
Supplementary information

**Circadian plasticity evolves through
regulatory changes in a neuropeptide gene**

In the format provided by the
authors and unedited

Supplementary Table 1. *Drosophila* strains.

Stock Name	Genotype	Species	Reference	Hybridised
Canton-S (CS)	<i>wt</i>	<i>D. melanogaster</i>	RRID:BDSC_64349	N/A
Oregon-R (OR)	<i>wt</i>	<i>D. melanogaster</i>	RRID:BDSC_2376	N/A
LZV L72	<i>wt</i>	<i>D. melanogaster</i>	PMID:24920013	N/A
LZV L76	<i>wt</i>	<i>D. melanogaster</i>	PMID:24920013	N/A
LZV A10	<i>wt</i>	<i>D. melanogaster</i>	PMID:24920013	N/A
MD221	<i>wt</i>	<i>D. simulans</i>	PMID:24920013	N/A
MD242	<i>wt</i>	<i>D. simulans</i>	PMID:24920013	N/A
LZV L47	<i>wt</i>	<i>D. simulans</i>	PMID:24920013	N/A
<i>Dsim04</i>	<i>wt</i>	<i>D. simulans</i>	DSSC 140210251.004	N/A
<i>Dsim196</i>	<i>wt</i>	<i>D. simulans</i>	DSSC 140210251.196	N/A
<i>Dsec07</i>	<i>wt</i>	<i>D. sechellia</i>	DSSC 140210248.07	N/A
<i>Dsec13</i>	<i>wt</i>	<i>D. sechellia</i>	DSSC 140210248.13	N/A
<i>Dsec28</i>	<i>wt</i>	<i>D. sechellia</i>	DSSC 140210248.28	N/A
<i>Dsec19</i>	<i>wt</i>	<i>D. sechellia</i>	DSSC 140210248.19	N/A
<i>Dsec21</i>	<i>wt</i>	<i>D. sechellia</i>	DSSC 140210248.21	N/A
<i>Dsec30</i>	<i>wt</i>	<i>D. sechellia</i>	DSSC 140210248.30	N/A
<i>Dsec31</i>	<i>wt</i>	<i>D. sechellia</i>	DSSC 140210248.31	N/A
<i>Dsec32</i>	<i>wt</i>	<i>D. sechellia</i>	DSSC 140210248.32	N/A
<i>Dmau90</i>	<i>wt</i>	<i>D. mauritiana</i>	DSSC 140210241.90	N/A
<i>Dmau91</i>	<i>wt</i>	<i>D. mauritiana</i>	DSSC 140210241.91	N/A
<i>W1118</i>	<i>W1118</i>	<i>D. melanogaster</i>	RRID:BDSC_3605	07 and 28
Würzburg Canton-S (CS ^W)	<i>wt</i>	<i>D. melanogaster</i>	Gift of C. Förster	07 and 28
<i>Pdf⁰¹</i>	<i>Pdf⁰¹</i> (in CS ^W background)	<i>D. melanogaster</i>	Gift of C. Förster	07 and 28
<i>Hr38</i>	<i>w*</i> ; <i>dpy ov1 bw1 Hr38⁵⁶/CyO</i> , <i>P{GAL4-twi.G}2.2</i> , <i>P{UAS-2xEGFP}AH2.2</i>	<i>D. melanogaster</i>	RRID:BDSC_76590	07 and 28

<i>Clk</i>	<i>Clk^{out}</i>	<i>D. melanogaster</i>	RRID:BDSC_56754	07 and 28
<i>PDP1</i>	<i>w¹¹¹⁸; Pdp1³¹³⁵/TM3, Sb1</i>	<i>D. melanogaster</i>	RRID:BDSC_80925	07 and 28
<i>cyc</i>	<i>cyc⁰¹</i>	<i>D. melanogaster</i>	RRID:BDSC_80929	07 and 28
<i>scro</i>	<i>scro^{Z211}</i>	<i>D. melanogaster</i>	RRID:BDSC_81875	07 and 28
<i>cwo</i>	<i>W¹¹¹⁸; PBac{RB}cwo[e04207]/T M6B, Tb1</i>	<i>D. melanogaster</i>	RRID:BDSC_85593	07 and 28
<i>Cry</i>	<i>w¹¹¹⁸; ss cry^b</i>	<i>D. melanogaster</i>	RRID:BDSC_80921	07 and 28
<i>Df(vri)</i>	<i>w¹¹¹⁸; Df(2L)Exel6011, P{XP-U}Exel6011/CyO</i>	<i>D. melanogaster</i>	RRID:BDSC_7497	07 and 28
<i>Rh7</i>	<i>y[1]; Rh7⁰</i>	<i>D. melanogaster</i>	RRID:BDSC_83716	07, not 28
<i>Jet^c</i>	<i>y[1] w[*]; jet^c</i>	<i>D. melanogaster</i>	RRID:BDSC_27641	No
<i>Jet^f</i>	<i>y[1] w[*]; jet^f</i>	<i>D. melanogaster</i>	RRID:BDSC_27641	No
<i>Df(Jet)</i>	<i>w¹¹¹⁸; Df(2L)ED7853, P{3'.RS5+3.3}ED7853/S M6a</i>	<i>D. melanogaster</i>	RRID:BDSC_24124	07 and 28
<i>CCHa1</i>	<i>y[1] w[*]; Mi{y[+mDint2]=MIC}CCHa 1[MI09190]</i>	<i>D. melanogaster</i>	RRID:BDSC_51261	07, poorly with 28
<i>ITP</i>	<i>w¹¹¹⁸; PBac{w[+mC]=RB}ITP[e0 2889]/CyO</i>	<i>D. melanogaster</i>	RRID:BDSC_85570	28
<i>tim</i>	<i>y[1] w[*]; tim⁰¹</i>	<i>D. melanogaster</i>	RRID:BDSC_80922	No
<i>Df(tim)</i>	<i>y[1] w[*]; Df(2L)drm-P2, P{lacW}ND- PDSWk10101/SM6b</i>	<i>D. melanogaster</i>	RRID:BDSC_6507	No
<i>sr</i>	<i>y[1] w[*]; P{neoFRT}82B sr¹⁵⁵/TM3, Sb¹</i>	<i>D. melanogaster</i>	RRID:BDSC_36535	No
<i>Fer2</i>	<i>w¹¹¹⁸; PBac{RB}Fer2[e03248]</i>	<i>D. melanogaster</i>	RRID:BDSC_86028	07, poorly with 28
<i>DmelPdf-Gal4</i>	<i>y[1] w67c23;;P{Dmel-PdfGal4}attP2</i>	<i>D. melanogaster</i>	<i>This work</i>	N/A
<i>DsecPdf-Gal4</i>	<i>y[1] w67c23;;P{Dsec-PdfGal4}attP2</i>	<i>D. melanogaster</i>	<i>This work</i>	N/A
<i>DmelPdf-CD4:tdGFP</i>	<i>y[1] w67c23;;P{DmelPdfCD4:tdGFP}attP2</i>	<i>D. melanogaster</i>	<i>This work</i>	N/A
<i>DsecPdf-CD4:tdGFP</i>	<i>y[1] w67c23;;P{DsecPdfCD4:tdGFP}attP2</i>	<i>D. melanogaster</i>	<i>This work</i>	N/A
<i>UAS-Pdf^{RNAi}</i>	<i>y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF01820}att P2</i>	<i>D. melanogaster</i>	RRID:BDSC_25802	N/A
<i>Dmel-Pdf, Pdf⁰¹</i>	<i>y[1] w[*];; P{DmelPdf^f}attP2, Pdf⁰¹</i>	<i>D. melanogaster</i>	<i>This work (two independent strains)</i>	N/A

<i>Dsec-Pdf, Pdf⁰¹</i>	<i>y[1] w[*];; P{DsecPdf¹}attP2, Pdf⁰¹</i>	<i>D. melanogaster</i>	<i>This work (two independent strains)</i>	N/A
-----------------------------------	--	------------------------	--	-----

Supplementary Table 2. Antibodies.

Antibody	Dilution	Source	Identifier
Mouse anti-Pdf (C7)	1:400	Developmental Studies Hybridoma Bank	AB_760350 AB_2315084
Rabbit anti-GFP	1:1000	Molecular Probes, Jackson ImmunoResearch	AB_221570
Rat anti-Cadherin-N (DN-Ex#8)	1:25	Developmental Studies Hybridoma Bank	AB_528121
Goat Alexa488 anti-mouse	1:100	Molecular Probes, Jackson ImmunoResearch	AB_2338840
Goat Alexa488 anti-rabbit	1:100	Molecular Probes, Jackson ImmunoResearch	AB_2338049
Donkey Cy5 anti-rat	1:200	Molecular Probes, Jackson ImmunoResearch	AB_2340672

Supplementary Table 3. Oligonucleotides.

Purpose	PCR product	Sequences (5'-3') sense / antisense
<i>Pdf</i> gene sequencing	CDS + 5'regulatory region I	catttctctcgacgcacca / ccaactgccgagctagctat
	5'-regulatory region II	aaacttaatagctagctcggcag / aatgtggctgcatggaaagt
	5'-regulatory region III	aaacattgacccaactccgc / gttcatcctaccagcgcc
Entry clone for <i>Pdf</i> 5'-regulatory region (Gateway cloning)	5' regulatory region (~2.4 kb upstream of start codon)	ggggacaactttgtacaaaaaagtggcaccggtccacatagtgccagta / ggggacaactttgtacaagaaagtggcaatagtccgaggagctggaagg
<i>Pdf</i> rescue attP2 insertion (Gibson Assembly)	5' regulatory region (~2.4 kb upstream of start codon)	gtcgacactagtggatctctagagttgtaccagattcaagtcgaacaatcc / tcggactaatggctcgctacacgtacct
<i>Pdf</i> rescue attP2 insertion (Gibson Assembly)	CDS	cgagccattagtccgaggagctggaagga / cgcttcatggtgtgaagctggtccacatagtgccagtatatgat