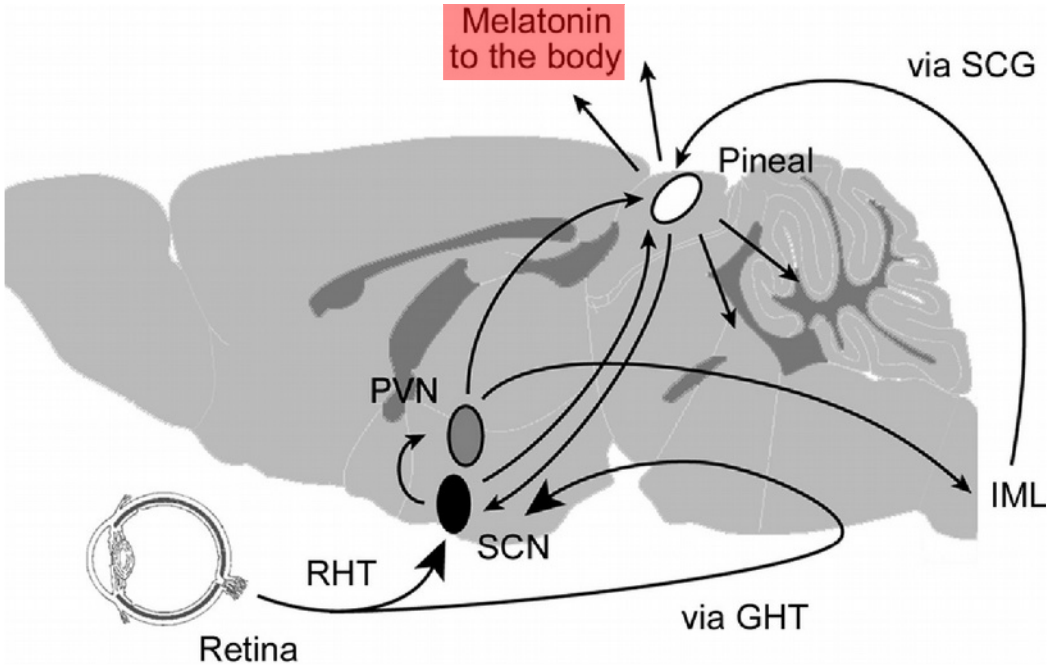


Modified from Numata et al., 2015

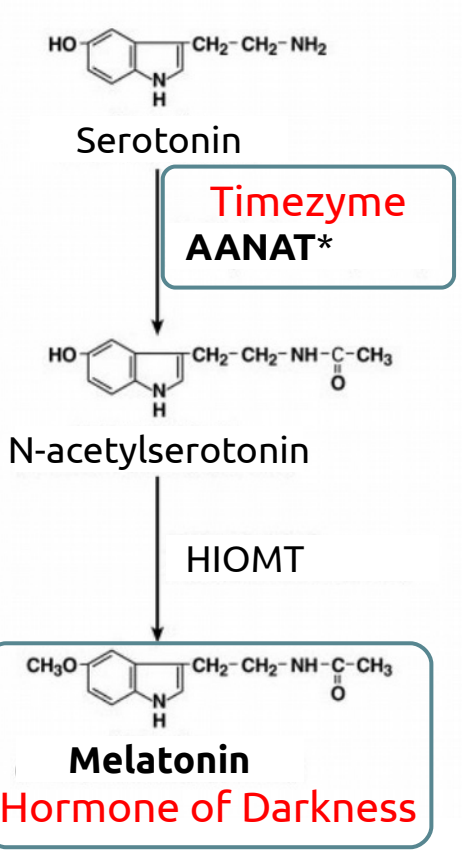
Circadian clok output



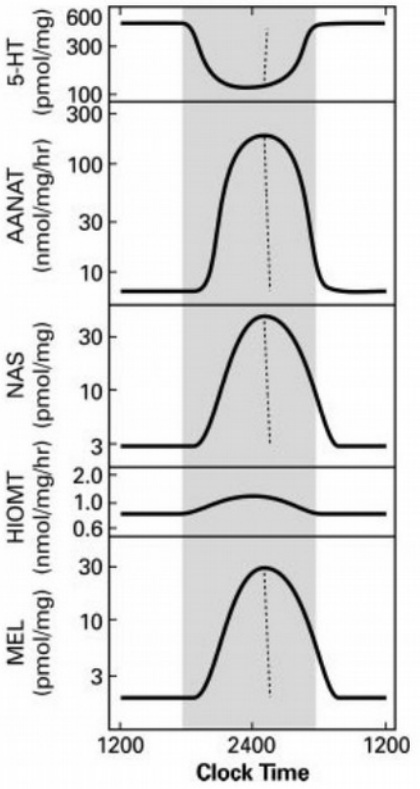
Mammals



Oster et al., 2002

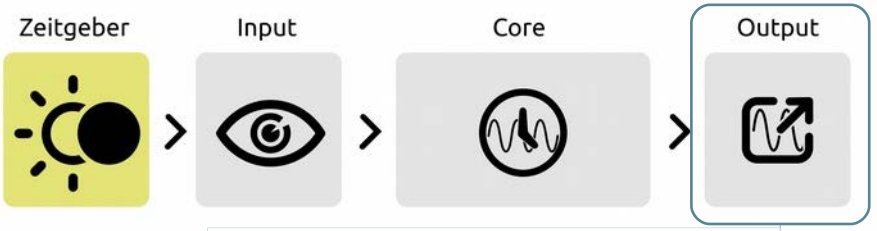


Klein, 2007



*AANAT = aryl alkylamine N-acetyl transferase

Circadian clock: output in insects

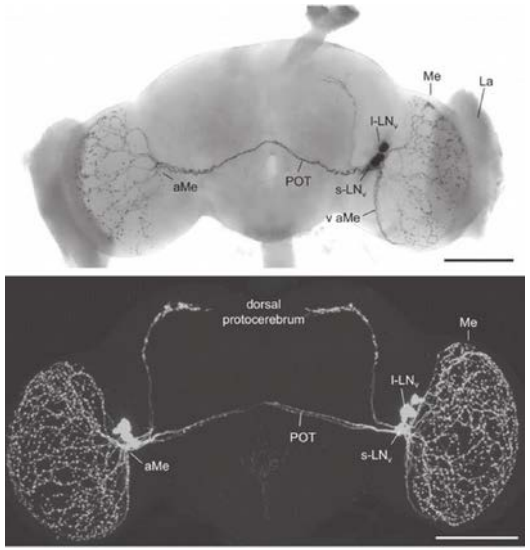


Insects

Pigment Dispersing Factor (PDF)

Crustaceans	N S E L I N S I L G L P K V M N D A – NH_2
	(L/I) (N/V/L/S/T)
Insects	N S E X I N S L L X L P K X X N D A – NH_2
	(G/S/A) (L/M)
Drosophila	N S E L I N S L L S L P K N M N D A – NH_2

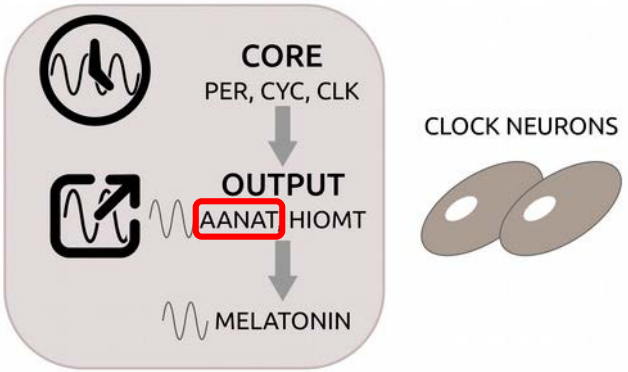
Shafer & Zao, 2014



Helfrich-Förster, 2009

AANAT & Melatonin

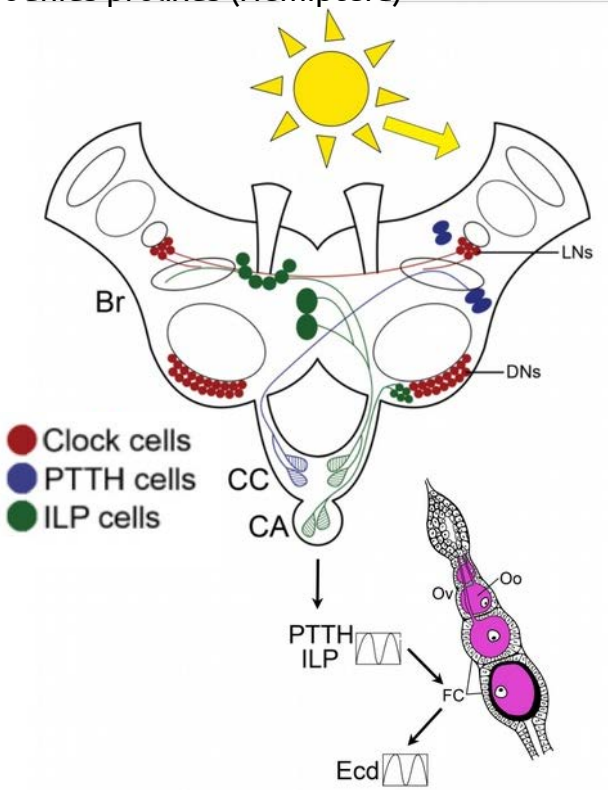
Antheraea pernyi (Lepidoptera)



Mohamed et al., 2014

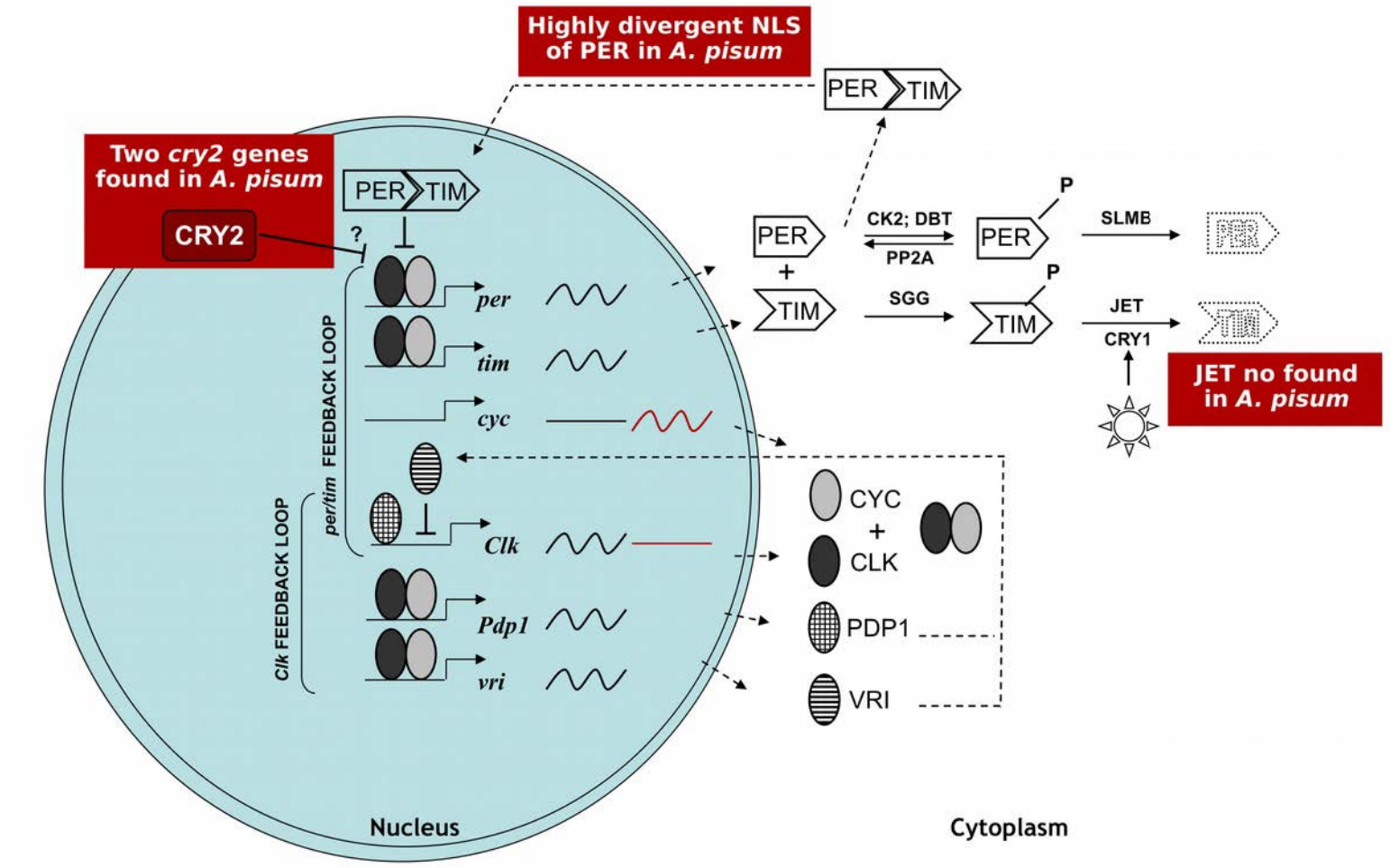
Prothoracicotropic Hormone (PTTH) & Ecdysteroids

Rhodnius prolixus (Hemiptera)



Cardinal-Aucoin, 2013

Circadian clock in aphids

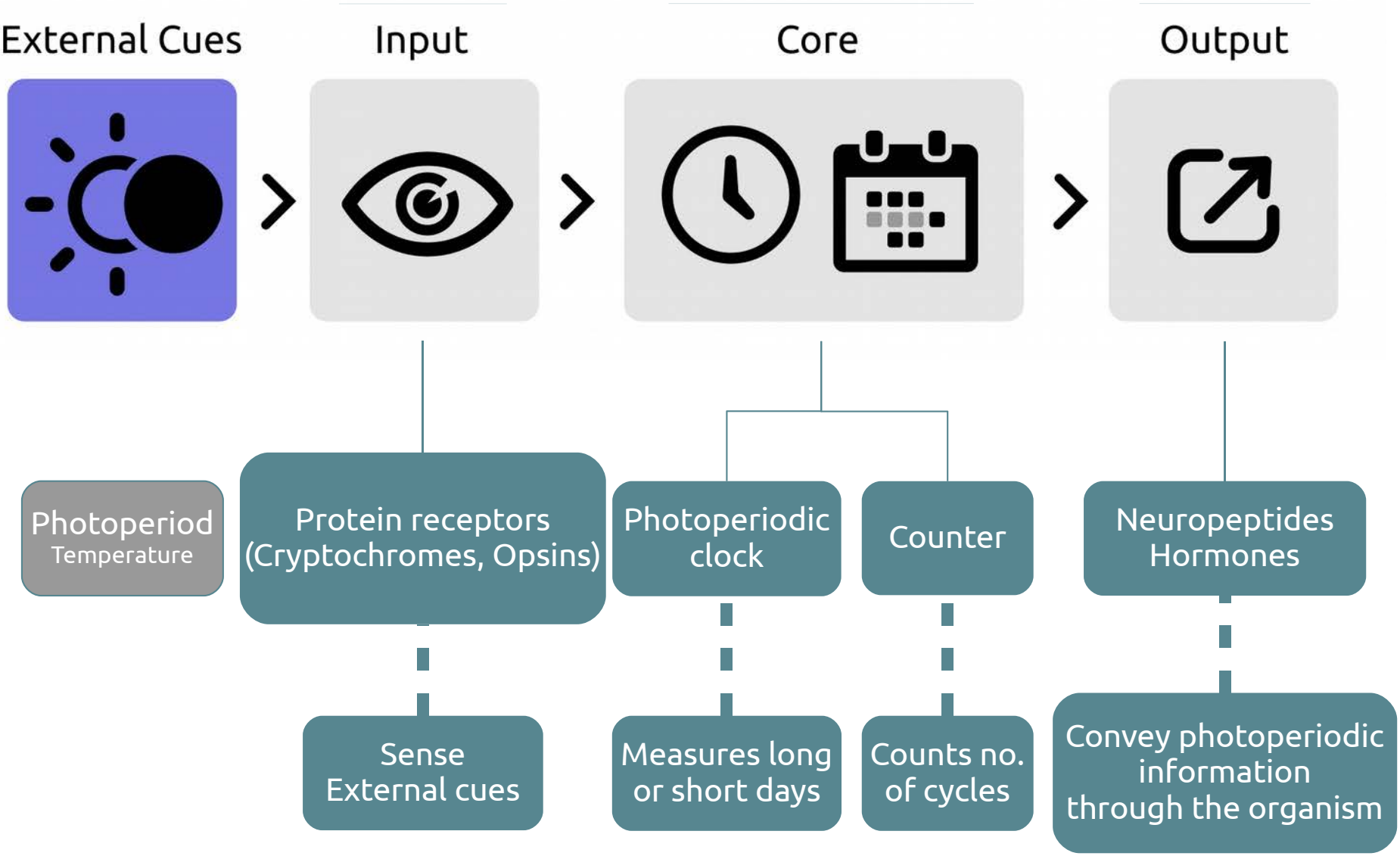


Cortés et al., 2010

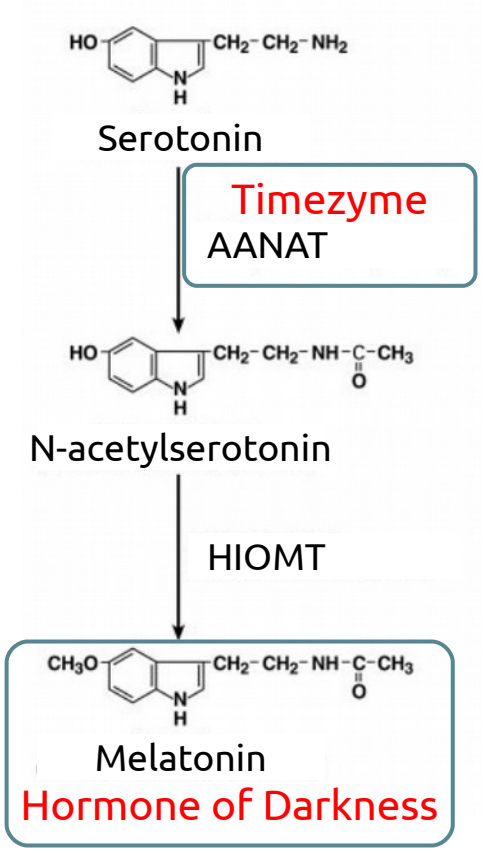
Seasonal rhythms and photoperiodism

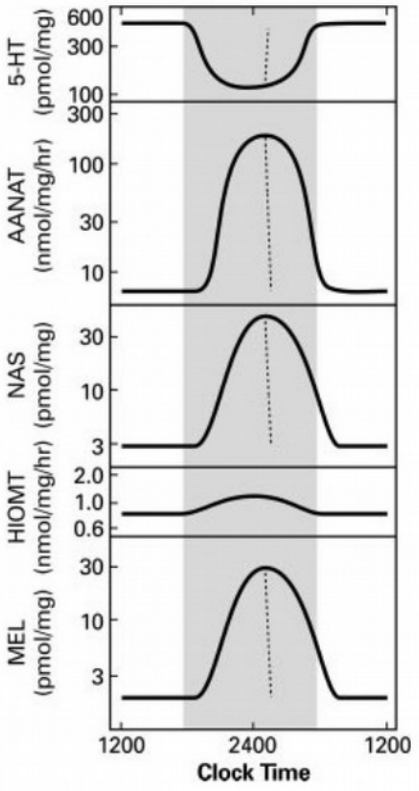
Photoperiodism: the use of changes in the day length on an annual basis to regulate seasonal behavioural or physiological processes.
(Dunlap et al., 2004)

Photoperiodic calendar

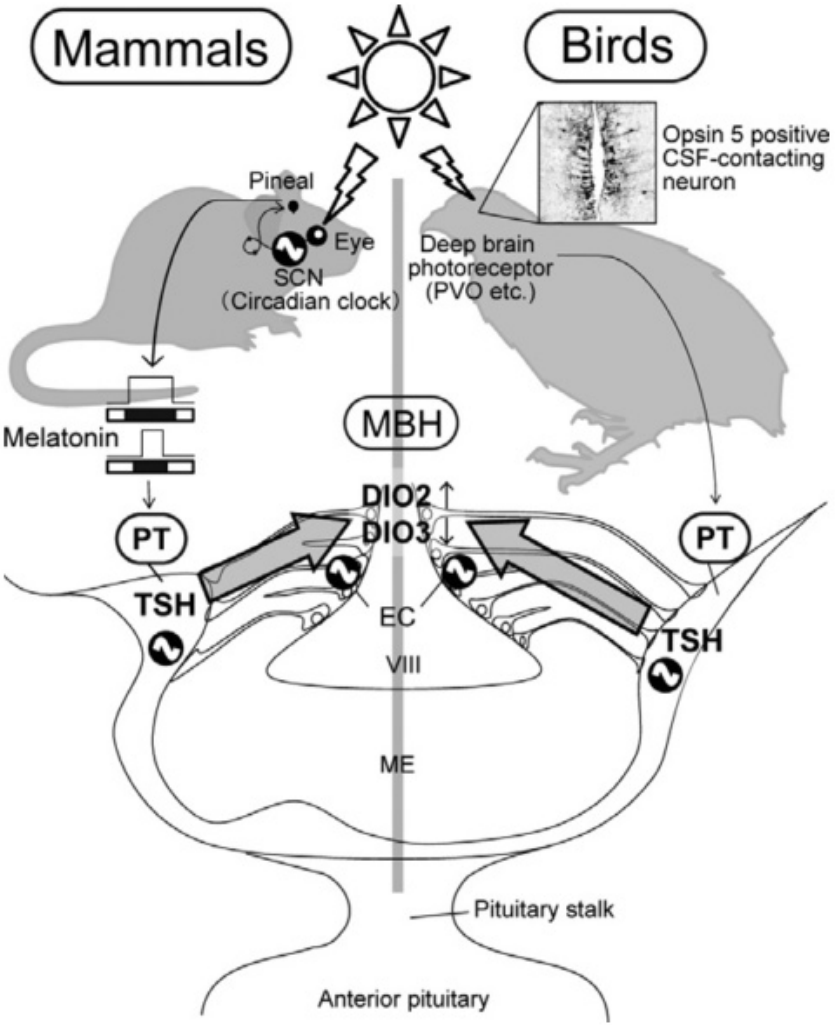


Photoperiodic calendar in mammals



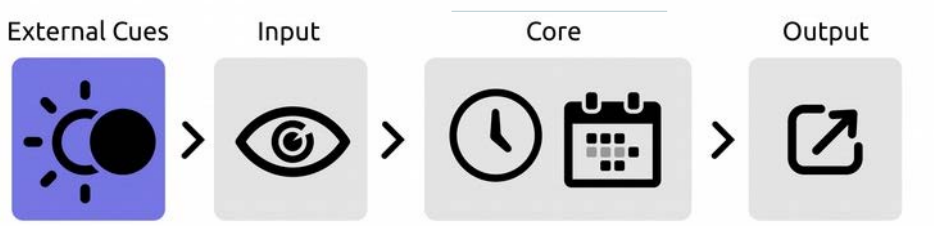


Klein, 2007



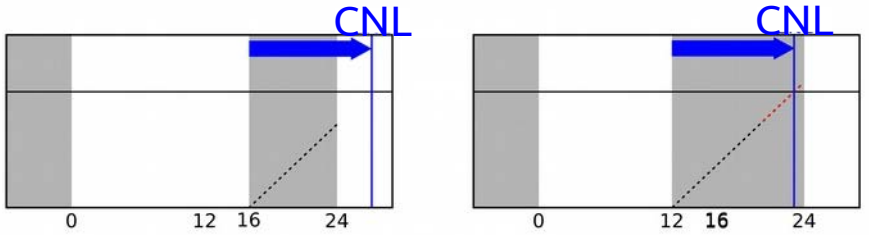
Ikegami & Yoshimura, 2012

Photoperiodic calendar: core



non circadian clock based

HOURGLASS

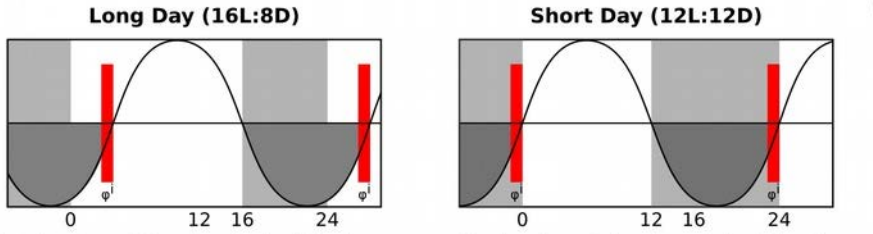


Nights shorter than CNL.
LD response.

Night longer than CNL.
SD response.

circadian clock based

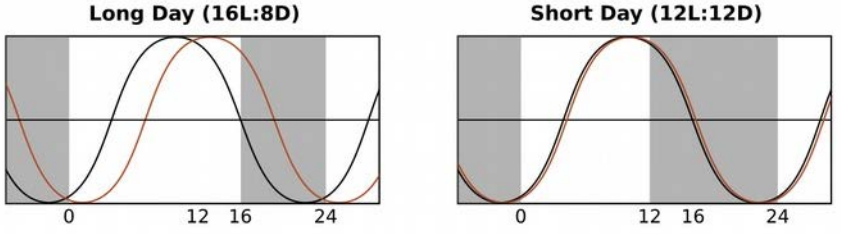
EXTERNAL COINCIDENCE



Illumination of the photoinducible phase.
LD response.

No ilumination of the photoinducible phase.
SD response.

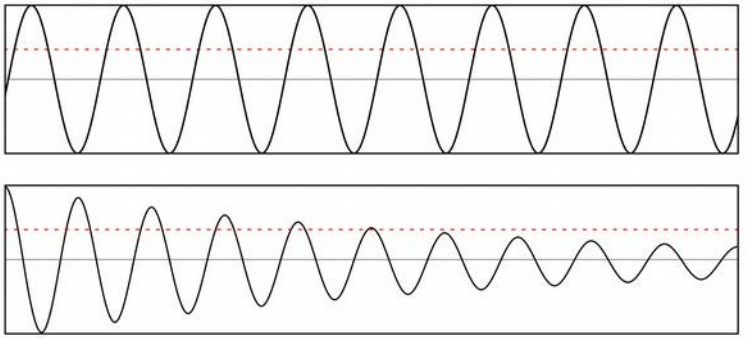
INTERNAL COINCIDENCE



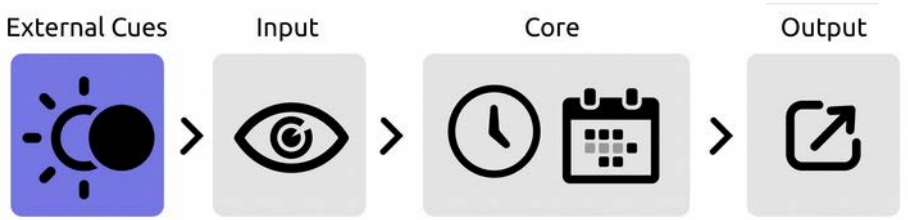
Two oscillators in asynchrony.
LD response.

Two oscillators in synchrony.
SD response.

HEAVILY DAMPENED OSCILLATOR

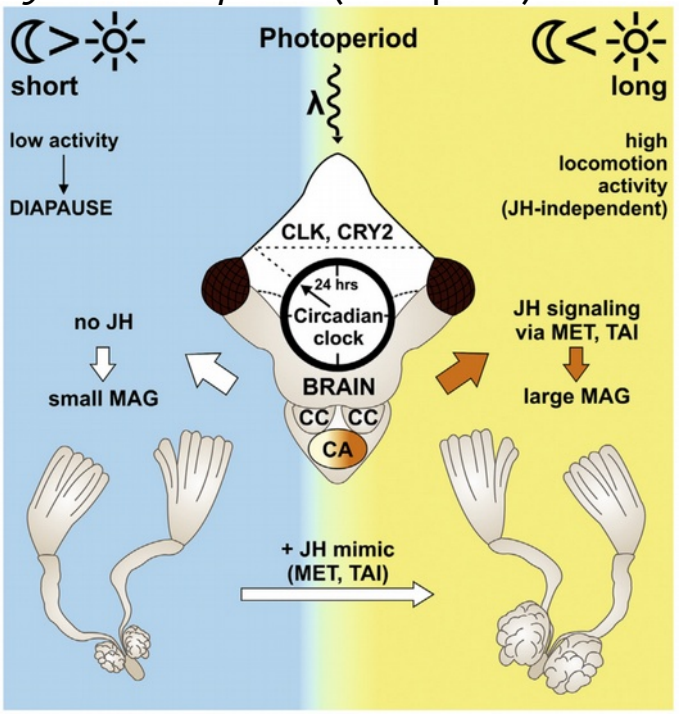


Photoperiodic calendar output in insects



Allatoregulatory Factors & Juvenile Hormone

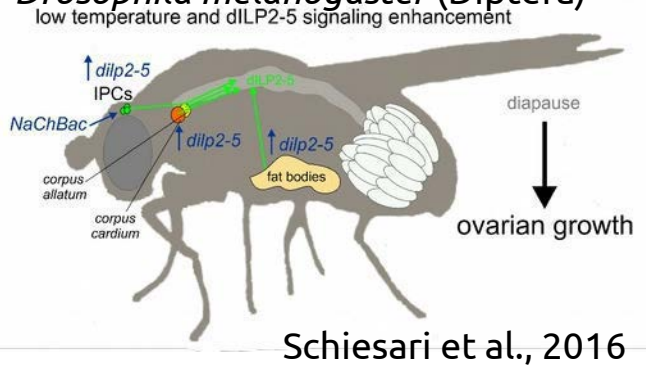
Pyrrhocoris apterus (Hemiptera)



Urbanová et al., 2016

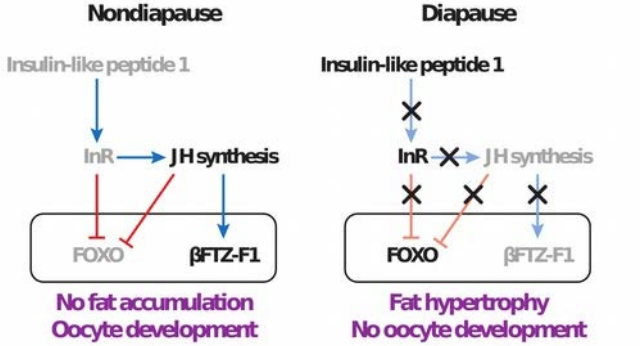
Insulin-like Peptides

Drosophila melanogaster (Diptera)



Schiesari et al., 2016

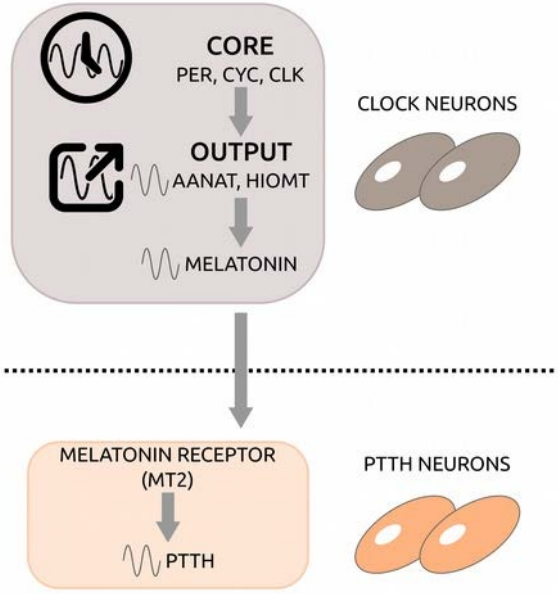
Culex pipiens (Diptera)



Denlinger & Armbruster, 2014

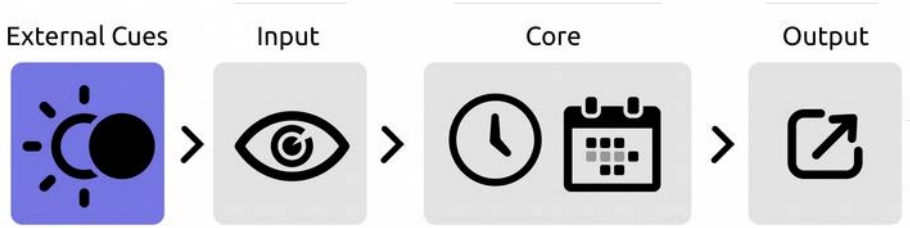
Melatonin, AANAT & PTTH

Antheraea pernyi (Lepidoptera)

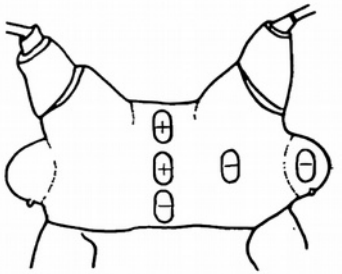


Mohamed et al., 2014

Photoperiodic calendar in aphids



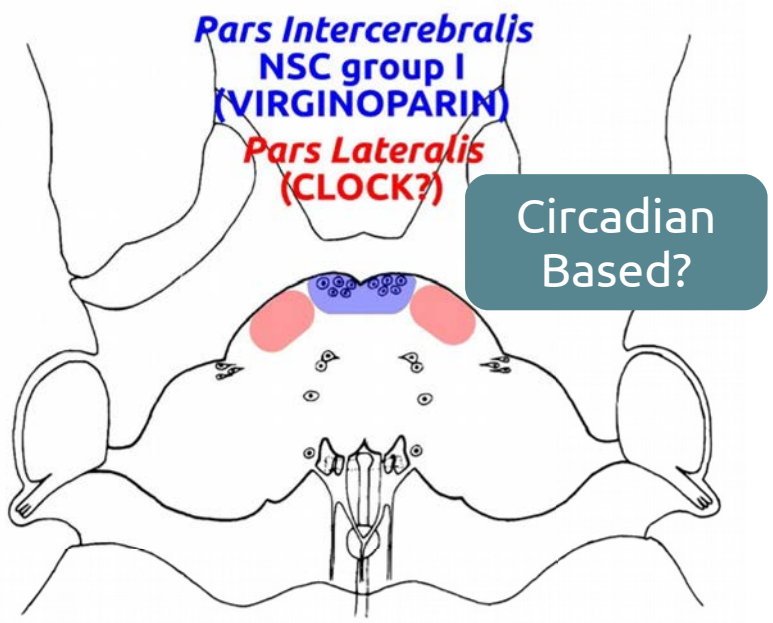
Photoperiodic Input



Lees, 1964

CRY?
Opsins?

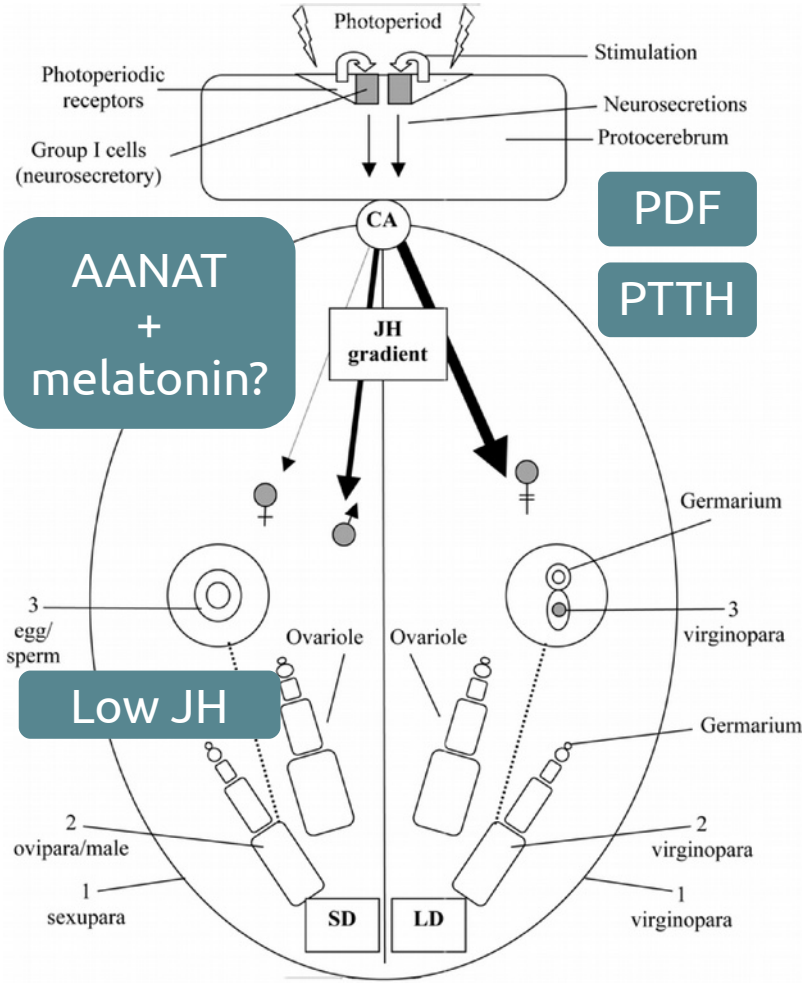
Core mechanism



Circadian Based?

Modified from Steel, 1977

Photoperiodic Output



PDF

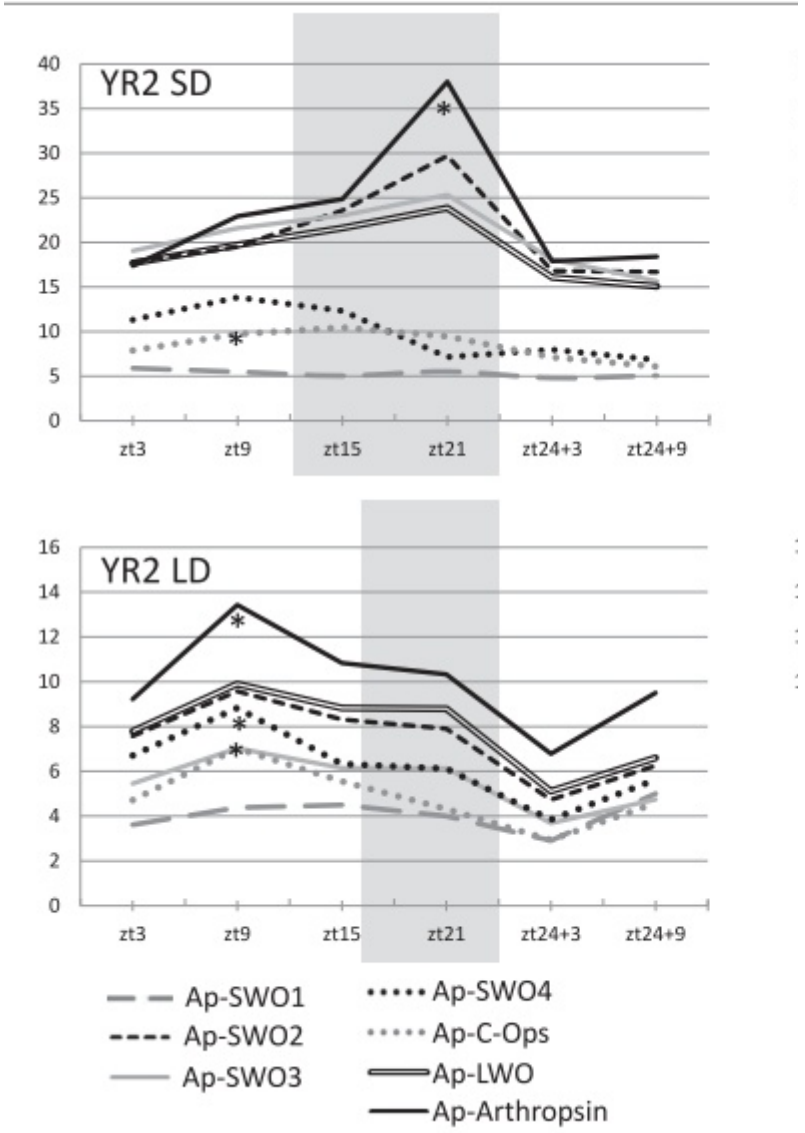
PTTH

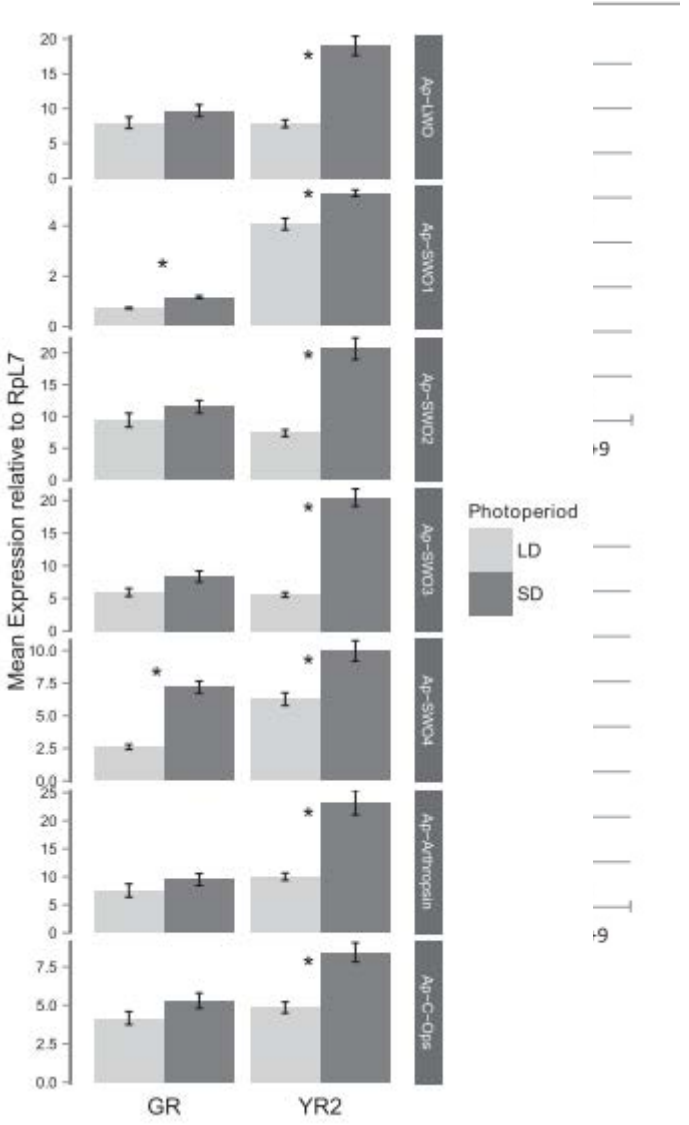
AANAT + melatonin?

Low JH

Tagu et al., 2005

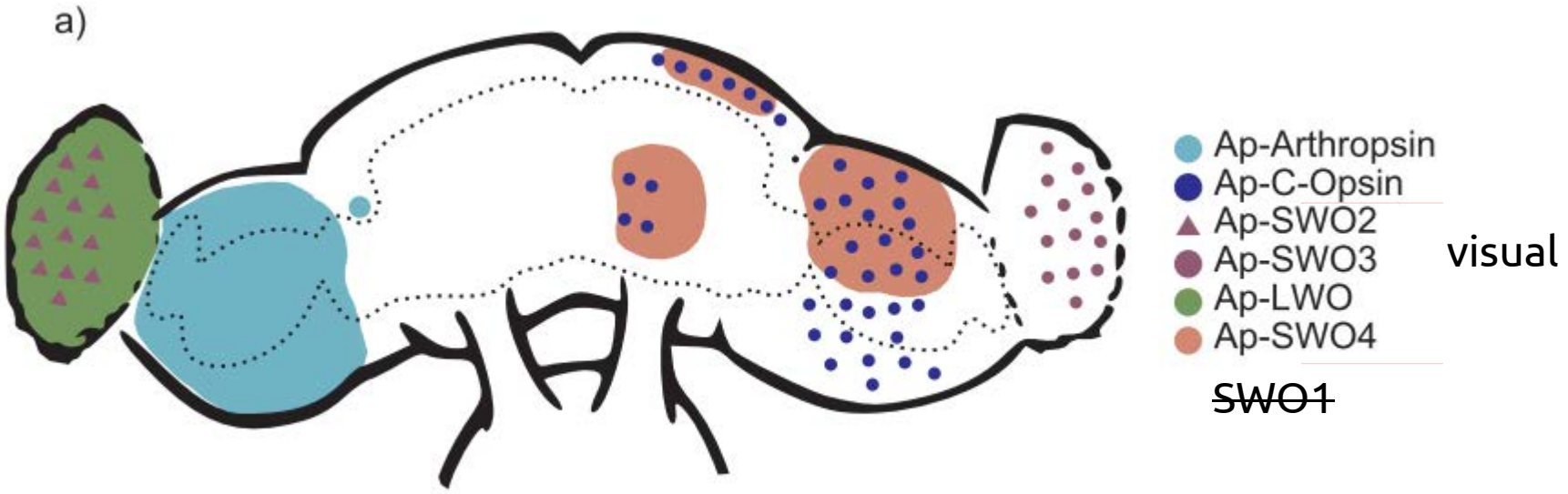
Circadian clock input in *A. pisum*





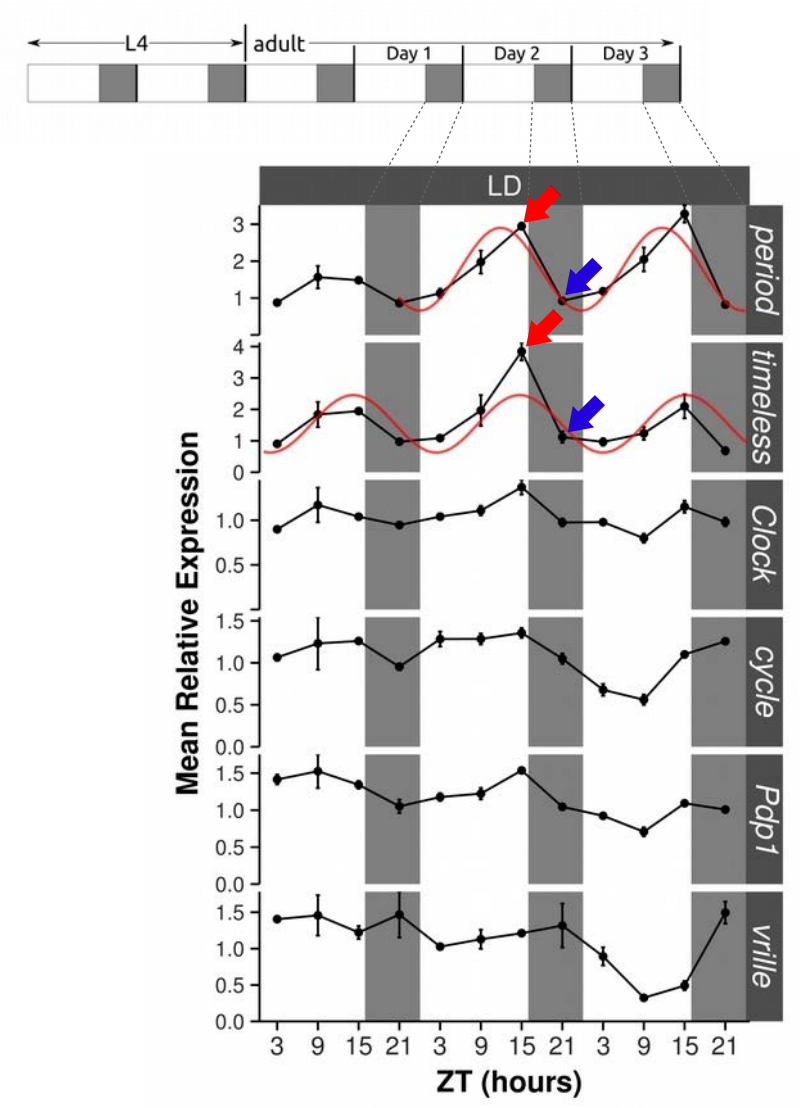
Collantes-Alegre et al., 2018

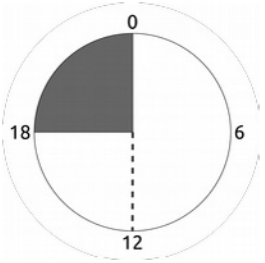
Circadian clock input in *A. pisum*



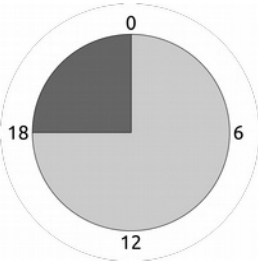
CRY1? Coming soon...

Quantification of expression: circadian analysis

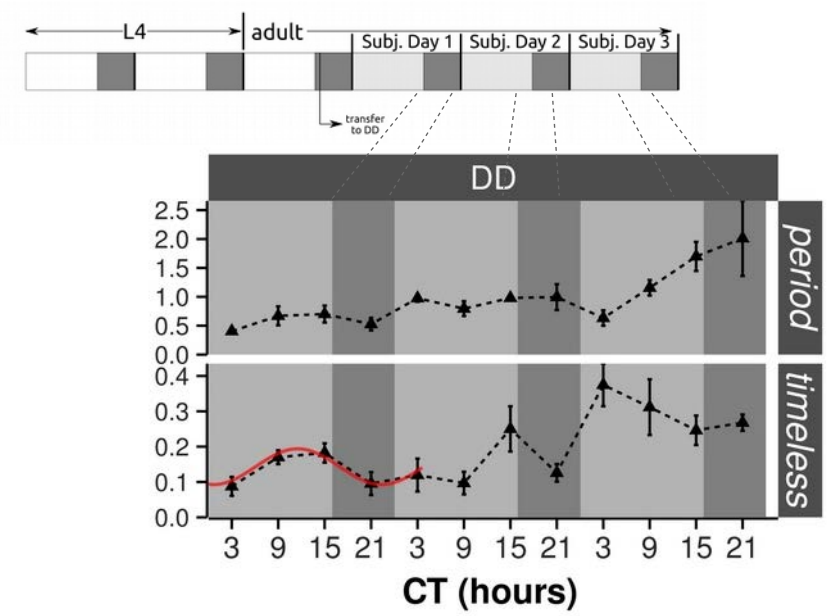




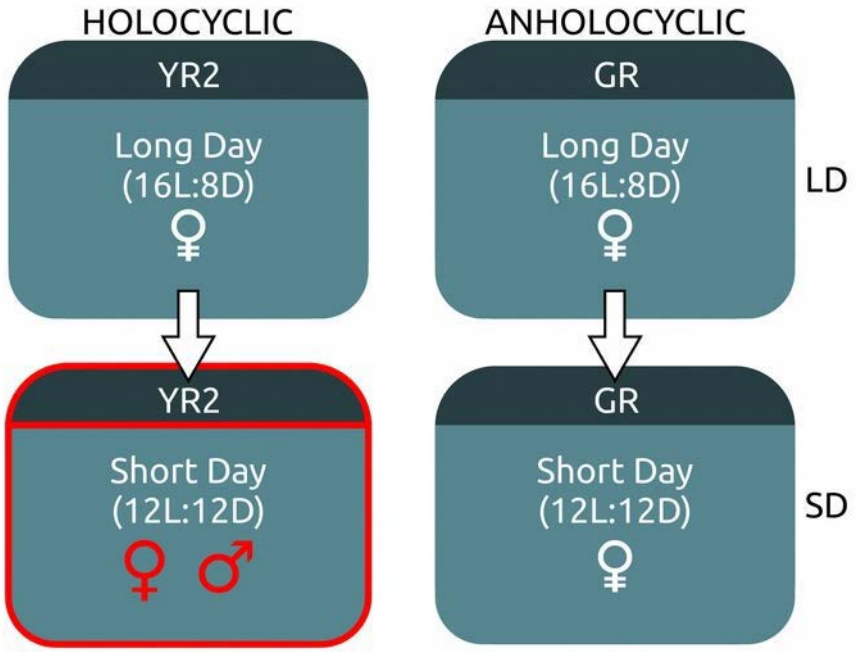
Long Day
(16L:8D)

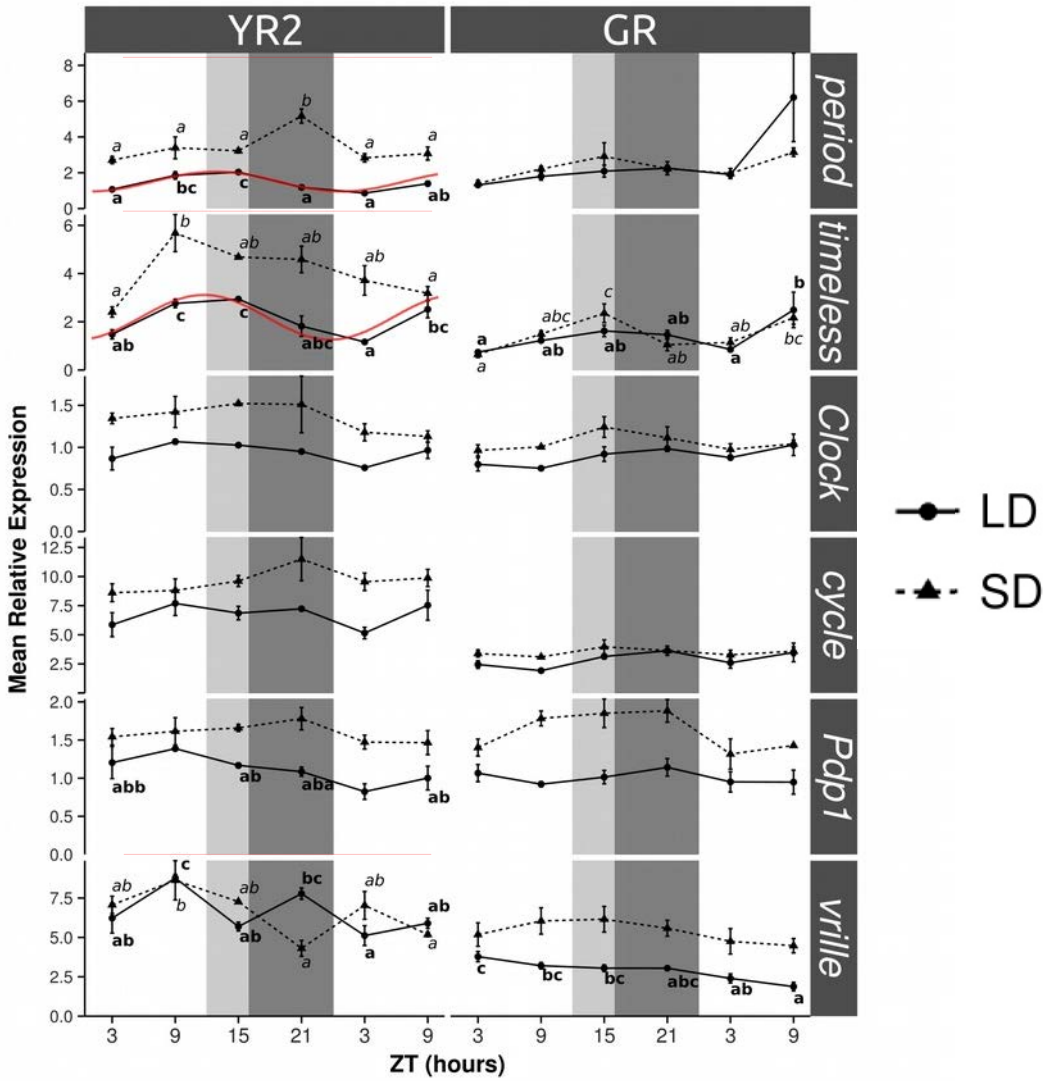


Constant
Darkness
(0L:24D)



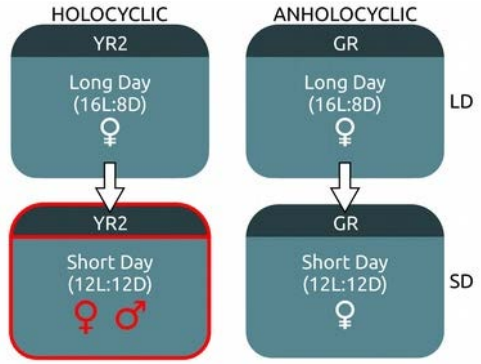
Quantification of expression: circadian analysis

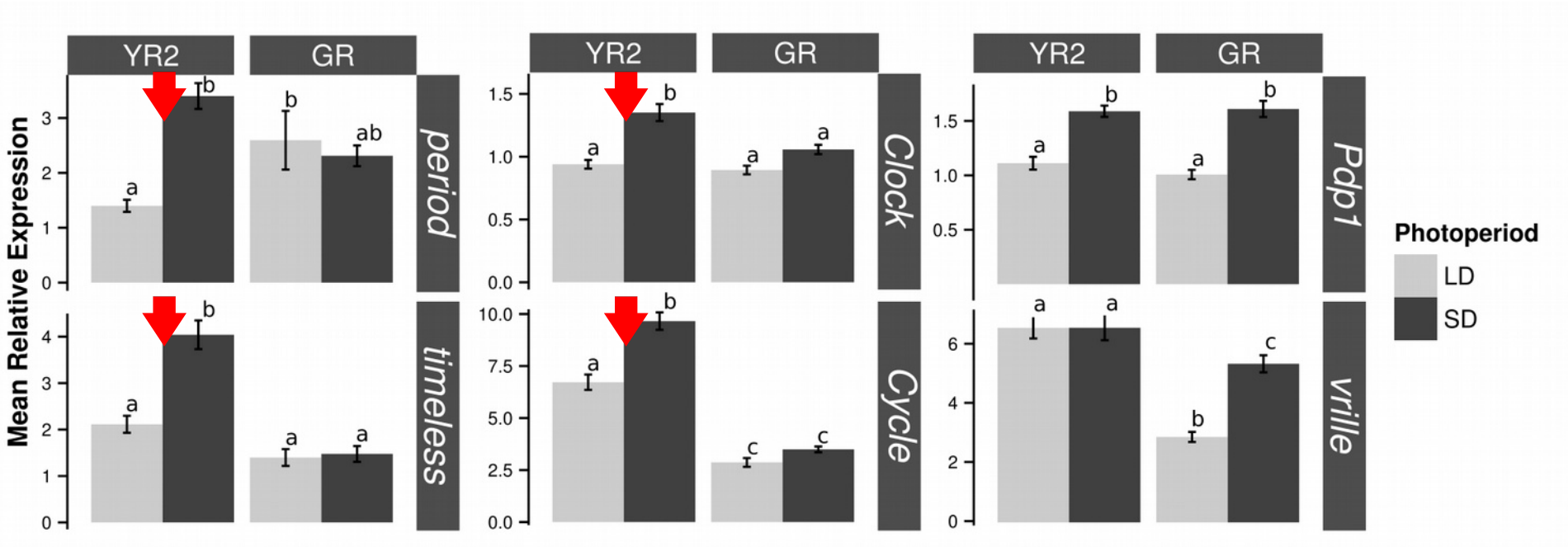




Differential patterns of expression of circadian clock genes between photoperiods have been observed in holocyclic but not in anholocyclic aphids.

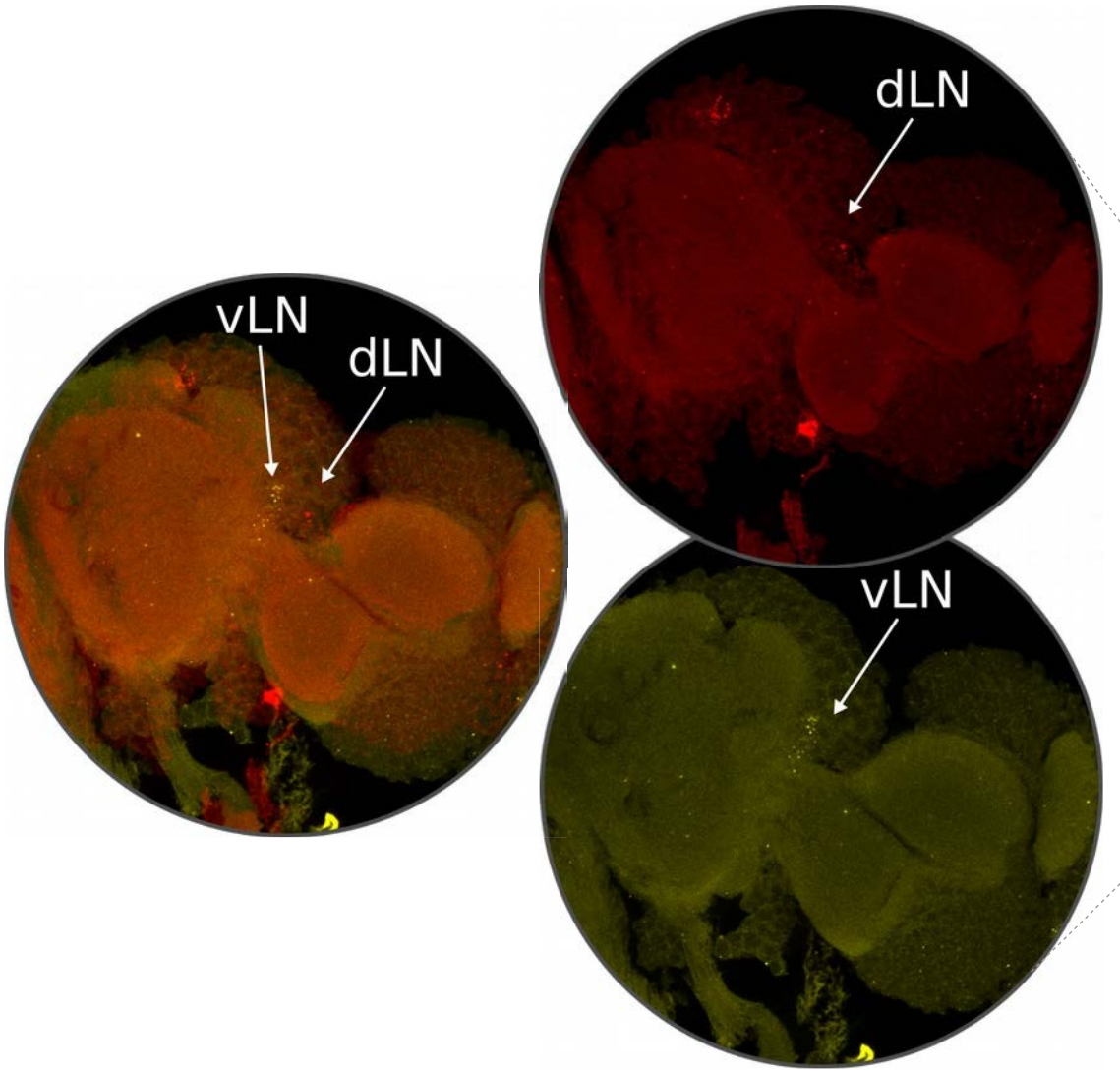
Quantification: photoperiodic analysis



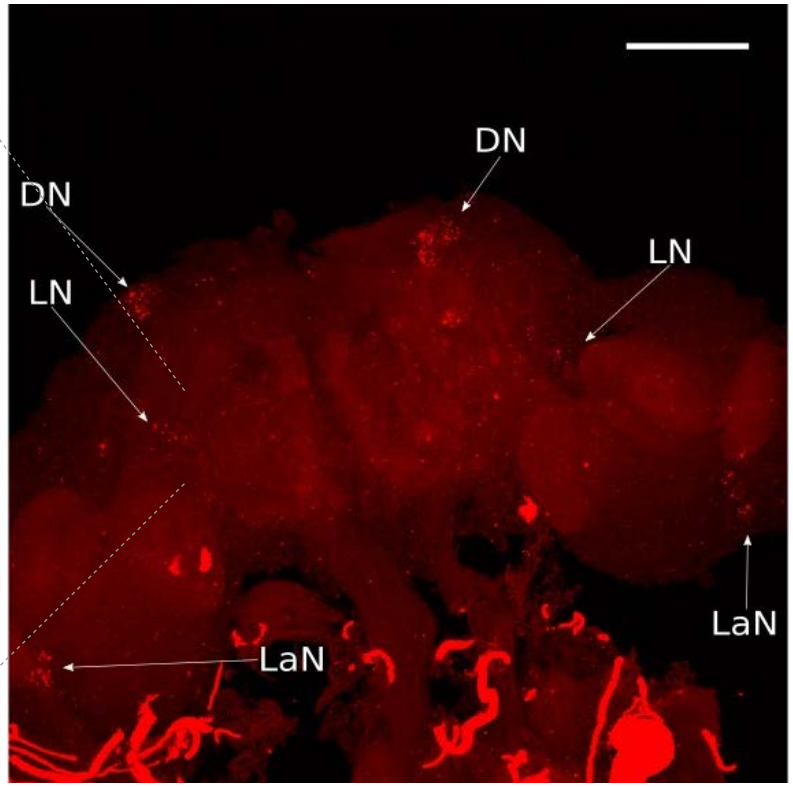


The genes that constitute the negative feedback loop of the circadian clock (*per*, *tim*, *Clk* and *cyc*) increase their expression in SD only in holocyclic aphids.

Localization of clock neurons in the aphid brain

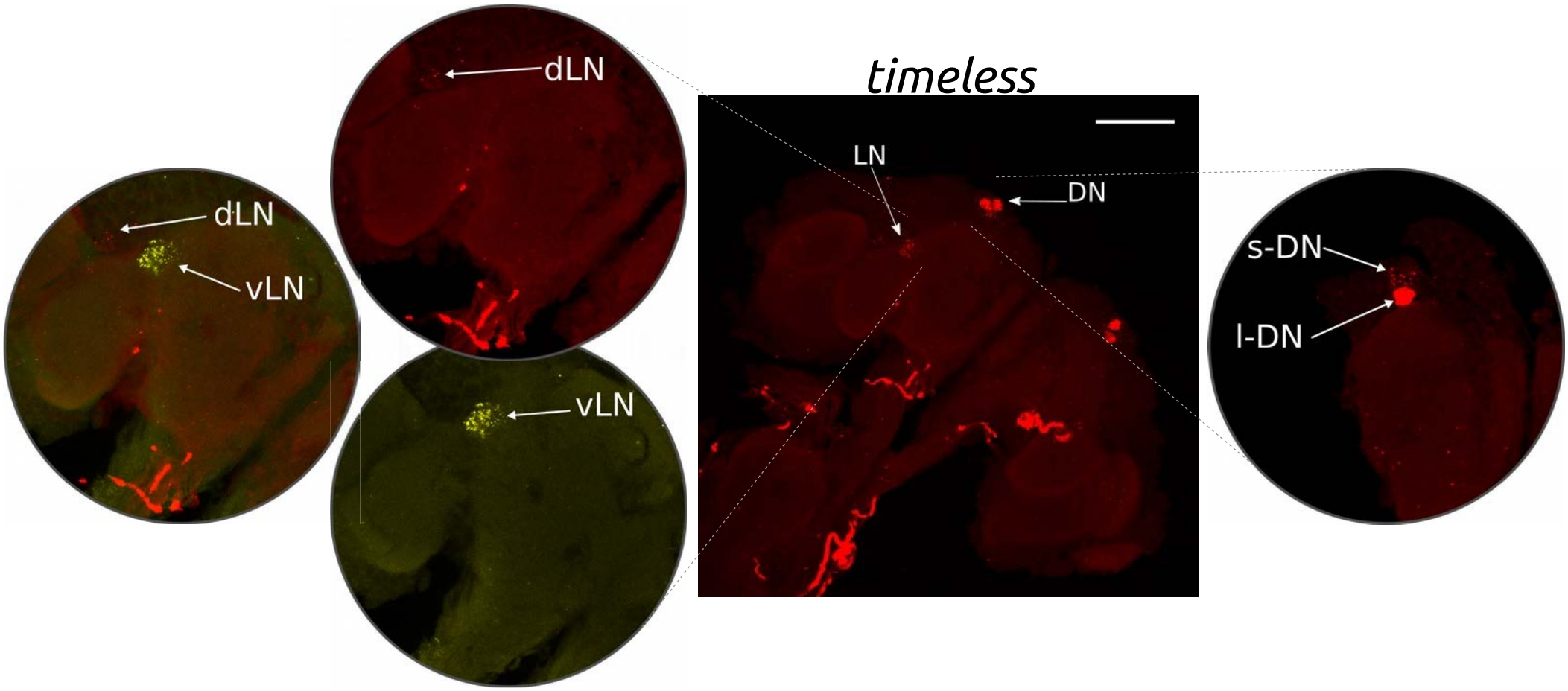


period



Period is expressed in three groups of neurons: DN, LN and LaN.
Within the LN cluster there two subgroups: the dLN and the vLN.

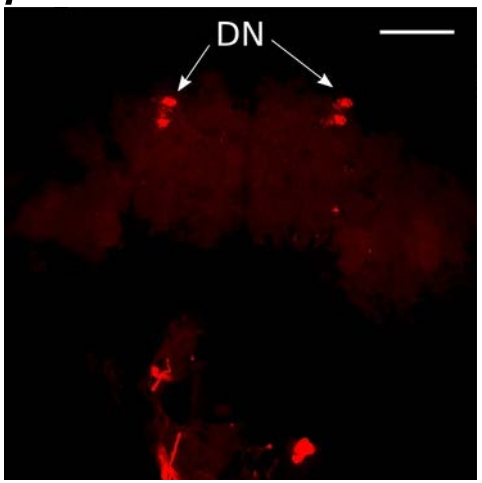
Localization of clock neurons in the aphid brain

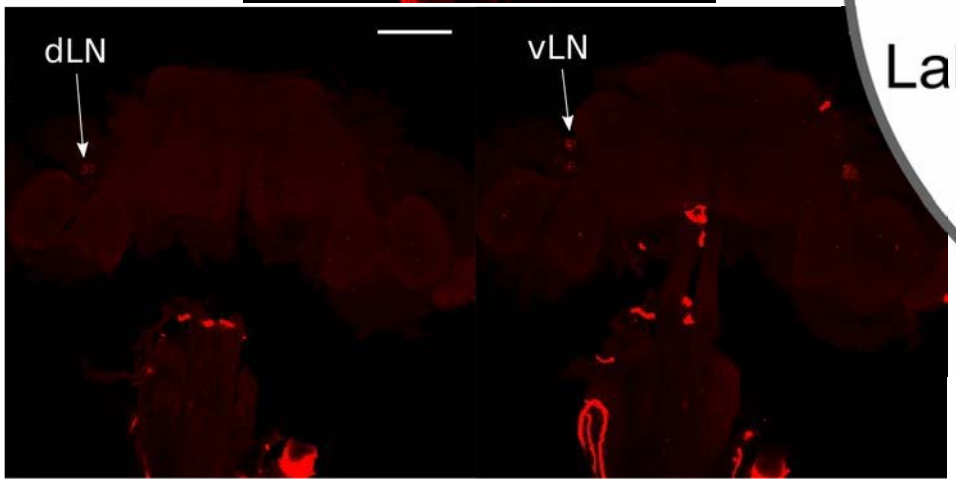


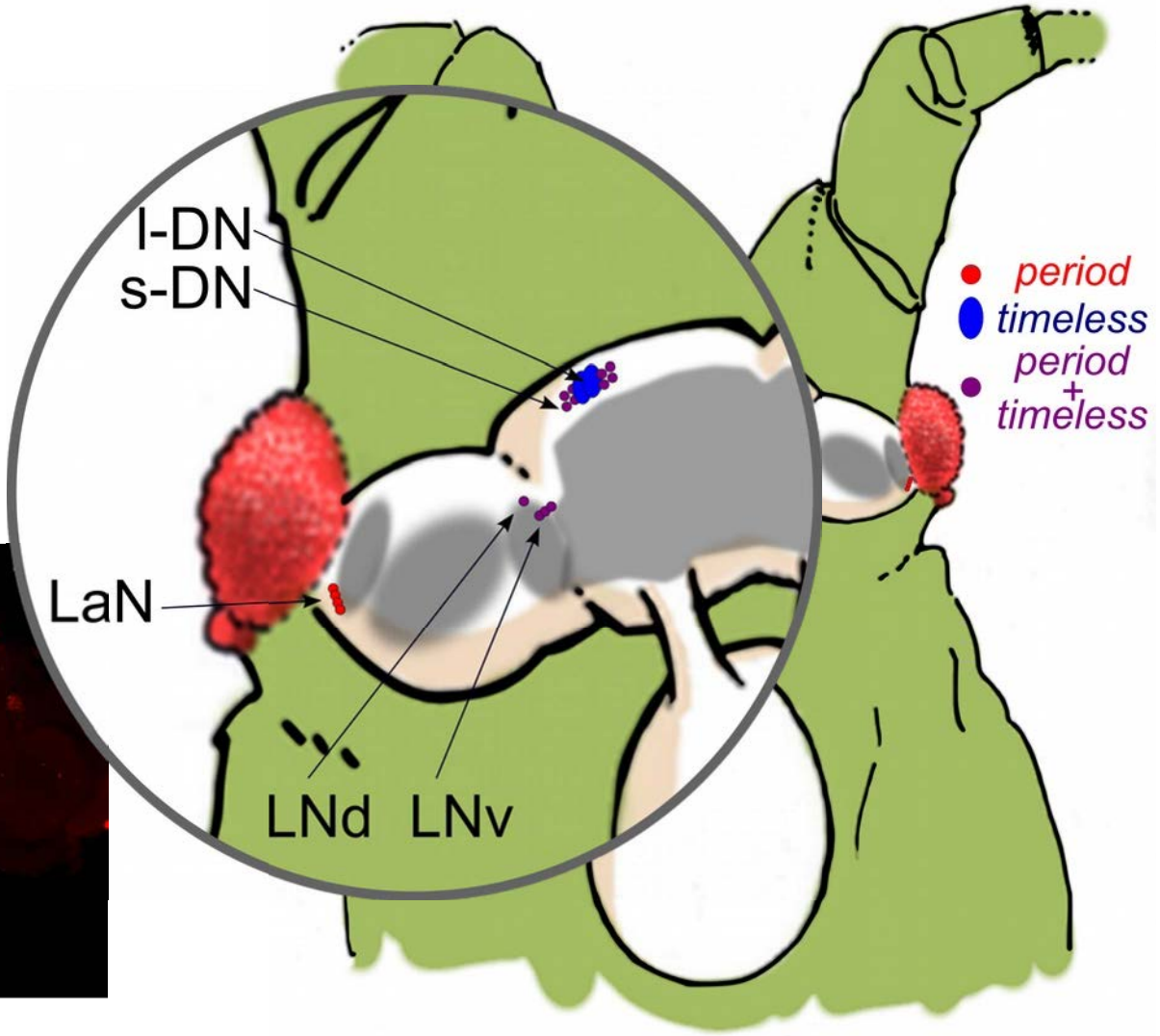
Timeless is also expressed in two groups of neurons: DN and LN.
The DN consists of two subgroups: l-DN and s-DN.
The LN cluster there two subgroups: the dLN and the vLN.

Localization of clock neurons in the aphid brain

period + *timeless*

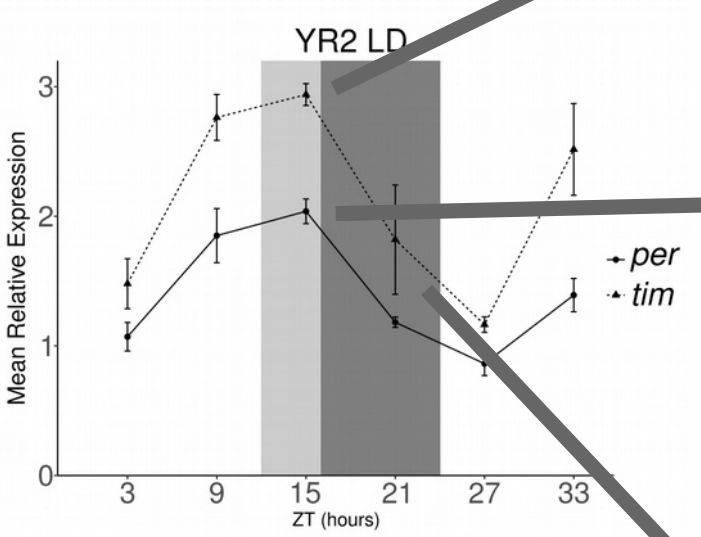




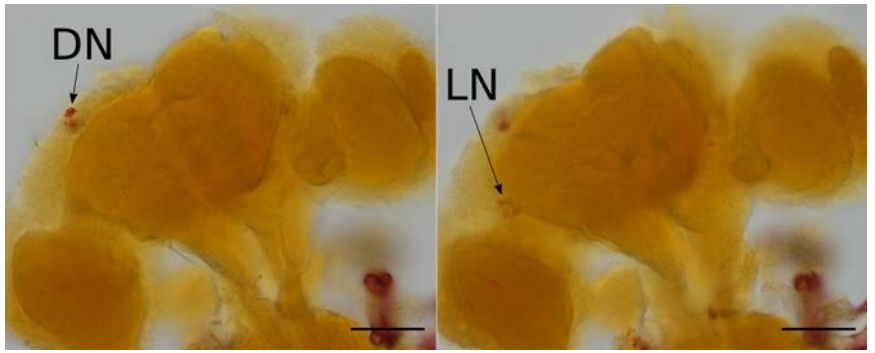


The aphid brain contains three groups of neurons expressing *period* and *timeless*: the DN cluster (l-DN and s-DN), the LN cluster (dLN and vLN) and the LaN.

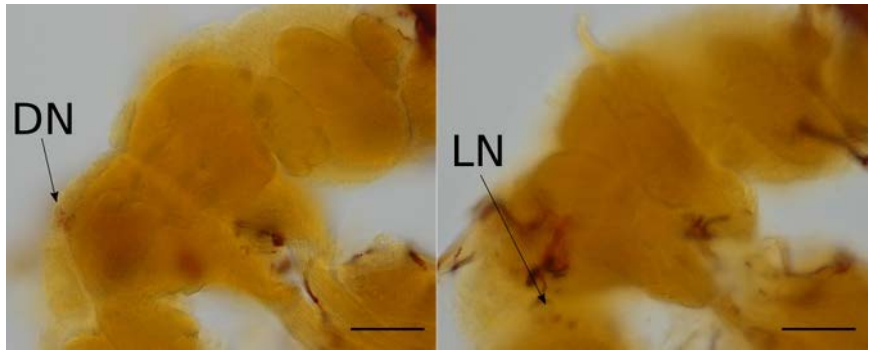
Consistency between localization and quantification



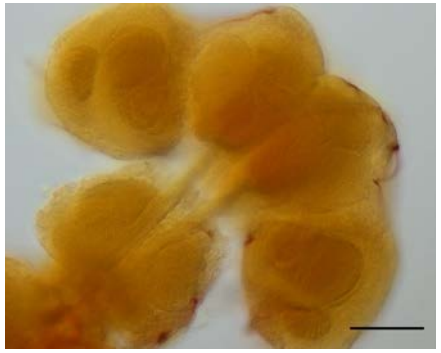
ZT15
timeless



ZT15
period

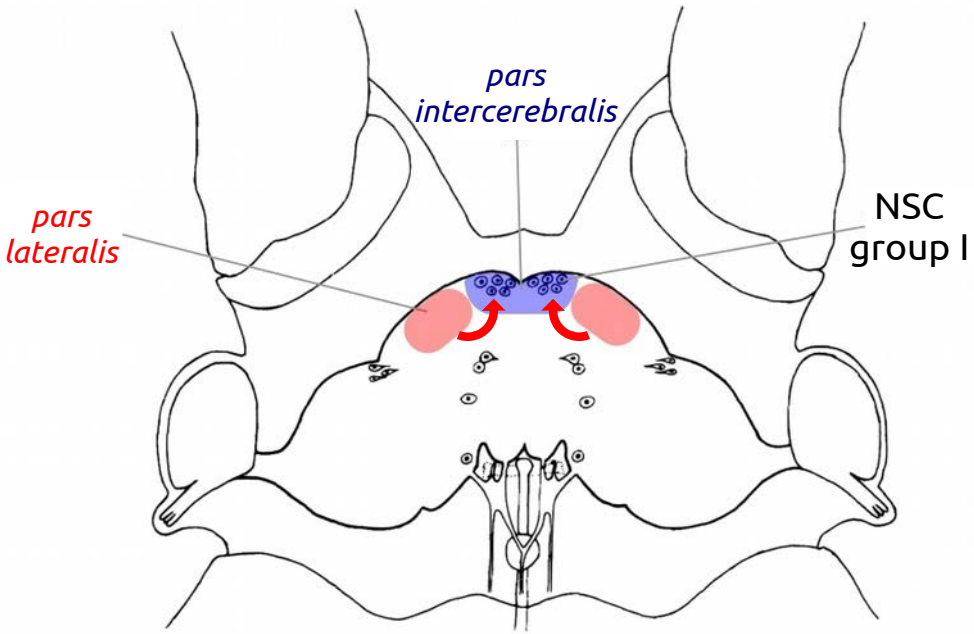


ZT21
period + timeless

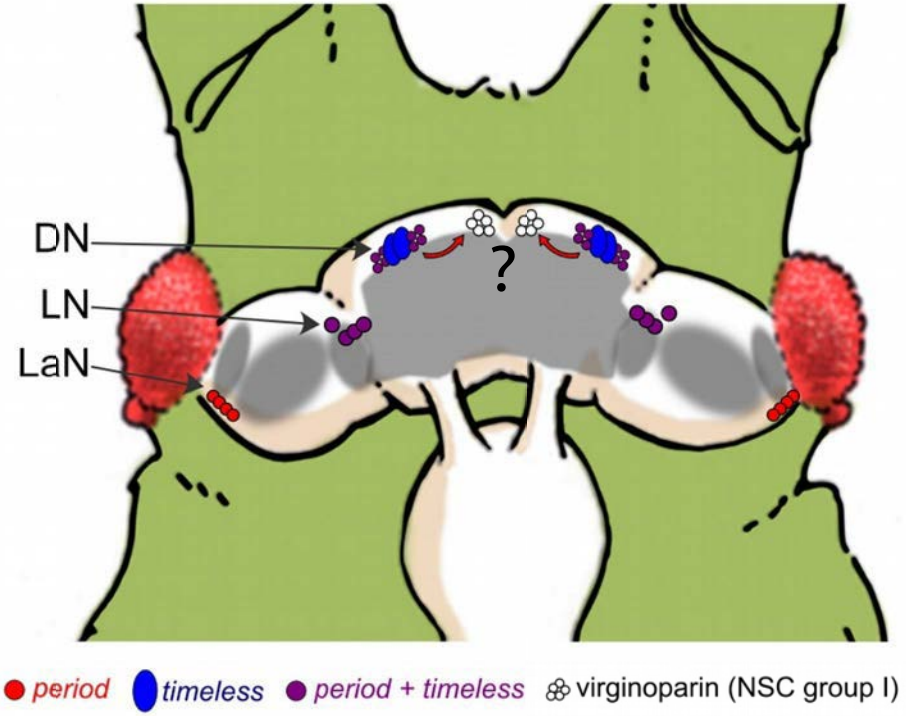


Period and *timeless* transcripts are localised at ZT15 when the peak of expression occurs, and are not detected at ZT21 when their expression is at minimum levels.

Are clock neurons controlling group I of NSC?



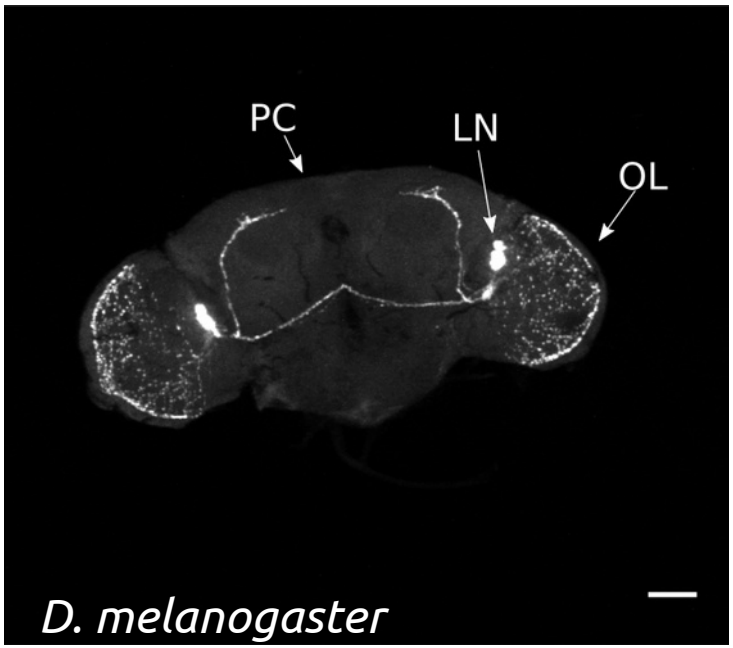
Modified from Steel (1977)

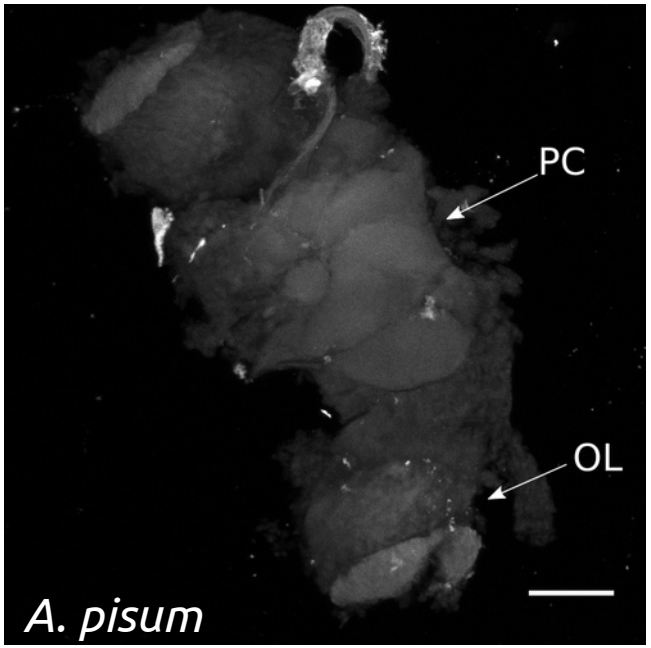


The photoperiodic calendar was suggested to reside in the *pars lateralis* of *protocerebrum*.
We localised clock neurons in this region.

Pigment Dispersing Factor/Hormone

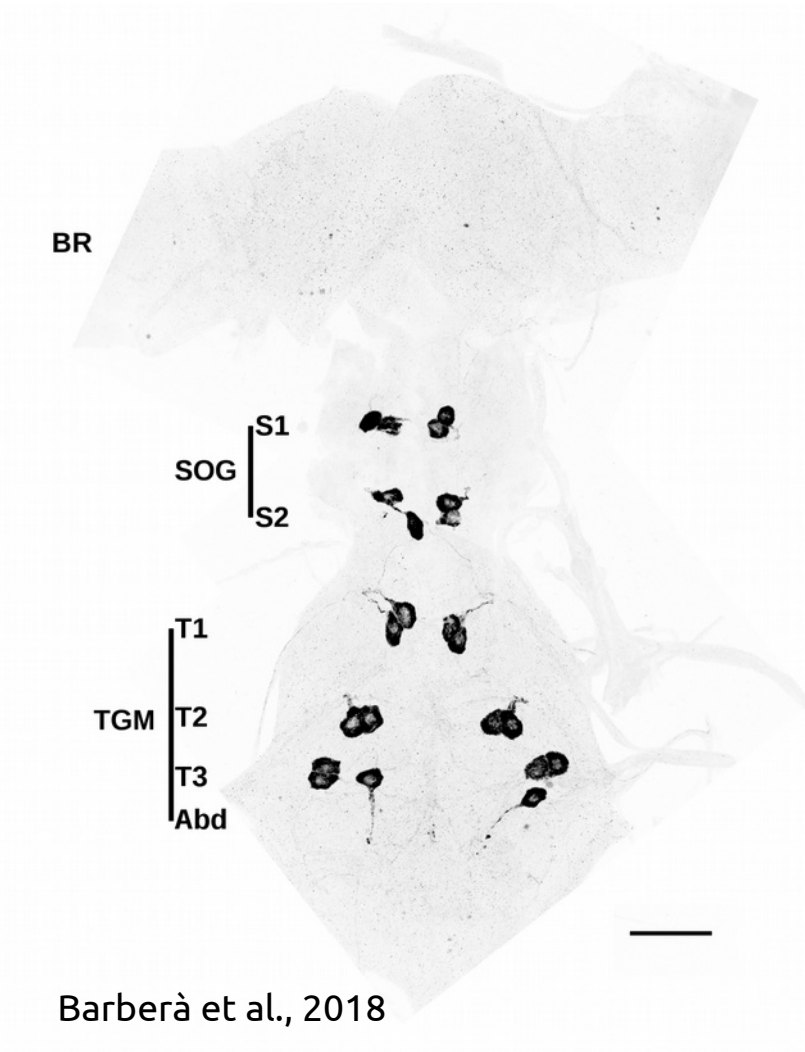
PDF detection with two antibodies





Two antibodies against *Drosophila* and crustacean PDF/PDH, widely used to detect PDF in arthropods, failed to detect PDF in the pea aphid brain.

Localization of melatonin in aphid CNSs

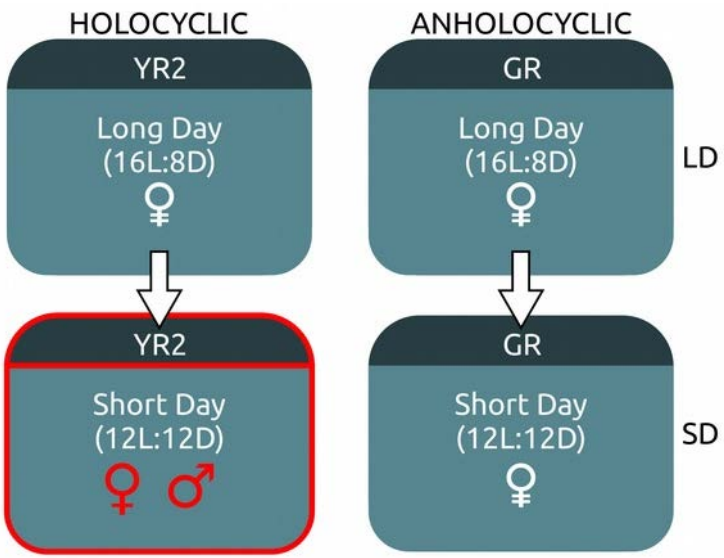


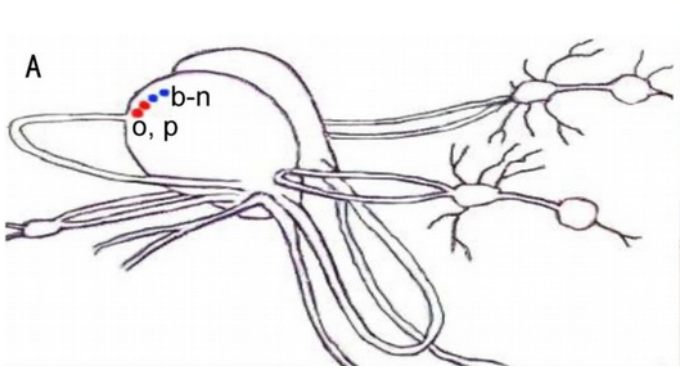
Barberà et al., 2018

Second *in situ* localisation in insects

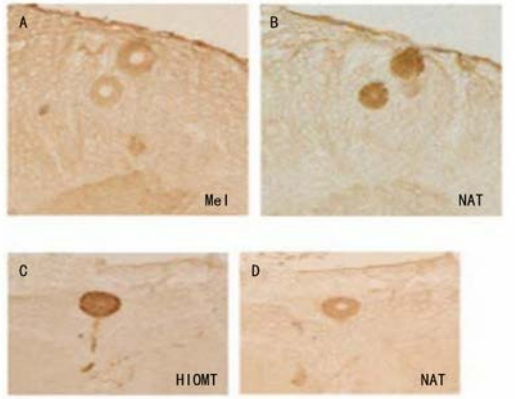
First *in situ* localisation in Hemimetabola

First *in situ* localisation in insect ganglia





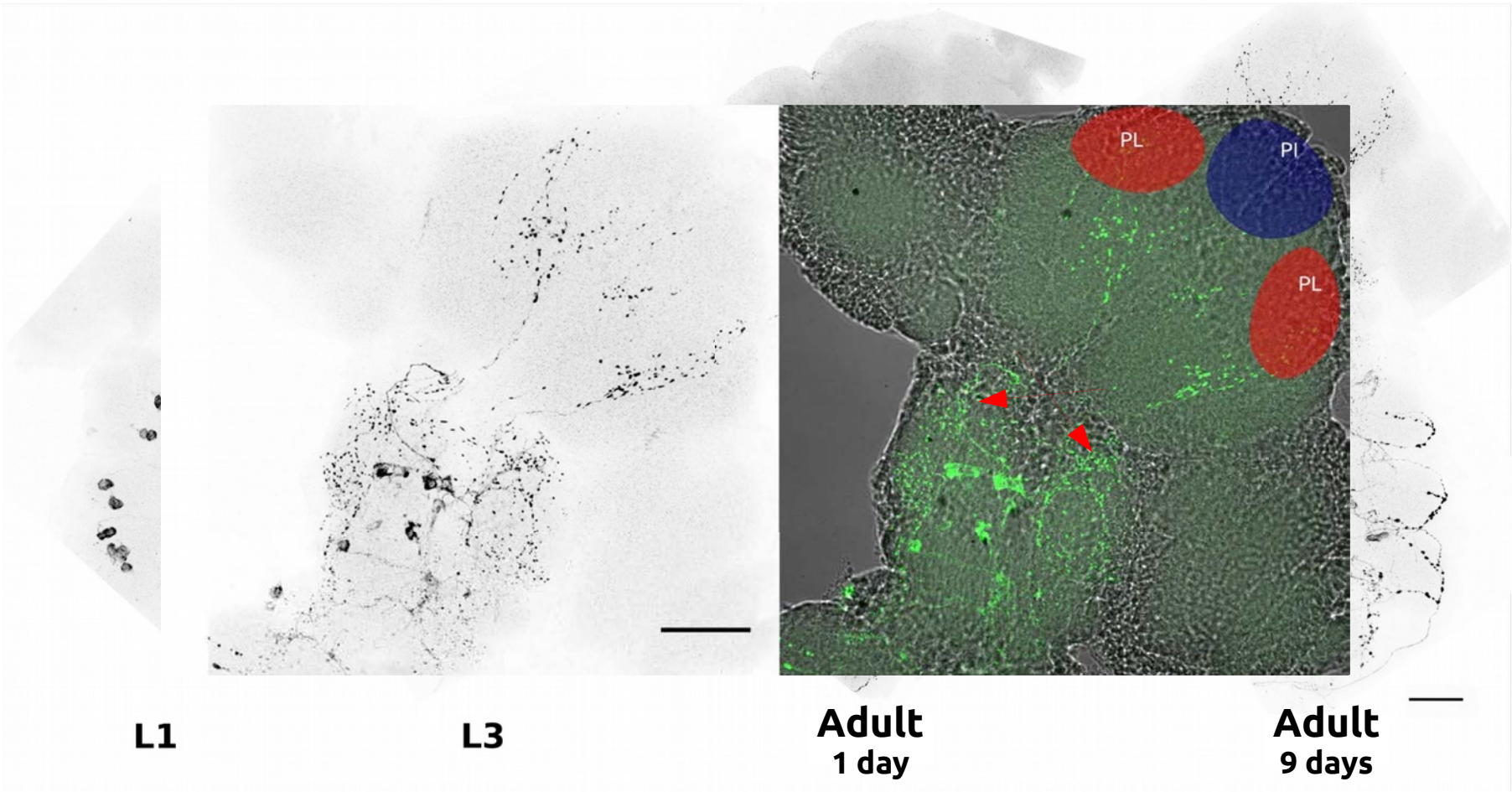
A. pernyi (Lepidoptera)



Mohamed et al., 2014

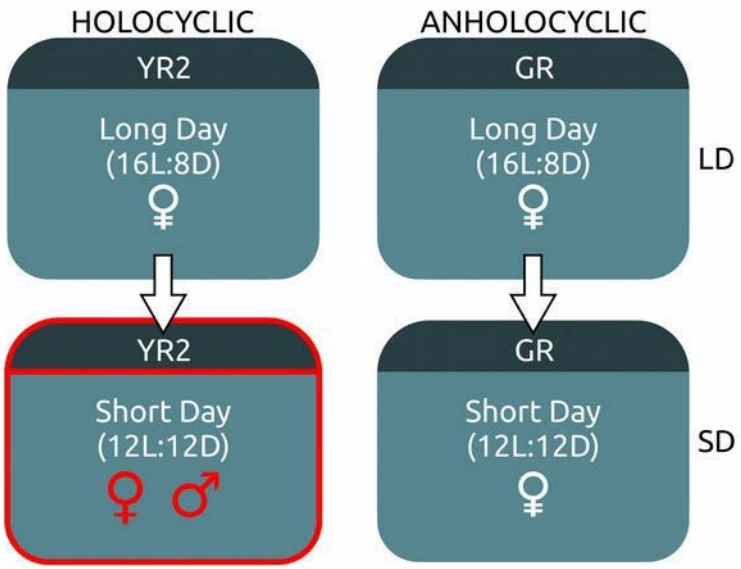
No evident differences in melatonin distribution were observed between the holocyclic and anholocyclic strains reared under LD and SD conditions.

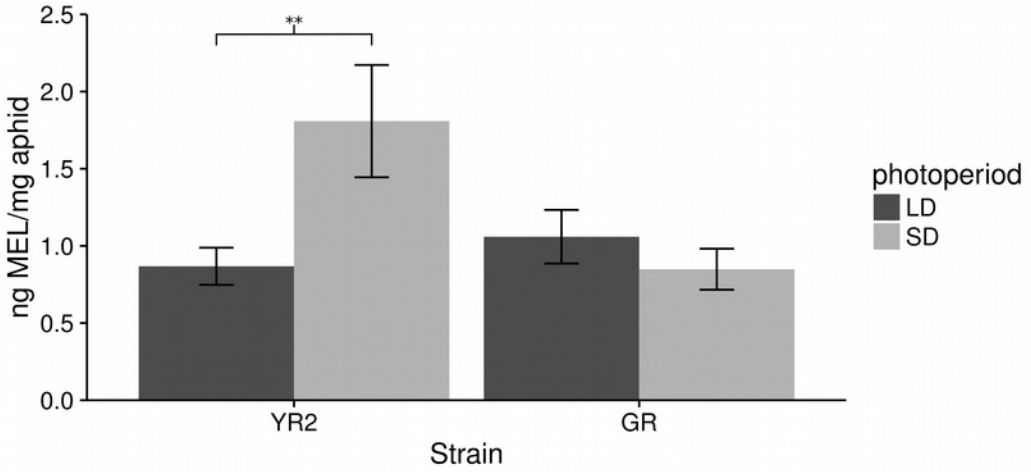
Melatonin dynamics through development

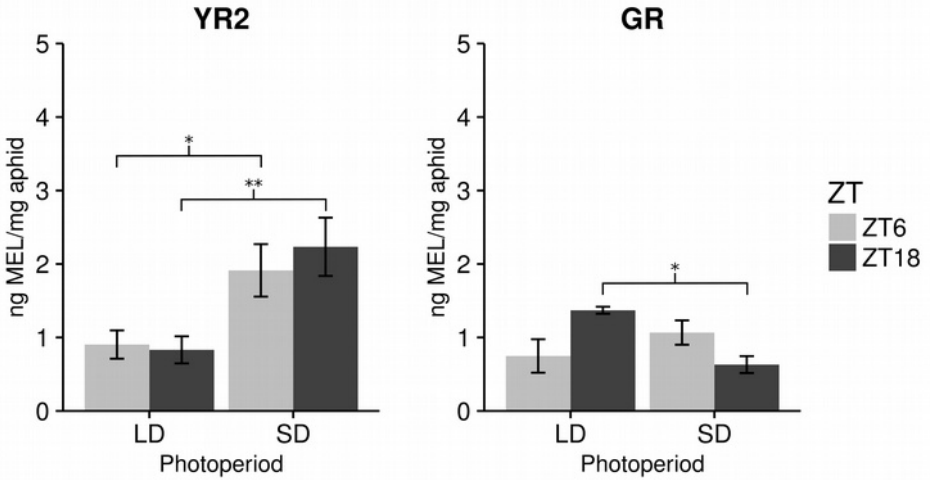


Melatonin is restricted within the neuron soma until L3.
From L3 on, melatonin is mobilised into the dendrites reaching contralateral regions of ganglia and the *protocerebrum*.

Melatonin quantification

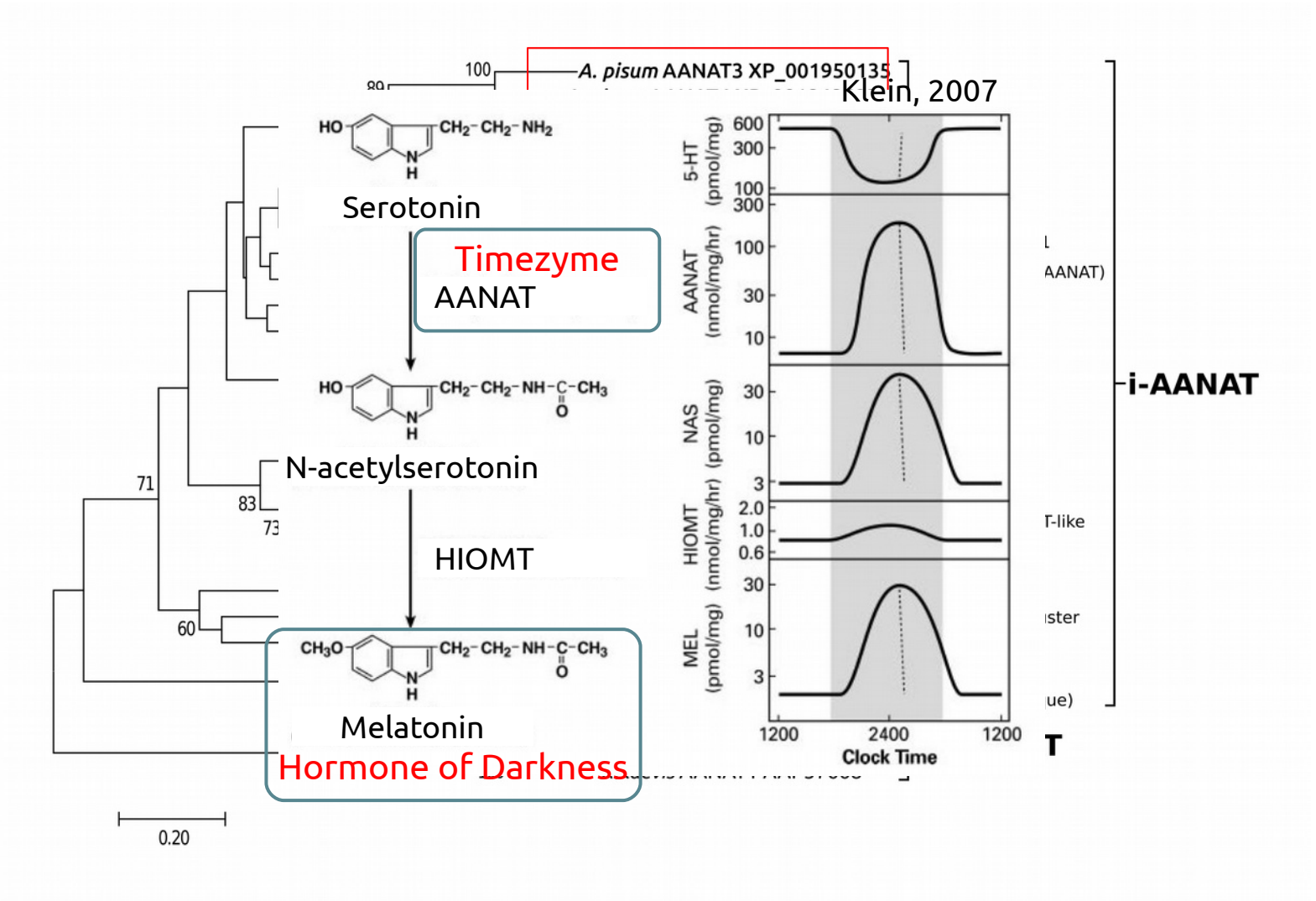






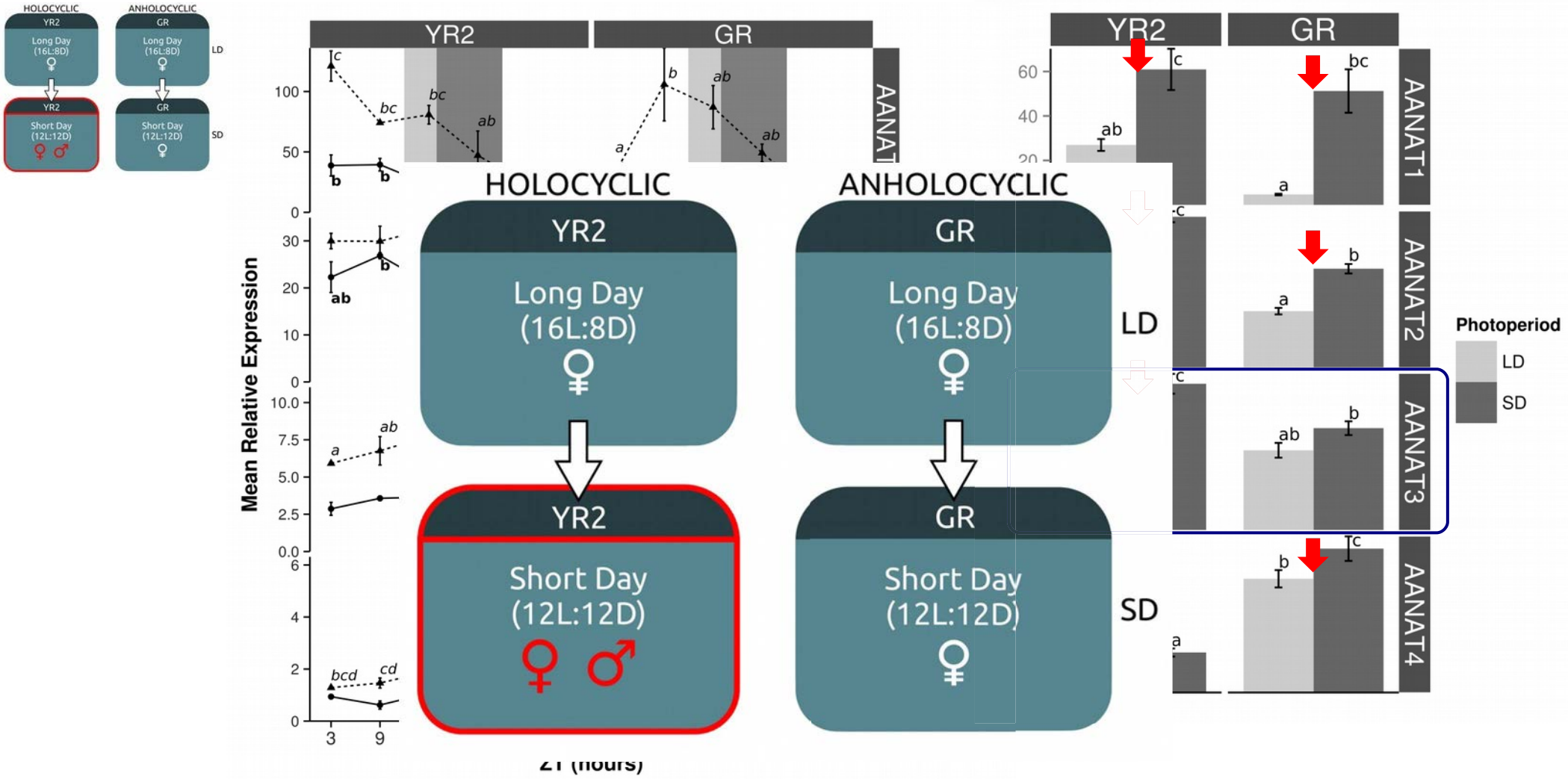
Only in aphids in which the seasonal response was induced (i.e. YR2 SD) there was a significant increase in melatonin content.

Pea aphid AANATs: identification



Four aphid AANAT genes were identified that grouped with typical insect AANAT.
All four encoded proteins with GNAT domain.

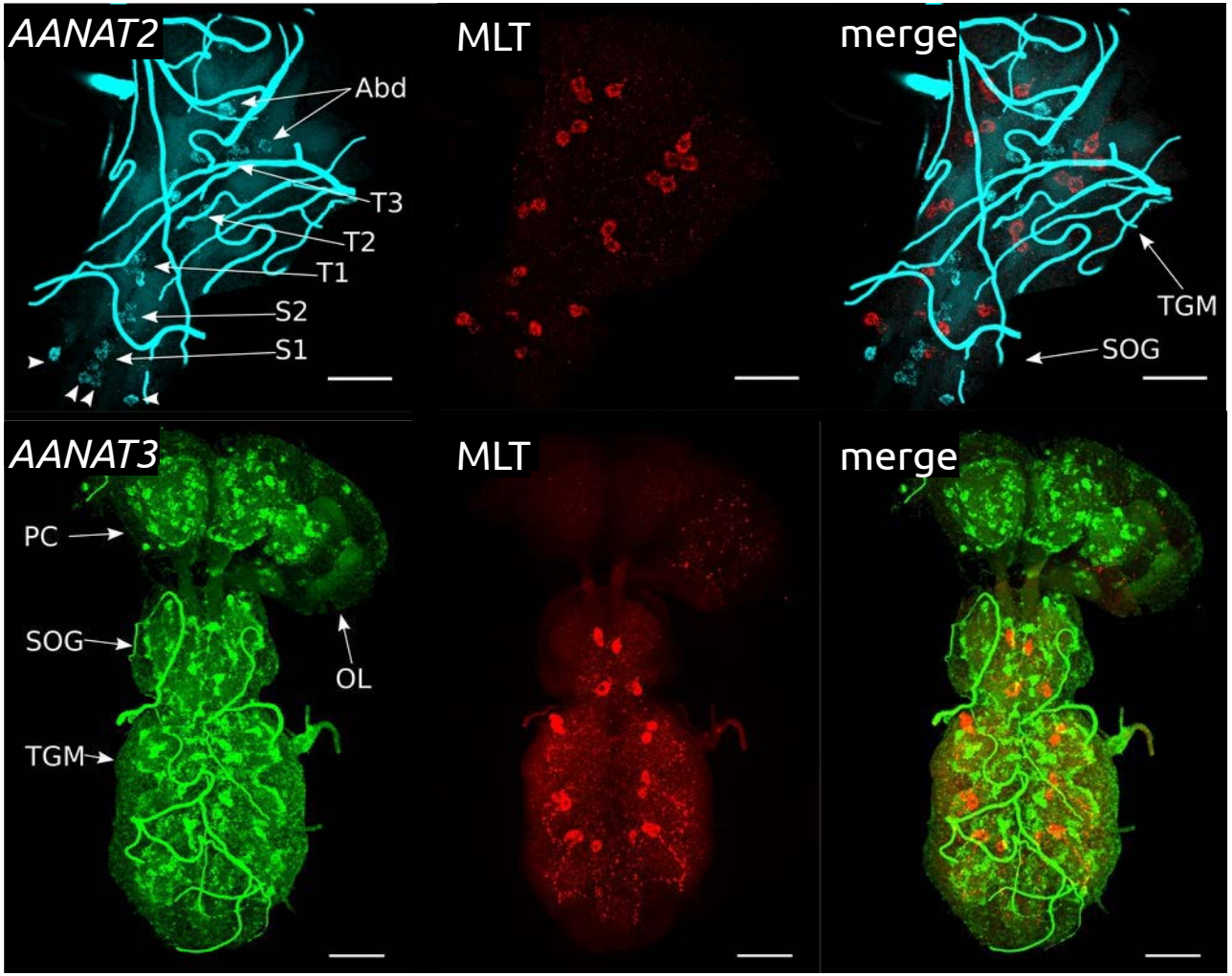
Pea aphid AANATs: quantification



No circadian expression, although there seems to be a rhythmic pattern in *AANAT3*

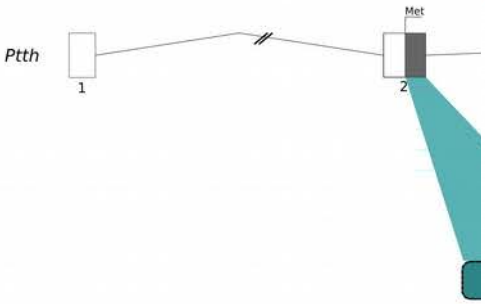
Higher expression in SD respect to LD specific of holocyclic aphids for genes *AANAT2* and *AANAT3*

Pea aphid AANATs: localization



AANAT2 and *AANAT3* transcripts were localised in the aphid ganglia.
However, none of them colocalised with melatonin.

Prothoracicotropic Hormone (PTTH)



Insect Molecular Biology

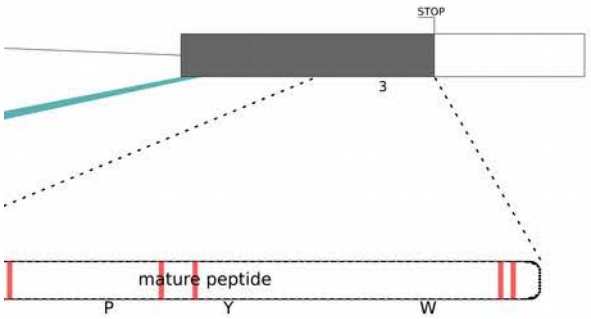
Insect Molecular Biology (2010), **19** (Suppl. 2), 87–95 doi: 10.1111/j.1365-2583.2009.00951.x

Neuropeptide and neurohormone precursors in the pea aphid, *Acyrthosiphon pisum*

J. Huybrechts*, J. Bonhomme†, S. Minoli†, N. Prunier-Leterme†, A. Dombrovsky‡, M. Abdel-Latief§, A. Robichon‡, J. A. Veenstra¶ and

Introduction

Aphids are insects that are in the centre of a network of



Research article

Open Access

Transcriptomic and proteomic analyses of seasonal photoperiodism in the pea aphid

G Le Trionnaire[1], F Francis[2], S Jaubert-Possamai[1], J Bonhomme[1], E De Pauw[3], J-P Gauthier[1], E Haubruge[2], F Legeai[1], N Prunier-Leterme[1], J-C Simon[1], S Tanguy[1] and D Tagu*[1]

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* Corresponding author

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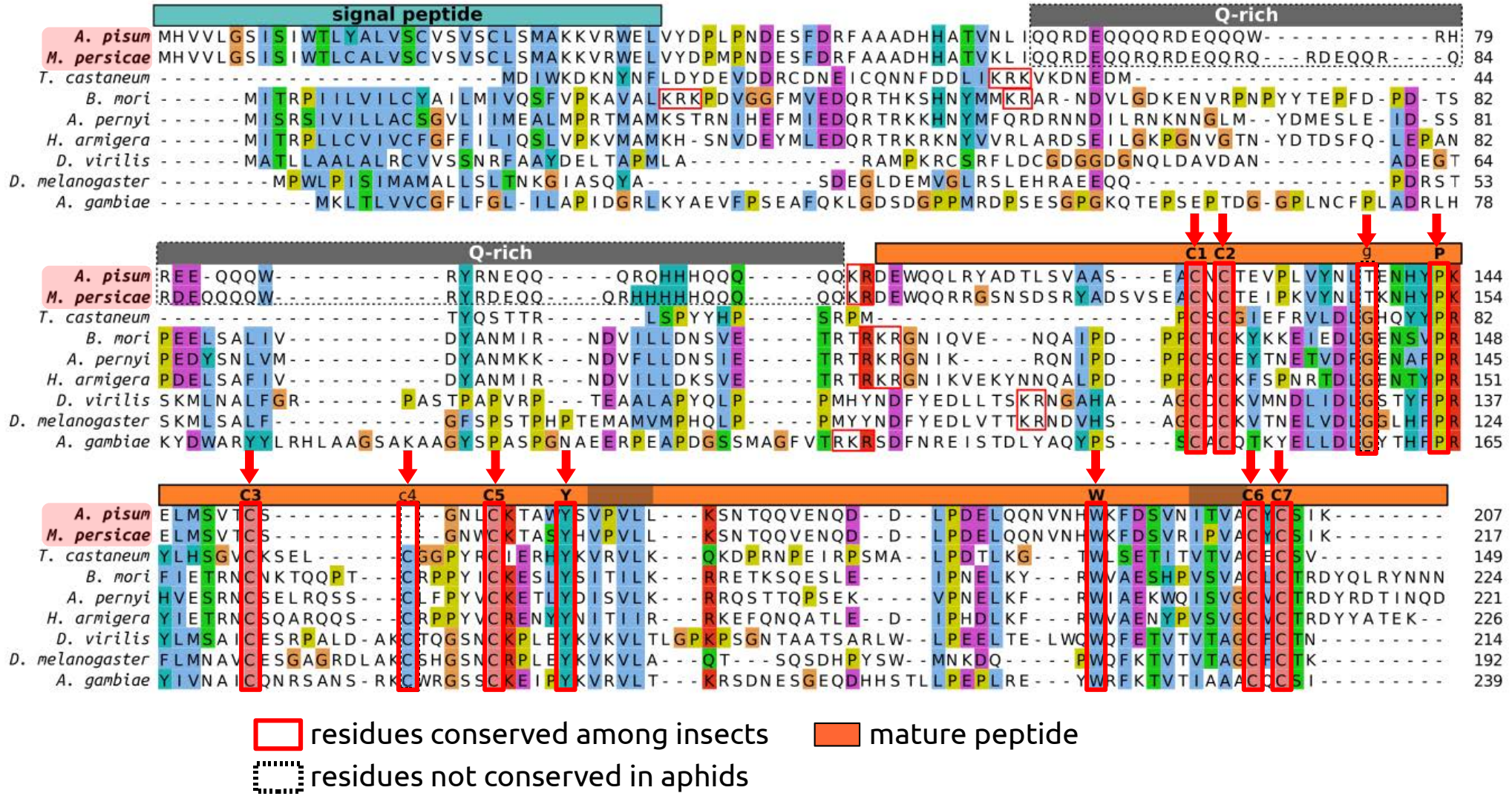
An aphid candidate *Ptth* gene was identified with features typical of other insect PTTH.

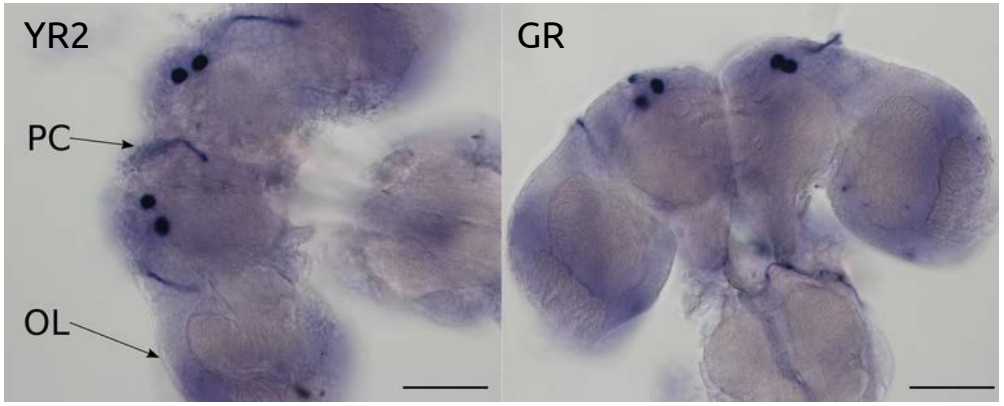
PTTH



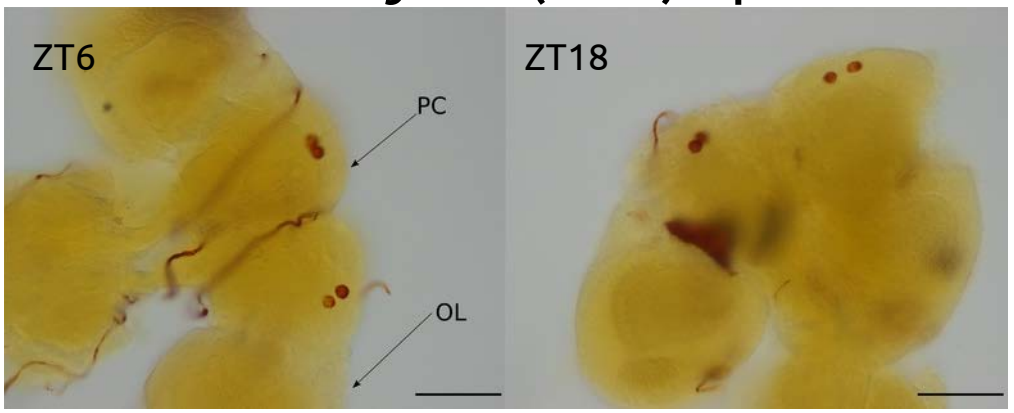
The putative mature peptide is the most conserved. Aphids only have six out of seven conserved Cys. The missing Cys could indicate a change in aphid PTTH structure.

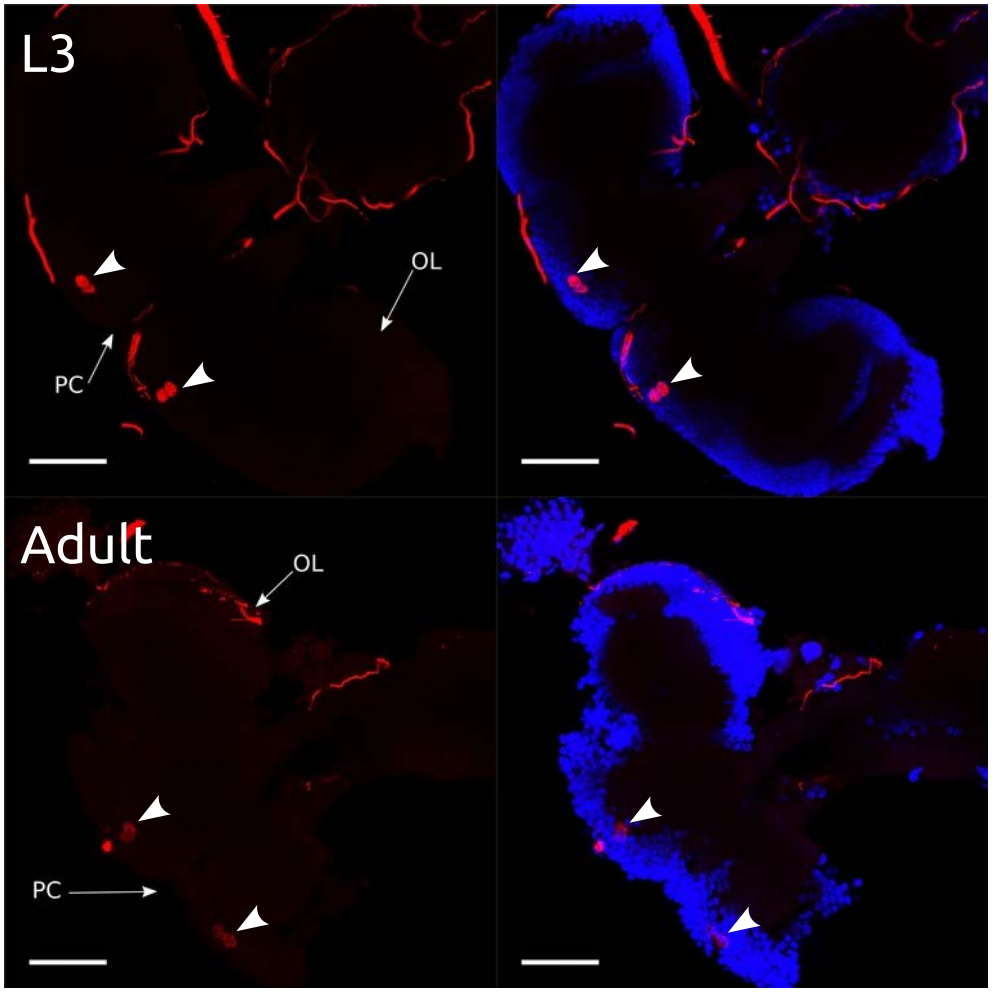
Localization of PTTH

L3 aphids



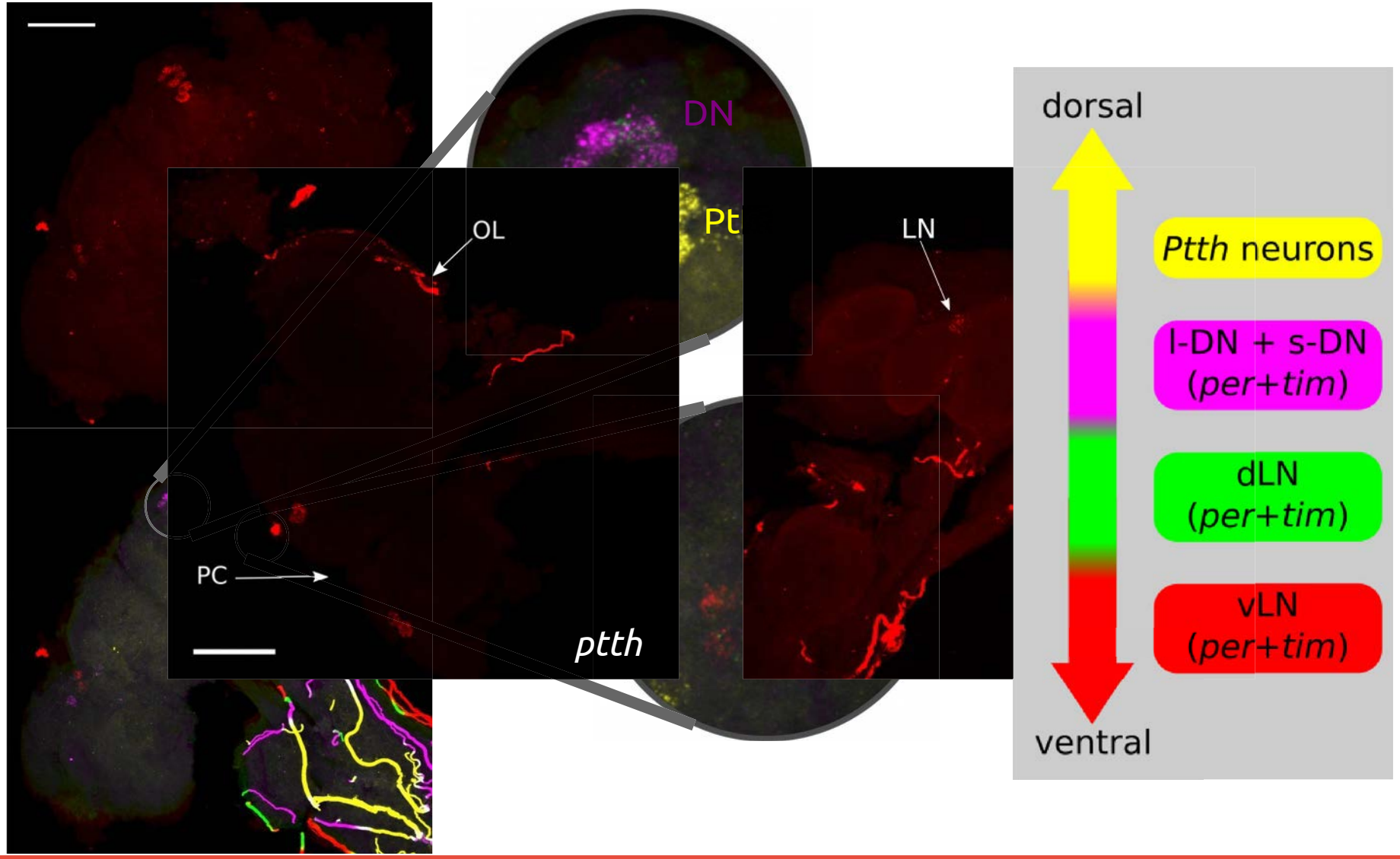
L3 holocyclic (YR2) aphids





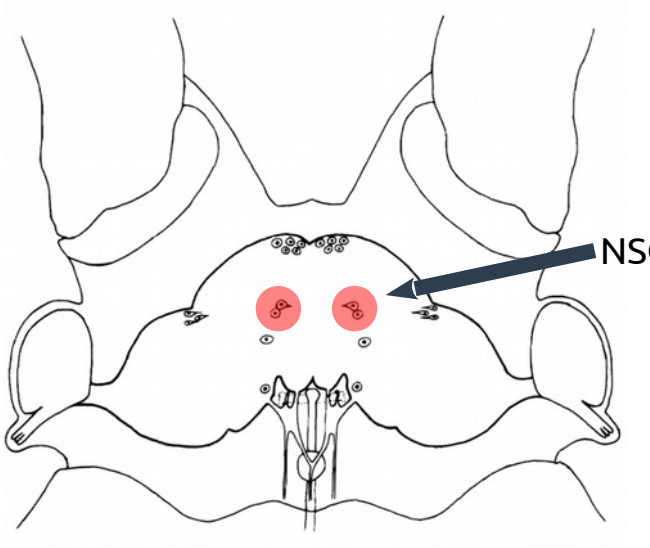
No differences in localisation of *Ptth* transcripts between holocyclic and anholocyclic aphids.
No evidence of a circadian rhythm in expression of *Ptth*.
Ptth is still expressed in the brain in adult aphids.

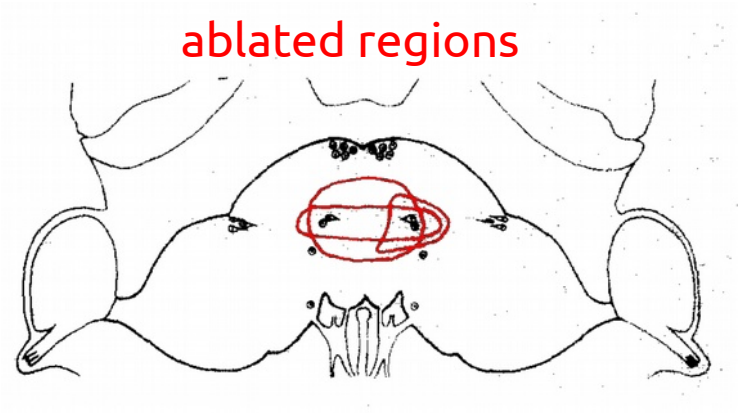
Localization of PTTH



Combined *in situ* hybridisation with *per*, *tim* and *Ptth* probes
Revealed the detailed position of clock neurons and *Ptth* neurons.

Localization of PTTH





Steel, 1978

Disruption of normal moulting process

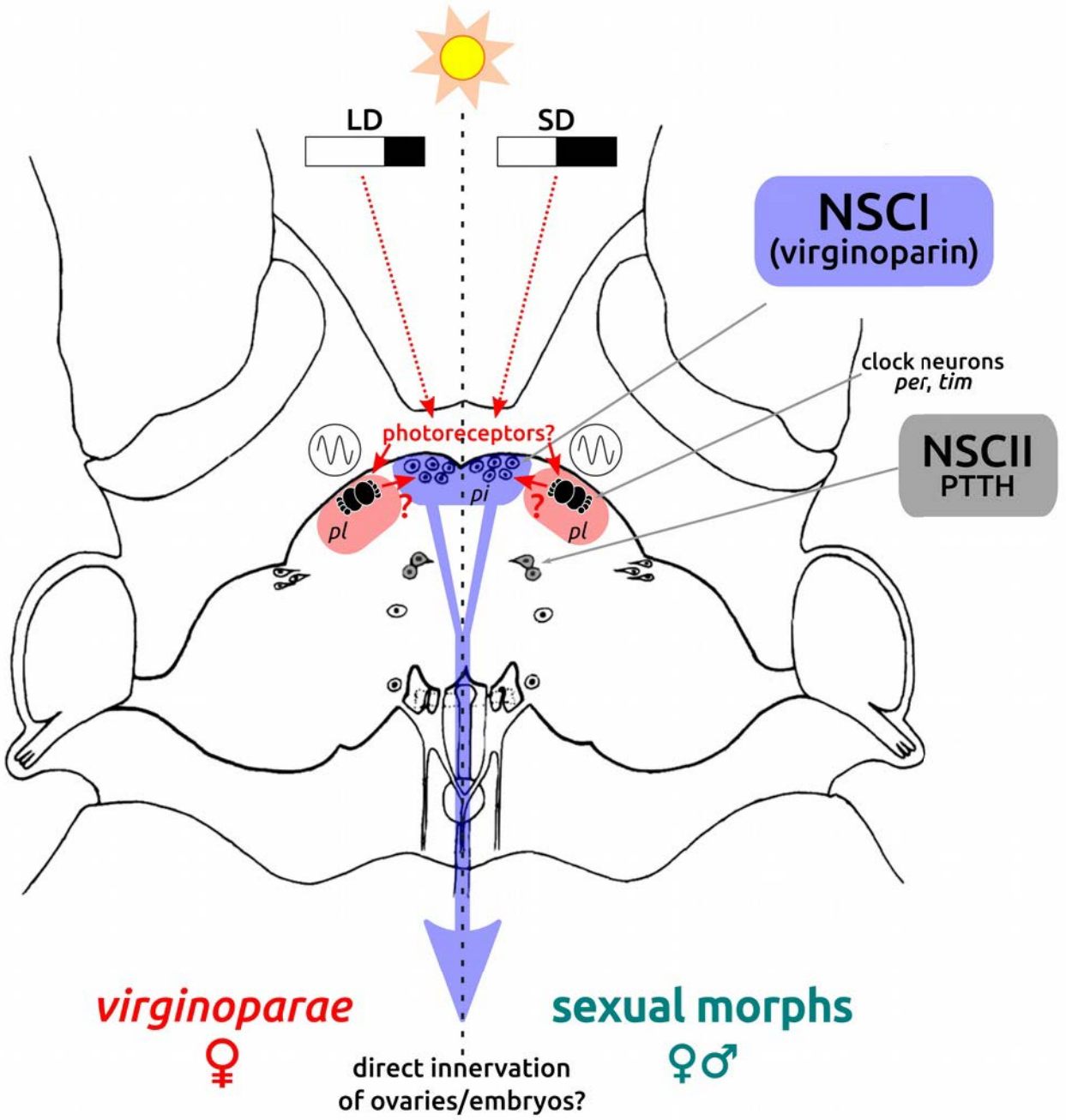
PTTH is expressed in the so called NSC group II.
Consequently, an essential function of PTTH in the aphid photoperiodic response is discarded.

Summary

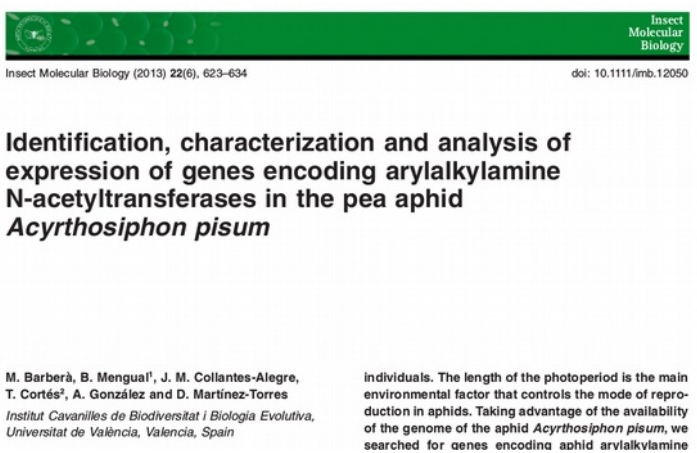
INPUT
SWO4, c-opsin, arthropsin
CRY1

CORE
Circadian clock based?
CRY2

OUTPUT
~~PTTH~~
Melatonin
JH



Articles



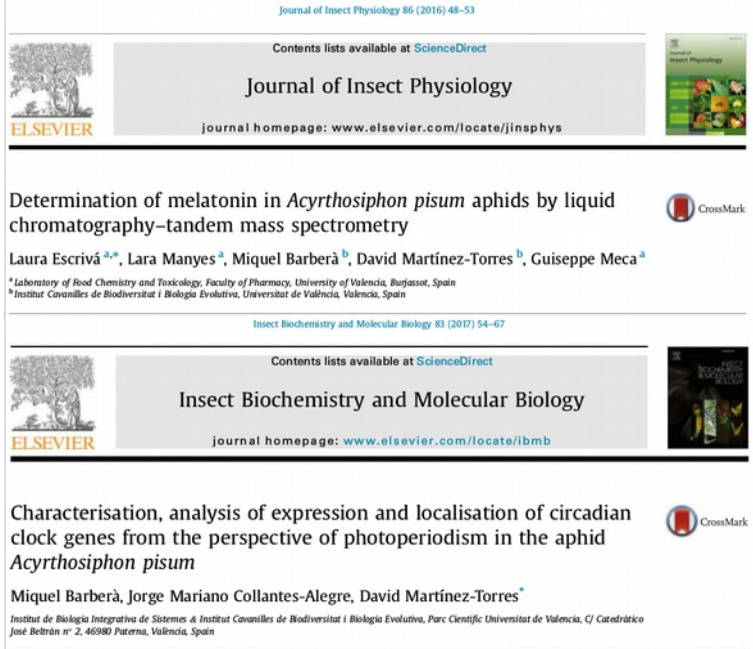
Insect Molecular Biology (2013) **22**(6), 623–634 doi: 10.1111/imb.12050

Identification, characterization and analysis of expression of genes encoding arylalkylamine N-acetyltransferases in the pea aphid *Acyrthosiphon pisum*

M. Barberà, B. Mengual[1], J. M. Collantes-Alegre, T. Cortés[2], A. González and D. Martínez-Torres

Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, Valencia, Spain

individuals. The length of the photoperiod is the main environmental factor that controls the mode of reproduction in aphids. Taking advantage of the availability of the genome of the aphid *Acyrthosiphon pisum*, we searched for genes encoding aphid arylalkylamine



Journal of Insect Physiology 86 (2016) 48–53

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Determination of melatonin in *Acyrthosiphon pisum* aphids by liquid chromatography–tandem mass spectrometry

Laura Escrivá [a,*], Lara Manyes [a], Miquel Barberà [b], David Martínez-Torres [b], Guiseppe Meca [a]

[a] Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Burjassot, Spain
[b] Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, Valencia, Spain

Insect Biochemistry and Molecular Biology 83 (2017) 54–67

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Characterisation, analysis of expression and localisation of circadian clock genes from the perspective of photoperiodism in the aphid *Acyrthosiphon pisum*

Miquel Barberà, Jorge Mariano Collantes-Alegre, David Martínez-Torres*

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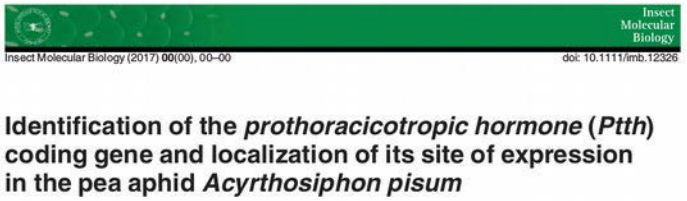
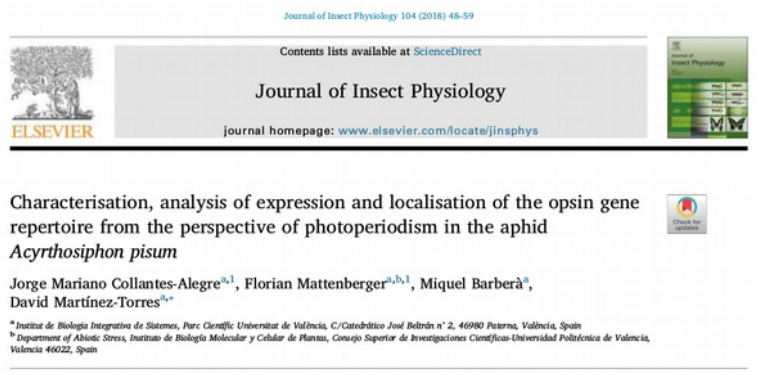


Insect Molecular Biology (2017) **00**(00), 00–00 doi: 10.1111/imb.12326

Identification of the *prothoracicotropic hormone* (*Ptth*) coding gene and localization of its site of expression in the pea aphid *Acyrthosiphon pisum*



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Characterisation, analysis of expression and localisation of the opsin gene repertoire from the perspective of photoperiodism in the aphid *Acyrthosiphon pisum*

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ORIGINAL ARTICLE

Melatonin in the seasonal response of the aphid *Acyrthosiphon pisum*

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QUESTIONS