

Supplementary Information for

Photoperiodic and clock regulation of the vitamin A pathway in the brain mediates seasonal responsiveness in the monarch butterfly

Samantha E. Iiams^{1,2}, Aldrin B. Lugena¹, Ying Zhang¹, Ashley N. Hayden¹, and Christine Merlin^{1,2*}

¹ Department of Biology and Center for Biological Clocks Research, Texas A&M University, College Station, Texas 77843, USA

² Genetics Interdisciplinary Program, Texas A&M University, College Station, Texas 77843, USA

*Corresponding author: Christine Merlin

Email: cmerlin@bio.tamu.edu

This PDF file includes:

Supplementary Material and Methods
Figures S1 to S4
Tables S1 to S5
Legends for Datasets S1 to S2
SI References

Other supplementary materials for this manuscript include the following:

Datasets S1 to S2

Supplementary Information

Material and Methods

Animal husbandry

For photoperiodic experiments, wild-type and mutant monarchs maintained in 15-hours light: 9-hours dark at 25°C were hand-paired. Fertilized females were placed in a cage to lay eggs on potted tropical milkweed plants (*Asclepias curassavica*). Eggs were transferred to petri dishes onto milkweed leaves and divided in two groups placed in Percival incubators respectively under 15-hours light: 9-hours dark (long photoperiod; LP) and under 10-hours light: 14-hours dark (short photoperiod; SP), both at constant temperature of 21°C and 70% humidity. Starting at the second instar, larvae were raised individually on semi-artificial diet until pupation. Post-eclosion, adult monarchs were housed in glassine envelopes and were manually fed a 25% honey solution daily until dissection.

For RNA-seq experiments, fall migratory monarch butterflies were captured on October 15, 2014 in College Station, Texas (latitude 30°37'N, longitude 96°20'W). After capture, migrants were housed indoors in glassine envelopes in a Percival incubator under fall-like conditions with light:dark (LD) cycle set to prevailing light conditions (11 h:13 h LD, 0730–1830 Central Standard Time), at a constant temperature of 21 °C with 70% humidity. They were manually fed a 25% honey solution every other day and dissected 8 days after capture. Monarchs used for the LP and SP data sets were raised in the same conditions as for the photoperiodic experiments, and were dissected 7 to 11 days post-eclosion.

Methoprene/vehicle treatment

Monarch wild-type and *Cry2*^{-/-} females were treated topically on their abdomens with 200µg of methoprene in 5µl of acetone or with 5µl of acetone alone on day 1 and day 3 after adult emergence, as previously described (1).

Compound eye painting and antennae removal

Painting of the compound eyes and removal of the antennae were performed on the

day of eclosion. For eye painting, the labial palps were first cut at the base to expose the entire compound eyes including the retina and the dorsal rim, which were then covered under a dissecting microscope with enamel based clear paint (Model master clear top coat; Testors no. 2736) or black paint (Glossy black; Testors no. 1147) using a fine paintbrush. Monarchs were harnessed to allow the paint to air-dry. The completeness of painting was verified the next day under a dissecting microscope, and touch ups were performed if needed. The light-permissive and light-blocking properties of the clear and black paints, respectively, had previously been characterized (2). Unpainted control monarchs were subjected to labial palps removal similar to painted monarchs. Antennae were removed by clipping them with scissors at the base of the flagellum just above the pedicel as described in (2).

RNA-sequencing experiments

Brains of monarchs raised indoors in LP and SP and of wild-caught migrants were dissected in 0.5X RNA later (Invitrogen) to prevent RNA degradation, the retinal pigmented photoreceptor layer was removed, and the brains were stored at -80°C until use. For each seasonal phenotype/photoperiodic condition, three pooled brains were collected in two replicates at Zeitgeber time (ZT)1, ZT4, ZT7, ZT10, ZT13, ZT16, ZT19, and ZT22. For each sample, total RNA was extracted using an RNeasy Mini kit (Qiagen). For samples from wild-caught migrants, polyA⁺ RNA was isolated from 2 μg of total RNA with NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs), and multiplexed libraries were prepared using the NEBNext[®] Ultra[™] II Directional RNA Library Prep Kit for Illumina and NEBNext Multiplex Oligos (New England Biolabs) and amplified with 12 PCR cycles, following the manufacturer's recommendations. RNA-seq datasets from brains of summer-like monarchs and clock-deficient mutants used in this study were already available (3). Butterflies were raised in 15:9 LD at 25°C , brains were collected at 3-hours intervals for summer-like monarchs starting at ZT1 and at 6-hours intervals for clock-deficient mutants starting at ZT4, and libraries were prepared as for wild-caught migrants. For samples from monarchs raised in LP and SP, multiplexed libraries were prepared by the Texas A&M AgriLife Genomics and Bioinformatics Facility using polyA⁺ RNA isolated from 1 μg of total RNA, and

multiplexed libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit for Illumina, following the manufacturer's recommendations. Libraries quality and size distribution was verified on a Bioanalyzer, libraries were quantified by real-time quantitative PCR, and 16 multiplexed libraries were mixed in equimolar ratios and sequenced on a Hi-seq 2500 (Illumina) using 50bp single end reads.

RNA-seq data processing, mapping and identification of cycling transcripts

The resulting sequencing files were checked for quality control and demultiplexed by the Texas A&M AgriLife Genomics and Bioinformatics Facility. Reads were mapped to the monarch genome (assembly v3; (4)) using TopHat2 (5) with parameters "--read-realign-edit-dist 2 -g 1 --b2-sensitive". On average across LP, SP, summer-like and migrant monarchs, 91.5% of the reads mapped to the monarch genome (Table S4). After mapping, expression levels of transcripts were quantified in each sample using Cufflinks (6, 7). To identify cycling transcripts, RAIN (8) and MetaCycle (9) were used with parameters "period=24, deltat=3, period.delta=3, nr.series=2, method='independent', peak.border=c(0.2, 0.8)" and "timepoints=seq(1, 46, by=3), adjustPhase='predictedPer', combinePvalue='fisher'", respectively. Only genes with three or more reads per kilo base per million mapped reads (RPKM) in at least one time point were considered as expressed and used for subsequent analysis. Rhythmically expressed genes were determined based on fold-change and *p*-values adjusted for multiple testing using Benjamini-Hochberg procedure to control for false discovery rate. Transcripts were considered rhythmically expressed when meeting the following criteria: (1) adjusted *p*-values ≤ 0.05 for both RAIN and MetaCycle; and (2) fold-change (maximal/minimal experimental values within a time series) ≥ 1.3 . Of the 15,130 genes in the monarch genome, 68.3%, 66.8%, 66.6%, and 68.4% were respectively expressed in the brains of LP, SP, summer-like, and migrant monarchs, of which 2.70%, 2.66%, 2.80% and 3.04% were respectively determined as rhythmically expressed (Table S5).

Comparisons of rhythmic expression were performed between LP and SP monarchs, and between summer-like monarchs and wild-caught migrants. Genes were sorted based on their rhythmic expression in a photoperiod condition or a seasonal form and classified in three groups: (1) rhythmic in both LP and SP or summer-like and migrant monarchs,

(2) rhythmic only in LP or summer-like monarchs, and (3) rhythmic only in SP or migrant monarchs. Heatmaps depicting all categories were produced using heatmap.2 in gplots package for R. To identify the genes that may be responsible for photoperiodic responses, genes present in both comparisons were identified. Results of this overlap analysis are summarized in Fig. 2. The complete list of genes is provided in Table S1. Using BLAST (10), homologous proteins from *Drosophila* and mouse that best matched the protein sequences of the overlapped genes were used to annotate them. Enriched gene ontology of biological processes and KEGG pathway were determined using Metascape (metascape.org).

gRNA design and construction

The gRNA site for CRISPR/Cas9-mediated targeted mutagenesis of *ninaB1* was selected within exon 3 of the 14 exons-containing *ninaB1* using CHOPCHOP target site finder (<http://chopchop.cbu.uib.no/index.php> target site finder; (11, 12)). The gRNA expression vector was constructed by inserting annealed synthetic oligomers into the DR274 plasmid from Addgene (13) at the *BsaI* cleavage site. Oligomer sequences were as follow (F, forward primer; R, reverse primer): F, 5'-TAGGAGTGACAACTATACGACCCG-3' and R, 5'-AAACCGGGTCGTATAGTTGTC-3'.

Synthesis of Cas9 mRNA and sgRNA

In vitro transcription of *Streptococcus pyogenes* Cas9 mRNA was performed using the mMessage mMachine T3 transcription kit (Invitrogen) and pCS2-nCas9n expression plasmid from Addgene (14), as previously described (15). The resulting capped PolyA mRNAs were purified by acid-phenol-chloroform extraction and resuspended in RNase-free water following isopropanol precipitation. The sgRNA was *in vitro* transcribed using T7 RNA polymerase (Promega) from purified PCR products containing the T7 promoter, gRNA and gRNA scaffold amplified from the DR274 vectors using the following primers; F: 5'-ATTGAGCCTCAGGAAACAGC-3' and R: 5'-AAAAGCACCGACTCGGTGCC-3'. The sgRNA was then treated with RQ1-DNase and purified by acid phenol-chloroform extraction and resuspended in RNase-free water after

isopropanol precipitation. Cas9 mRNAs and the sgRNA were quantitated by spectrophotometry (NanoDrop 1000) and diluted in RNase-free water to a final concentration of 0.25 µg/µl for the sgRNA and 0.5 µg/µl for Cas9 mRNAs.

Egg microinjections

Eggs were collected and microinjected as previously described (16) with a mix of Cas9 mRNA at 0.5 µg/µl, sgRNA at 0.25 µg/µl, and blue food coloring for visual tracking of the injection. After injection, embryos were placed in an incubator at 25°C and 70% humidity. Developing embryos were transferred into individual small petri dishes containing milkweed leaves until larvae hatched. Larvae were fed milkweed leaves until the second larval instar before being transferred onto semi-artificial diet.

Analysis of CRISPR/Cas9-induced mutations and generation of a *ninaB1* monarch loss-of-function line

PCR fragments flanking the targeted region were amplified from genomic DNA extracted from larval sensors of potential founders at the fifth instar with the following primers: *ninaB1F*, 5'-GTTTCACTTGTACCGTGACTTC-3' and *ninaB1R*, 5'-GGATACTGTTTAGCCAGGTACC-3. PCR products were purified using 2 X modified Sera-Mag Magnetic Speed-beads (GE Healthcare) as previously described (16, 17), and resuspended in 10 µl of RNase-free water. Cas9-based cleavage assays of PCR products (250-350 ng) were performed using a recombinant Cas9 protein (750 ng) and the sgRNA (400-600 ng), as previously described (16). Digested products were purified using 2 X modified Sera-Mag Magnetic Speed-beads before being resolved with agarose gel electrophoresis and EtBr staining. Larvae presenting a high degree of targeting in somatic cells, estimated based on the relative abundance of uncleaved fragments, were selected and reared to adulthood. Surviving adults of opposite sexes presenting the highest level of somaticism were hand-paired in individual cages to establish a mutant line. Eggs were collected and the hatched larvae were screened for the presence of mutated alleles as described above. Uncleaved fragments corresponding to mutated alleles were gel purified and sequenced using one of the primers used for PCR amplification. A 7-base pair deletion causing a frameshift and the introduction of a premature stop codon was selected

to establish a mutant line.

Proboscis extension reflex assay

The proboscis extension reflex (PER) is a response in which stimulation of the chemoreceptors on the middle legs with a sugar solution (unconditioned stimulus, US) results in full extension of the proboscis (unconditioned response). Proboscis extension tests were conducted as previously described (2) by touching the middle leg with a cotton-tipped applicator soaked in 50% sucrose solution (wt/wt), with slight modifications. Individual butterflies were fed daily with 150 μ l of a 25% honey solution prior to the experiment. The day prior to stimulus conditioning, individuals were harnessed in 15ml polypropylene conical tubes as previously described (2) and starved for 24 hours. On the day of testing, individuals were checked for a positive proboscis extension reflex. Wild-type monarchs with eyes covered with either clear or black paint, and *ninaB1* $-/-$ monarchs were conditioned to a colored stimulus (red flag; conditioned stimulus, CS) by presenting the stimulus for 5 sec (CS only), contacting the middle legs with sucrose solution for 15 sec (CS +US), and removing the stimulus after 5 sec (US only). Individuals were then held for 5 min and this pairing procedure was performed again. US-CS pairing continued in this way for 13 to 14 trials a day for three consecutive days until the individual extended its proboscis upon the initial presentation of the CS, in which case a sucrose reward was given. Monarchs were considered to have a positive PER (conditioned response) if they fully extended their proboscis in response to the CS. On the second and third day of testing, butterflies were fed 50 μ l of a 25% honey solution to prevent extreme starvation.

Real-time qPCR

To test for the presence of a functional circadian clock in *ninaB1* loss-of-function monarchs, brains of adult wild-type and *ninaB1* homozygous mutant monarchs entrained to seven 15:9 LD cycles were dissected under red light on the first day of transfer into DD at circadian time (CT) 0, CT4, CT8, CT12, CT16, and CT20. Dissections were performed in 0.5X RNA later (Invitrogen) to avoid RNA degradation, and brains free of eye photoreceptors were stored at -80°C until use. Total RNA was extracted using

RNeasy Mini kit (Qiagen), treated with RQ1 Dnase (Promega), and random hexamers (Promega) were used to prime reverse transcription with Superscript II Reverse Transcriptase (Thermo Scientific), all according to the manufacturers' instructions. Quantifications of gene expression were performed on a QuantStudio™ 6 Flex Real-Time PCR System (Thermo Scientific) using iTaq Universal SYBR Green Supermix (Bio-Rad). PCR reactions were assembled by combining two master mixes: the first mix contained 5 µl of iTaq Universal SYBR® Green Supermix (Bio-Rad) and forward and reverse primers (5 µmol each) per reaction and the second mix contained approximately 7 ng of cDNA template and the water needed to bring each reaction to a final volume of 10 µl. The monarch *per* and control *rp49* primers were as follows (F, forward primer; R, reverse primer): *perF*, 5'-AGTGAAGCGTCCCTCAAACA-3'; *perR*, 5'-TGGCGACGAGCATCTGTGT-3'; *rp49F*, 5'-TGCGCAGGCGTTTTAAGG-3'; *rp49R*, 5'-TTGTTTGATCCGTAACCAATGC-3'. The near 100% efficiency of each primer set was validated by determining the slope of Ct versus dilution plot on a dilution series. Individual reactions were used to quantify each RNA level in a given cDNA sample, and the average Ct from duplicated reactions within the same run was used for quantification. The data were normalized to *rp49* as an internal control, and normalized to the mean of one sample within a set for statistics.

Supplementary Figures

Fig. S1

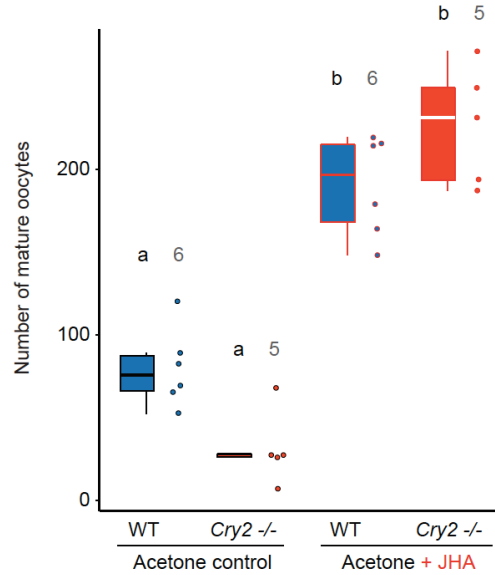


Fig. S1. Application of a juvenile hormone analogue restores high levels of oocyte production in monarch *Cry2*^{-/-}. Number of mature oocytes produced 10 days post adult emergence in wild-type or *Cry2*^{-/-} female monarchs treated with either an acetone vehicle or with methoprene, a juvenile hormone analogue (JHA). Monarch were raised in LP at 25°C. Legends as in Figure 1. Interaction genotype x treatment, Two-way ANOVA, Tukey's pairwise comparisons, $p < 0.05$.

Fig. S2

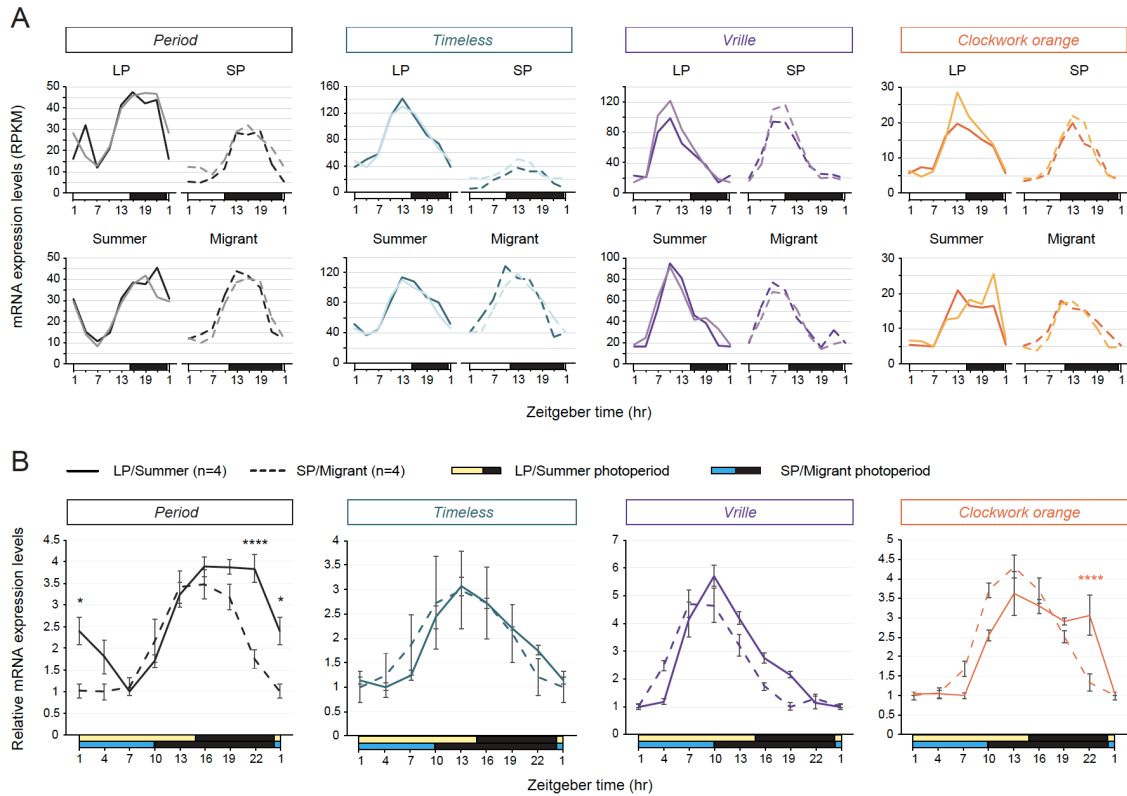


Fig. S2. Temporal mRNA expression profiles of *period*, *timeless*, *vrille*, and *clockwork orange* in brains of monarchs in different photoperiods. A) mRNA expression levels in brains of monarchs raised in LP and SP (*top*) and of summer-like and migrant monarchs (*bottom*). For each condition, two biological replicates are plotted. White bars: light; black bars: dark. B) Relative mRNA expression levels from pooled biological replicates of LP and summer-like monarchs (solid line), and SP and migrants (dashed line). Data represent the mean \pm s.e.m. Yellow bars: long day; blue bars: short day; black bars: dark. Interactions seasonal morph \times photoperiod, Two-way ANOVAs: *period*, $p < 0.0001$; *timeless*, non significant; *vrille*, $p < 0.005$; *clockwork orange*, $p < 0.0001$. Tukey's pairwise comparisons, *, $p < 0.05$; ****, $p < 0.0001$.

Fig. S3

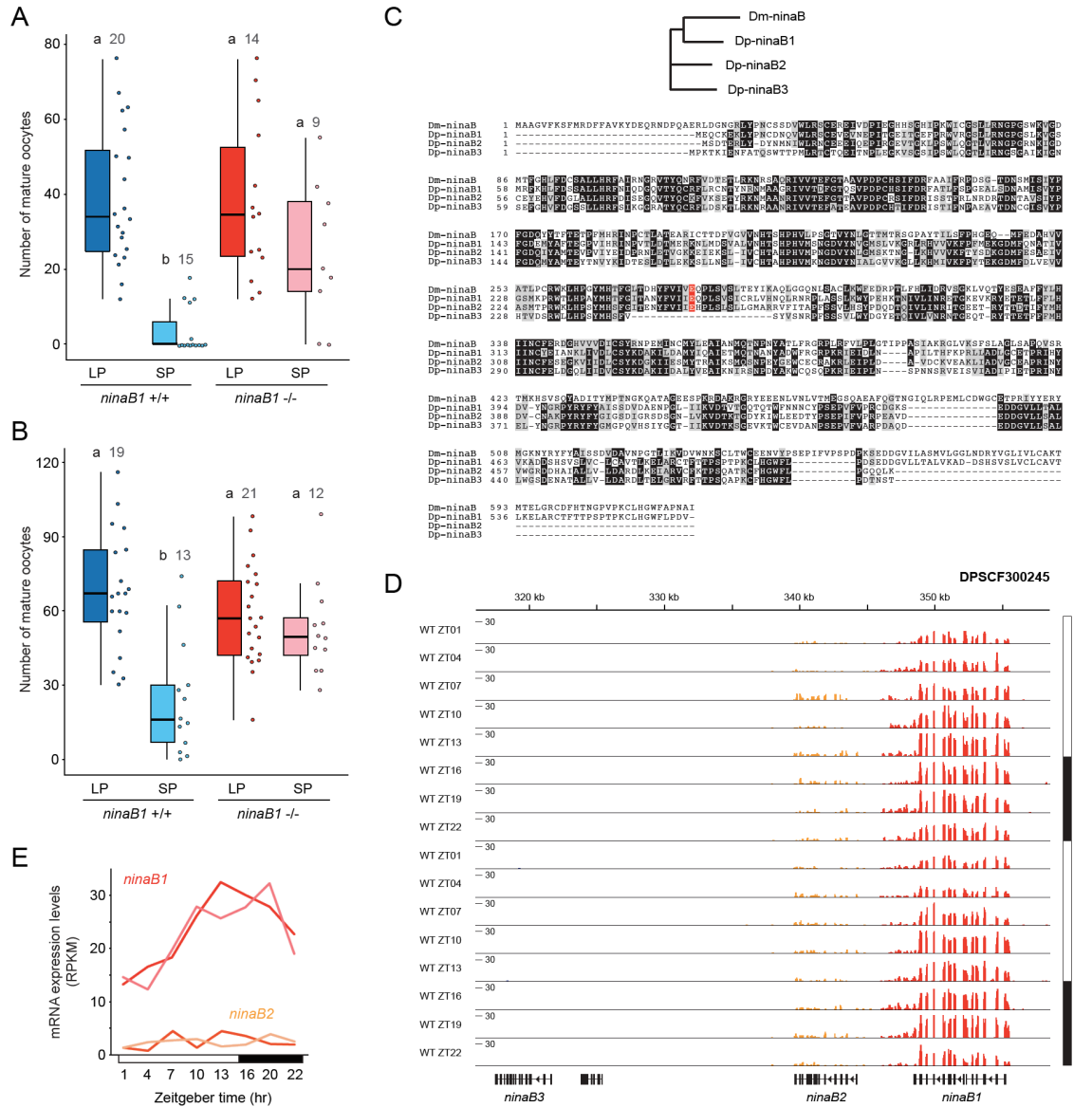


Fig. S3. The reproductive state exhibited by photoperiod-impaired *ninaB1* loss-of-function could be caused by redundant function of *ninaB1* and *ninaB2*. (A, B) Number of mature oocytes produced 10 days post adult emergence in *ninaB1* homozygous loss-of-function and wild-type sibling females, all raised in LP and SP at 21 °C after either one backcross (A) or three backcrosses (B). Legends as in Figure 4B. Interaction genotype x photoperiod, Two-way ANOVA, Tukey's pairwise comparisons, $p < 0.05$. (C) Comparisons of *Drosophila ninaB* (Dm; AAF54978) and monarch (Dp) *ninaB1* (DPOGS212590), *ninaB2* (DPOGS212591), and *ninaB3* (DPOGS212592) sequences. *Top*, phylogenetic relationship. *Bottom*, sequences alignment. Identical and similar residues found in at least three out of four sequences are shaded in black and gray,

respectively. The glutamic acid residue highlighted in red, which is conserved between Dm-ninaB, Dp-ninaB1 and Dp-ninaB2 but absent in Dp-ninaB3, corresponds to the residue providing the enzymatic activity of Dm-ninaB (18). (D) Visualization of RNA-seq signal in brains of wild-type monarchs over 24 hours in LP on scaffold DPSCF300245 that contains the three copies of *ninaB* present in the monarch genome. Of the two detectable expressed copies, only *ninaB1* displays rhythmic expression. Two biological replicates are plotted consecutively from top to bottom. White bar: light; dark bar: night. (E) mRNA expression levels of *ninaB1* and *ninaB2* in the brain of wild-type monarchs over the course of the day in LP. For each gene, two biological replicates are plotted. White bar: light; black bar.

Fig. S4

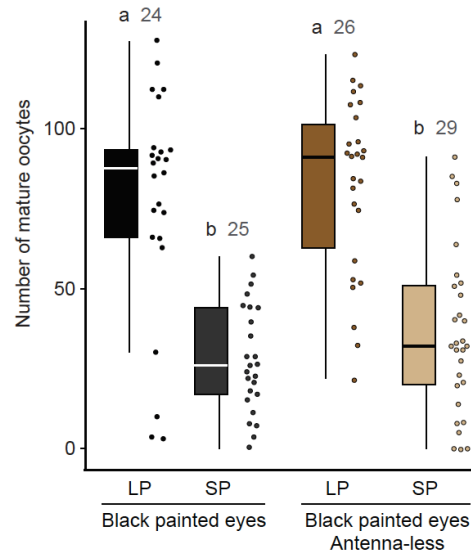


Fig. S4. The antennae are not necessary for photoperiodic responses. Number of mature oocytes produced 10 days post adult emergence in wild-type female monarchs with eyes painted black and with or without antennae, all raised in LP and SP at 21°C. Legends as in Figure 4C. Interaction genotype x photoperiod, Two-way ANOVA, Tukey's pairwise comparisons, $p < 0.05$.

Table S1. List of genes with similar or differential temporal expression patterns in the brain of monarchs raised in LP and SP. R-R: Genes rhythmic in both conditions; R-AR: Genes rhythmic in LP monarchs and arrhythmic in SP monarchs; AR-R: Genes arrhythmic in LP monarchs and rhythmic in SP monarchs. The annotation is based on *Drosophila* (no shading) or mouse (gray shading) orthologues. NA, monarch genes without orthologues in *Drosophila* or the mouse.

Gene ID	R-R
DPOGS211878	Cyclin D
DPOGS200190	similar
DPOGS214070	stumps
DPOGS205548	Ubiquitin conjugating enzyme 7
DPOGS207780	multiple edematous wings
DPOGS209874	solute carrier family 2, (facilitated glucose transporter), member 8
DPOGS207730	CG10082
DPOGS205355	Ucp4A
DPOGS213007	Cytochrome P450-18a1
DPOGS207942	synaptotagmin XI
DPOGS206908	Glucose dehydrogenase
DPOGS205549	G protein-coupled receptor 158
DPOGS205086	Multidrug-Resistance like Protein 1
DPOGS209797	Vacuolar H ⁺ ATPase 100kD subunit 2
DPOGS215479	monooxygenase, DBH-like 1
DPOGS210745	transmembrane protein 135
DPOGS201345	tocopherol (alpha) transfer protein-like
DPOGS215439	lethal (2) essential for life
DPOGS211121	ubiquitin-conjugating enzyme E2Q family-like 1
DPOGS204644	phospholipid phosphatase 1
DPOGS209511	solute carrier family 17 (anion/sugar transporter), member 5
DPOGS215865	THUMP domain containing 1
DPOGS213900	Heat shock protein 68
DPOGS208883	Glycerol 3 phosphate dehydrogenase
DPOGS215738	eiger
DPOGS213901	Heat shock protein 68
DPOGS215489	6-phosphofructo-2-kinase
DPOGS205929	sprite
DPOGS202078	Cyp9f2
DPOGS204253	calsyntenin 2

DPOGS202178	Glutamate oxaloacetate transaminase 1
DPOGS207058	choline dehydrogenase
DPOGS215494	1,4-Alpha-Glucan Branching Enzyme
DPOGS210128	PAPS synthetase
DPOGS201488	Mid1 interacting protein 1 (gastrulation specific G12-like (zebrafish))
DPOGS213925	Heat shock protein 68
DPOGS204642	GTPase regulator associated with FAK
DPOGS205928	sprite
DPOGS213114	Choline transporter-like 2
DPOGS205386	phosphoglucose mutase
DPOGS214409	solute carrier family 22 (organic cation transporter), member 1
DPOGS201746	Carbonic anhydrase 1
DPOGS202237	DnaJ heat shock protein family (Hsp40) member B4
DPOGS200817	alkaline phosphatase, liver/bone/kidney
DPOGS214695	meteorin, glial cell differentiation regulator-like
DPOGS200691	Enhancer of split mbeta, helix-loop-helix
DPOGS213804	Inositol 1,4,5-triphosphate kinase 1
DPOGS213896	Mitf
DPOGS200684	Enhancer of split mbeta, helix-loop-helix
DPOGS200409	Integrin betanu subunit
DPOGS203128	argos
DPOGS210886	rau
DPOGS208606	vriille
DPOGS201013	high mobility group box transcription factor 1
DPOGS205643	solute carrier family 36 (proton/amino acid symporter), member 4
DPOGS215160	Trehalose transporter 1-2
DPOGS214408	solute carrier family 22 (organic cation transporter), member 21
DPOGS211073	cysteine-rich secretory protein 2
DPOGS207391	clavesin 2
DPOGS204091	Salt-inducible kinase 3
DPOGS215159	Trehalose transporter 1-1
DPOGS215378	chondroitin sulfate synthase 1
DPOGS205105	ATP binding cassette subfamily G member 4
DPOGS201012	Leucine-rich pentatricopeptide repeat containing 2
DPOGS214162	Na ⁺ /H ⁺ hydrogen antiporter 1
DPOGS211050	Insulin-like receptor
DPOGS209785	Aminolevulinatase synthase
DPOGS207504	Farnesyl pyrophosphate synthase

DPOGS214179	timeless
DPOGS209175	phosphatidylinositol-specific phospholipase C, X domain containing 3
DPOGS203974	Calcium-dependent secretion activator
DPOGS209926	hattifattener
DPOGS214215	Na ⁺ /H ⁺ hydrogen antiporter 1
DPOGS205855	solute carrier family 46, member 3
DPOGS200290	penguin
DPOGS212590	neither inactivation nor afterpotential B
DPOGS207444	phospholipase A2, group III
DPOGS201101	solute carrier family 2 (facilitated glucose transporter), member 6
DPOGS209146	solute carrier family 22 (organic cation transporter), member 3
DPOGS209925	clockwork orange
DPOGS211474	pastrel
DPOGS204463	Matrix metalloproteinase 1
DPOGS213552	Ecdysone-induced protein 28/29kD
DPOGS216102	lethal (2) k09913
DPOGS201925	solute carrier family 2, (facilitated glucose transporter), member 8
DPOGS215419	dumpy
DPOGS203908	period
DPOGS208959	Vacuolar H ⁺ ATPase 100kD subunit 2
DPOGS203797	Heat shock factor
DPOGS209585	FCH domain only 2
DPOGS212685	sarcoplasmic calcium-binding protein
DPOGS208406	CG30069
DPOGS204552	solute carrier family 2, (facilitated glucose transporter), member 8
Gene ID	R-AR
DPOGS215700	primo-2
DPOGS207634	tribbles
DPOGS214920	Multicopper oxidase-1
DPOGS201668	short gastrulation
DPOGS205889	Rab40
DPOGS202087	Cytochrome P450-9b2
DPOGS215481	no mechanoreceptor potential C
DPOGS213661	stearoyl-Coenzyme A desaturase 1
DPOGS208470	CG13272
DPOGS215992	PDGF- and VEGF-receptor related
DPOGS210839	solute carrier family 16 (monocarboxylic acid transporters), member 14

DPOGS209237	Slowpoke binding protein
DPOGS206903	SIK family kinase 3
DPOGS206136	LON peptidase N-terminal domain and ring finger 2
DPOGS207671	branchless
DPOGS209909	carboxylesterase 2F
DPOGS214094	mysospheroid
DPOGS202126	very low density lipoprotein receptor
DPOGS208999	retinol dehydrogenase 13 (all-trans and 9-cis)
DPOGS213594	Glycogen binding subunit 76A
DPOGS203211	Cuticular protein 97Ea
DPOGS201245	CAP
DPOGS215826	solute carrier family 46, member 3
DPOGS203379	periaxin
DPOGS213639	xin actin-binding repeat containing 2
DPOGS200414	twist
DPOGS201544	Organic anion transporting polypeptide 74D
DPOGS203582	CAP
DPOGS214692	phosphoribosyl pyrophosphate synthetase-associated protein 2
DPOGS207676	lethal (3) 73Ah
DPOGS200195	transmembrane protein 135
DPOGS212327	F-box protein 32
DPOGS201486	windpipe
DPOGS201894	Maltase A4
DPOGS212552	Cyp9f2
DPOGS205180	lipase, member H
DPOGS210627	carbonic anhydrase 3
DPOGS200316	globin 1
DPOGS214402	Glycogen synthase
DPOGS205764	U2A
DPOGS206008	microtubule associated monooxygenase, calponin and LIM domain containing 3
DPOGS214921	Multicopper oxidase-1
DPOGS210848	starvin
DPOGS204087	ribbon
DPOGS210850	leucine rich repeat containing G protein coupled receptor 5
DPOGS207651	UDP glucuronosyltransferase 2 family, polypeptide B1
DPOGS212108	straw
DPOGS201241	CAP

DPOGS204250	phenazine biosynthesis-like protein domain containing 2
DPOGS207000	Myosin heavy chain-like
DPOGS212996	Ecdysone-inducible gene E1
DPOGS215099	transmembrane protease, serine 11f
DPOGS208825	Protostome-specific GEF
DPOGS202077	Cyp9f2
DPOGS209025	LIM domain only 7
DPOGS207287	selenium binding protein 1
DPOGS207994	family with sequence similarity 193, member A
DPOGS202356	mutagen-sensitive 312
DPOGS205888	Serine hydroxymethyltransferase 2
DPOGS213632	pallbearer
DPOGS215384	RIO kinase 2
DPOGS208881	DnaJ-like-2
DPOGS200771	stall
DPOGS215969	Transaldolase
DPOGS202029	membrane steroid binding protein
DPOGS205833	amylo-1,6-glucosidase, 4-alpha-glucanotransferase
DPOGS200067	Trehalose transporter 1-2
DPOGS208971	leucine rich melanocyte differentiation associated
DPOGS213899	Heat shock protein 83
DPOGS209508	phosphoglucose mutase
DPOGS206382	lethal (2) essential for life
DPOGS202781	Hsp70/Hsp90 organizing protein
DPOGS203810	Phosphofructokinase
DPOGS210890	Autophagy-related 3
DPOGS214304	spenito
DPOGS215007	Brahma associated protein 170kD
DPOGS215703	firelighter
DPOGS203355	string
DPOGS210704	COP9 signalosome subunit 7B
DPOGS201784	MEP-1
DPOGS201296	Cyclic nucleotide-gated ion channel subunit B
DPOGS207433	dumpy
DPOGS210130	folliculin interacting protein 2
DPOGS201291	Ecdysone-induced protein 93F
DPOGS209745	Reversion-inducing-cysteine-rich protein with kazal motifs

DPOGS204036	crowded by cid
DPOGS200075	Hexokinase A
DPOGS205799	Desaturase 1
DPOGS205647	ATP binding cassette subfamily G member 4
DPOGS202409	dally-like
DPOGS208054	ENL/AF9-related
DPOGS211554	Partner of paired
DPOGS202573	Phosphoenolpyruvate carboxykinase
DPOGS214934	distal antenna
DPOGS210087	unc-5
DPOGS207462	Beadex
DPOGS212184	lysyl oxidase-like 2
DPOGS209609	MLF1-adaptor molecule
DPOGS203228	Cln7
DPOGS203167	solute carrier family 22 (organic cation transporter), member 3
DPOGS207999	MYC-associated zinc finger protein (purine-binding transcription factor)
DPOGS201447	Chitin deacetylase-like 5
DPOGS202150	Chondrocyte-derived ezrin-like domain containing protein
DPOGS202152	Chondrocyte-derived ezrin-like domain containing protein
DPOGS211888	otopetrin 2
DPOGS209166	Bre1
DPOGS208905	kuzbanian
DPOGS201136	Ecdysone-induced protein 74EF
DPOGS202153	Chondrocyte-derived ezrin-like domain containing protein
DPOGS200402	Prosap
DPOGS205510	CG42304
DPOGS208589	Adherens junction protein p120
DPOGS208870	cadherin 15
DPOGS203088	solute carrier family 5 (sodium/glucose cotransporter), member 12
DPOGS204939	fibronectin type III domain containing 5
DPOGS210490	pyroglutamyl-peptidase I
DPOGS203127	Pyruvate kinase
DPOGS214140	Fatty acid (long chain) transport protein
DPOGS206803	Ubiquitin conjugating enzyme 4
DPOGS207157	Formin homology 2 domain containing

DPOGS202825	fuseless
DPOGS205514	happyhour
DPOGS200399	schlank
DPOGS205915	folded gastrulation
DPOGS201347	tocopherol (alpha) transfer protein
DPOGS212492	CG31324
DPOGS215237	Mid1
DPOGS207272	ADP ribosylation factor-like 4
DPOGS211190	minidisks
DPOGS208314	Mitochondrial pyruvate carrier
DPOGS213044	mediator complex subunit 14
DPOGS202609	Acetylcholine esterase
DPOGS208868	Membrin
DPOGS209828	jaguar
DPOGS214045	transmembrane and tetratricopeptide repeat containing 2
DPOGS200370	discs overgrown
DPOGS214228	Protein kinase, cAMP-dependent, catalytic subunit 3
DPOGS216090	ral guanine nucleotide dissociation stimulator-like 3
DPOGS210086	Prp18
DPOGS205077	neurotrimin
DPOGS209183	sevenless
DPOGS208166	Fimbrin
DPOGS203456	Fasciclin 3
DPOGS208790	proline rich 22
DPOGS203229	solute carrier family 22 (organic cation transporter), member 3
DPOGS204749	solute carrier family 22 (organic cation transporter), member 1
DPOGS214622	BIR repeat containing ubiquitin-conjugating enzyme
DPOGS203436	RIKEN cDNA 3425401B19 gene
DPOGS212948	BCL2/adenovirus E1B interacting protein 3-like
DPOGS208674	GLI pathogenesis-related 2
DPOGS204941	hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)
DPOGS210475	Adipokinetic hormone receptor
DPOGS201412	CG11370
DPOGS201957	carboxylesterase 2A
DPOGS209128	carboxylesterase 4A
DPOGS202827	scavenger receptor acting in neural tissue and majority of rhodopsin is absent
DPOGS203890	scavenger receptor acting in neural tissue and majority of rhodopsin

	is absent
DPOGS213662	Pyrokinin 1 receptor
DPOGS214271	Sodium/solute co-transporter-like 5A11
DPOGS213185	missing-in-metastasis
DPOGS201183	Peptidoglycan recognition protein LC
DPOGS210599	Cytochrome b5
DPOGS206439	glutaryl-Coenzyme A dehydrogenase
DPOGS201885	nervana 2
DPOGS201896	NA
DPOGS208079	cryptochrome 2
DPOGS205727	Glycoprotein hormone alpha 2
DPOGS205014	red Malpighian tubules
DPOGS202323	CG12947
DPOGS206847	I'm not dead yet
DPOGS215688	solute carrier family 34 (sodium phosphate), member 1
DPOGS214174	RIKEN cDNA 1110008P14 gene
DPOGS202921	puffeye
DPOGS213305	Trehalose transporter 1-2
DPOGS203808	arc
DPOGS200805	Chondrocyte-derived ezrin-like domain containing protein
DPOGS208132	CG8854
DPOGS207346	NA
DPOGS208658	Multiple C2 domain and transmembrane region protein
Gene ID	AR-R
DPOGS215693	Kinesin-like protein at 31E
DPOGS205193	pita
DPOGS213910	dawdle
DPOGS213990	Valyl-tRNA synthetase, mitochondrial
DPOGS209800	absent, small, or homeotic discs 2
DPOGS204630	Cytochrome b5
DPOGS213168	thickveins
DPOGS209055	breast carcinoma amplified sequence 3
DPOGS201195	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2
DPOGS208537	fritz
DPOGS213902	Unc-89
DPOGS213038	RNA-binding protein 6

DPOGS211622	purine-nucleoside phosphorylase 2
DPOGS213604	family with sequence similarity 205, member A2
DPOGS215784	narrow
DPOGS215002	Tetraspanin 97E
DPOGS208998	ethanolamine phosphate phospholyase
DPOGS205152	Ecdysone-inducible gene L2
DPOGS205265	sulfotransferase family 2A, dehydroepiandrosterone (DHEA)-preferring, member 6
DPOGS208044	sodium potassium chloride cotransporter
DPOGS210295	Phosphoglucose isomerase
DPOGS210772	ubiquitin protein ligase E3 component n-recogin 5
DPOGS215272	SET and MYND domain containing, arthropod-specific, member 4
DPOGS213903	solute carrier family 2, (facilitated glucose transporter), member 8
DPOGS200764	wunen
DPOGS200490	Malate dehydrogenase 1
DPOGS211592	Mucin 14A
DPOGS206048	mannan-binding lectin serine peptidase 1
DPOGS209724	transmembrane protease, serine 9
DPOGS211461	family with sequence similarity 43, member A
DPOGS201483	Rrp42
DPOGS213101	Collagen type IV alpha 1
DPOGS203208	Novel nucleolar protein 3
DPOGS200182	Dual oxidase
DPOGS203734	Nckx30C
DPOGS208305	lethal (1) G0020
DPOGS213282	zelda
DPOGS210429	Rab23
DPOGS209220	takeout
DPOGS209293	ACAT-related protein required for viability 1
DPOGS203267	ELF1 homolog, elongation factor 1
DPOGS209941	glutamate receptor, ionotropic, kainate 2 (beta 2)
DPOGS203459	Sphingosine kinase 1
DPOGS215663	DIM1 dimethyladenosine transferase 1-like (<i>S. cerevisiae</i>)
DPOGS213088	Rrp4
DPOGS215556	PSEA-binding protein 95kD
DPOGS210537	ATP synthase mitochondrial F1 complex assembly factor 1
DPOGS208596	geminin
DPOGS211313	JNK1/MAPK8-associated membrane protein

DPOGS207276	karyopherin alpha1
DPOGS207451	Glutaminyl-tRNA synthetase
DPOGS212326	tRNA isopentenyltransferase 1
DPOGS211640	dehydrogenase/reductase (SDR family) member 4
DPOGS202064	mitochondrial ribosomal protein L32
DPOGS201773	Regulatory particle non-ATPase 12
DPOGS203161	merry-go-round
DPOGS211728	jitterbug
DPOGS202638	PWP2 periodic tryptophan protein homolog (yeast)
DPOGS210122	Phospholipase A2 activator protein
DPOGS213171	Mitochondrial trifunctional protein alpha subunit
DPOGS202243	ribosomal RNA processing 15 homolog (<i>S. cerevisiae</i>)
DPOGS210477	Glutathione S transferase D3
DPOGS204283	transmembrane protein 256
DPOGS207617	noisette
DPOGS204316	Isocitrate dehydrogenase
DPOGS213570	methyltransferase like 22
DPOGS201067	Vacuolar protein sorting 33B
DPOGS208233	Phenylalanyl-tRNA synthetase, beta-subunit
DPOGS214668	arsA arsenite transporter, ATP-binding, homolog 1 (bacterial)
DPOGS205156	NAD synthetase
DPOGS203689	ariadne 2
DPOGS203457	Fasciclin 3
DPOGS206624	mitochondrial ribosomal protein S2
DPOGS204467	rhomboid-7
DPOGS209768	Signal recognition particle receptor beta
DPOGS200790	motile sperm domain containing 2
DPOGS205949	GTP-binding protein 10 (putative)
DPOGS208966	SPOUT domain containing methyltransferase 1
DPOGS206283	beta-4-galactosyltransferase 7
DPOGS213207	transmembrane protein 173
DPOGS208468	coiled-coil domain containing 97
DPOGS209268	thioredoxin domain containing 9
DPOGS215730	kurtz
DPOGS212615	SLC22A family member
DPOGS210816	nth (endonuclease III)-like 1 (<i>E.coli</i>)
DPOGS203217	Chaperonin containing TCP1 subunit 8
DPOGS207298	thioredoxin 2

DPOGS214837	dynein cytoplasmic 2 heavy chain 1
DPOGS200566	RIKEN cDNA 0610037L13 gene
DPOGS213209	URB1 ribosome biogenesis 1 homolog (<i>S. cerevisiae</i>)
DPOGS211595	Yip1 domain family, member 7
DPOGS200382	transducin (beta)-like 3
DPOGS214128	acid phosphatase 2, lysosomal
DPOGS201455	mitochondrial ribosomal protein L42
DPOGS206673	DnaJ heat shock protein family (Hsp40) member C17
DPOGS213506	Gdap1
DPOGS213027	Obg-like ATPase 1
DPOGS205508	CCAAT/enhancer binding protein zeta
DPOGS200102	epidermal growth factor receptor pathway substrate 8
DPOGS207615	pitchoune
DPOGS213587	Myb/SANT-like DNA-binding domain containing 3
DPOGS209577	Receptor component protein
DPOGS213219	RIO kinase 3
DPOGS215914	Esa1-associated factor 6
DPOGS210215	Protoporphyrinogen oxidase
DPOGS209063	taxilin alpha
DPOGS207888	smg-9 homolog, nonsense mediated mRNA decay factor (<i>C. elegans</i>)
DPOGS207088	heterogeneous nuclear ribonucleoprotein H3
DPOGS203418	Nucleoporin 107kD
DPOGS215076	syntaxin 18
DPOGS212345	small nuclear ribonucleoprotein E
DPOGS213025	Sp1
DPOGS204880	COP9 signalosome subunit 1b
DPOGS212049	short coiled-coil protein
DPOGS207464	Mediator complex subunit 7
DPOGS212376	telomerase RNA component interacting RNase
DPOGS205969	larsen
DPOGS216155	PSEA-binding protein 45kD
DPOGS211976	coiled-coil domain containing 22
DPOGS213675	Bekka
DPOGS205848	NAD(P)HX epimerase
DPOGS202592	DnaJ heat shock protein family (Hsp40) member B2
DPOGS204859	mitochondrial ribosomal protein S34
DPOGS200836	Ribosomal RNA processing 40

DPOGS214256	Zizimin
DPOGS216147	solute carrier family 2 (facilitated glucose transporter), member 9
DPOGS201420	acyl-Coenzyme A oxidase 1, palmitoyl
DPOGS213908	ATP-binding cassette, sub-family D (ALD), member 2
DPOGS215178	solute carrier organic anion transporter family, member 6c1
DPOGS202770	ubiquitin protein ligase E3 component n-recognin 7 (putative)
DPOGS213674	Chaperonin containing TCP1 subunit 4
DPOGS205058	Ciao1
DPOGS203048	FtsJ RNA methyltransferase homolog 3 (E. coli)
DPOGS205401	Chaperonin containing TCP1 subunit 3
DPOGS212825	transmembrane protein 179
DPOGS216039	dumpy
DPOGS213135	will die slowly
DPOGS212137	pescadillo ribosomal biogenesis factor 1
DPOGS203037	malectin
DPOGS213133	mitochondrial ribosomal protein L30
DPOGS208977	Dynactin 6, p27 subunit
DPOGS202415	Glutamyl-tRNA synthetase, mitochondrial
DPOGS207758	RNA binding motif protein 19
DPOGS212995	Isocitrate dehydrogenase
DPOGS205462	NA
DPOGS203662	Regulator of cullins 2
DPOGS207772	signal sequence receptor, gamma
DPOGS201718	THO complex 2
DPOGS212484	NA
DPOGS209419	zinc finger, MYND-type containing 8
DPOGS204681	Autophagy-related 8a
DPOGS209186	Cadherin 86C
DPOGS207380	leucine-rich repeats and guanylate kinase domain containing
DPOGS210994	Cellular Repressor of E1A-stimulated Genes
DPOGS215977	Dpr-interacting protein eta
DPOGS210673	Eclosion hormone
DPOGS207305	ATP binding cassette subfamily G member 4
DPOGS214673	Hepatocyte nuclear factor 4
DPOGS207246	Rho guanine nucleotide exchange factor at 64C
DPOGS202067	RAS-like, estrogen-regulated, growth-inhibitor
DPOGS212324	tRNA isopentenyltransferase 1
DPOGS210097	Limpet

DPOGS204377	beta-1,4-glucuronyltransferase 1
DPOGS215770	SH2 ankyrin repeat kinase
DPOGS212331	lethal (1) G0469
DPOGS211865	branched chain aminotransferase 1, cytosolic
DPOGS206255	Chromatin assembly factor 1, p180 subunit
DPOGS212259	JmjC domain-containing histone demethylase 2
DPOGS203004	CNMamide Receptor
DPOGS213560	nuclear fallout
DPOGS208273	erb-b2 receptor tyrosine kinase 3
DPOGS206025	Peroxidasin
DPOGS204181	Fermitin 1
DPOGS211900	anterior open
DPOGS206135	Multi drug resistance 65

Table S2. List of genes with similar or differential temporal expression patterns in the brain of summer-like monarchs and wild-caught migrants. R-R: Genes rhythmic in both conditions; R-AR: Genes rhythmic in summer-like monarchs and arrhythmic in migrants; AR-R: Genes arrhythmic in summer-like monarchs and rhythmic in migrants. The annotation is based on *Drosophila* (no shading) or mouse (gray shading) orthologues. NA, monarch genes without orthologues in *Drosophila* or the mouse.

Gene ID	R-R
DPOGS212327	F-box protein 32
DPOGS211121	ubiquitin-conjugating enzyme E2Q family-like 1
DPOGS204552	solute carrier family 2, (facilitated glucose transporter), member 8
DPOGS209874	solute carrier family 2, (facilitated glucose transporter), member 8
DPOGS207730	CG10082
DPOGS209797	Vacuolar H[+] ATPase 100kD subunit 2
DPOGS206136	LON peptidase N-terminal domain and ring finger 2
DPOGS208537	fritz
DPOGS204644	phospholipid phosphatase 1
DPOGS212996	Ecdysone-inducible gene E1
DPOGS214070	stumps
DPOGS205823	polypeptide N-acetylgalactosaminyltransferase 10
DPOGS215384	RIO kinase 2
DPOGS200190	similar
DPOGS205549	G protein-coupled receptor 158
DPOGS212022	klarsicht
DPOGS210186	Pyruvate dehydrogenase kinase
DPOGS204253	calsyntenin 2
DPOGS203791	Semaphorin 1b
DPOGS215738	eiger
DPOGS202608	maternal gene required for meiosis
DPOGS207058	choline dehydrogenase
DPOGS207000	Myosin heavy chain-like
DPOGS209025	LIM domain only 7
DPOGS213925	Heat shock protein 68
DPOGS202993	solute carrier family 36 (proton/amino acid symporter), member 1
DPOGS213900	Heat shock protein 68
DPOGS202607	maternal gene required for meiosis
DPOGS208881	DnaJ-like-2
DPOGS213901	Heat shock protein 68

DPOGS214402	Glycogen synthase
DPOGS213114	Choline transporter-like 2
DPOGS213326	CG7110
DPOGS204626	transmembrane protein 86B
DPOGS210128	PAPS synthetase
DPOGS213327	predicted gene 973
DPOGS210295	Phosphoglucose isomerase
DPOGS212829	carboxyesterase 2B
DPOGS207764	Enolase
DPOGS202781	Hsp70/Hsp90 organizing protein
DPOGS215969	Transaldolase
DPOGS203810	Phosphofructokinase
DPOGS205152	Ecdysone-inducible gene L2
DPOGS206692	pudgy
DPOGS202237	DnaJ heat shock protein family (Hsp40) member B4
DPOGS208606	vriille
DPOGS200811	hairy
DPOGS215159	Trehalose transporter 1-1
DPOGS209166	Bre1
DPOGS201013	high mobility group box transcription factor 1
DPOGS215160	Trehalose transporter 1-2
DPOGS201012	Leucine-rich pentatricopeptide repeat containing 2
DPOGS203088	solute carrier family 5 (sodium/glucose cotransporter), member 12
DPOGS212605	ATP binding cassette subfamily G member 4
DPOGS209175	phosphatidylinositol-specific phospholipase C, X domain containing 3
DPOGS214215	Na ⁺ /H ⁺ hydrogen antiporter 1
DPOGS212590	neither inactivation nor afterpotential B
DPOGS215394	NAD-dependent methylenetetrahydrofolate dehydrogenase
DPOGS214162	Na ⁺ /H ⁺ hydrogen antiporter 1
DPOGS214179	timeless
DPOGS205105	ATP binding cassette subfamily G member 4
DPOGS205647	ATP binding cassette subfamily G member 4
DPOGS215419	dumpy
DPOGS209925	clockwork orange
DPOGS212884	sushi, nidogen and EGF-like domains 1
DPOGS203908	period
DPOGS212885	Lysine (K)-specific demethylase 2

DPOGS207088	heterogeneous nuclear ribonucleoprotein H3
DPOGS201668	short gastrulation
DPOGS213282	zelda
DPOGS208079	cryptochrome 2
DPOGS203797	Heat shock factor
DPOGS206718	ArfGAP with SH3 domain, ankyrin repeat and PH domain1
Gene ID	R-AR
DPOGS206156	hyaluronoglucosaminidase 2
DPOGS201294	crinkled
DPOGS208747	cacophony
DPOGS203552	acyl-CoA synthetase family member 3
DPOGS211223	transcriptional Adaptor 1-1
DPOGS212916	ferric-chelate reductase 1
DPOGS210716	ariadne 2
DPOGS212858	atlastin
DPOGS215318	short spindle 7
DPOGS212482	gemini
DPOGS211034	Optic atrophy 1
DPOGS211138	Ral guanine nucleotide dissociation stimulator-like
DPOGS209507	S-adenosylmethionine decarboxylase
DPOGS210627	carbonic anhydrase 3
DPOGS207651	UDP glucuronosyltransferase 2 family, polypeptide B1
DPOGS212685	sarcoplasmic calcium-binding protein
DPOGS208406	CG30069
DPOGS201345	tocopherol (alpha) transfer protein-like
DPOGS201488	Mid1 interacting protein 1 (gastrulation specific G12-like (zebrafish))
DPOGS215783	hook
DPOGS216126	Gcn5 acetyltransferase
DPOGS207942	synaptotagmin XI
DPOGS215488	fatty acid desaturase 1
DPOGS203813	sphingomyelin phosphodiesterase 1, acid lysosomal
DPOGS202434	ebony
DPOGS211900	anterior open
DPOGS201894	Maltase A4
DPOGS209585	FCH domain only 2
DPOGS208044	sodium potassium chloride cotransporter
DPOGS204181	Fermitin 1

DPOGS210257	ebony
DPOGS201241	CAP
DPOGS205824	pickled eggs
DPOGS208999	retinol dehydrogenase 13 (all-trans and 9-cis)
DPOGS201544	Organic anion transporting polypeptide 74D
DPOGS203103	epithelial membrane protein
DPOGS201445	integrator complex subunit 13
DPOGS202439	NA
DPOGS201195	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2
DPOGS213560	nuclear fallout
DPOGS201376	Neuralized E3 ubiquitin protein ligase 4
DPOGS215489	6-phosphofructo-2-kinase
DPOGS211148	unzipped
DPOGS213546	dual specificity phosphatase 22
DPOGS215479	monooxygenase, DBH-like 1
DPOGS213594	Glycogen binding subunit 76A
DPOGS215439	lethal (2) essential for life
DPOGS201194	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2
DPOGS212594	Trehalose-6-phosphate synthase 1
DPOGS201446	CG10527
DPOGS214041	beta Spectrin
DPOGS200407	midline uncoordinated
DPOGS202602	Minichromosome maintenance 2
DPOGS200764	wunen
DPOGS205265	sulfotransferase family 2A, dehydroepiandrosterone (DHEA)-preferring, member 6
DPOGS215494	1,4-Alpha-Glucan Branching Enzyme
DPOGS201573	cabut
DPOGS202178	Glutamate oxaloacetate transaminase 1
DPOGS214921	Multicopper oxidase-1
DPOGS212113	straw
DPOGS209035	transketolase-like 2
DPOGS205929	sprite
DPOGS209948	regucalcin
DPOGS215720	Arginine kinase
DPOGS214139	Fatty acid (long chain) transport protein
DPOGS206348	Picot

DPOGS213878	synaptic vesicle glycoprotein 2 b
DPOGS205027	Glycogen phosphorylase
DPOGS212608	androgen dependent TFPI regulating protein
DPOGS202126	very low density lipoprotein receptor
DPOGS211169	mitochondrial ribosomal protein L3
DPOGS212844	Copper transporter 1A
DPOGS200490	Malate dehydrogenase 1
DPOGS200089	Triose phosphate isomerase
DPOGS202687	ATP-binding cassette, sub-family C (CFTR/MRP), member 4
DPOGS208998	ethanolamine phosphate phospholyase
DPOGS213690	beat-IIIc
DPOGS205927	split ends
DPOGS206646	Salt-inducible kinase 2
DPOGS204296	Poly(ADP-ribose) glycohydrolase
DPOGS206596	Megalin
DPOGS205557	Centromeric protein-C
DPOGS207241	5-hydroxytryptamine (serotonin) receptor 1A
DPOGS211921	Myb-interacting protein 130
DPOGS211191	Zinc/iron regulated transporter-related protein 71B
DPOGS213064	Phosphoglycerate kinase
DPOGS207671	branchless
DPOGS207964	CG13024
DPOGS212595	Trehalose-6-phosphate synthase 1
DPOGS202111	Tob
DPOGS215458	glutathione S-transferase, C-terminal domain containing
DPOGS215868	family with sequence similarity 107, member B
DPOGS200356	HEAT repeat containing 2
DPOGS208542	meiotic 9
DPOGS215460	Glyceraldehyde 3 phosphate dehydrogenase 2
DPOGS201746	Carbonic anhydrase 1
DPOGS208579	tektin 4
DPOGS214349	wunen
DPOGS207621	Leucine-rich-repeats and calponin homology domain protein
DPOGS210992	Mig-2-like
DPOGS208578	Vesicular glutamate transporter
DPOGS215853	NMDA receptor 1
DPOGS209910	Sox box protein 14

DPOGS215947	methyltransferase like 4
DPOGS203786	male fertility factor kl3
DPOGS215377	chondroitin sulfate synthase 1
DPOGS213804	Inositol 1,4,5-triphosphate kinase 1
DPOGS204494	metabotropic GABA-B receptor subtype 1
DPOGS205706	CG42588
DPOGS200691	Enhancer of split mbeta, helix-loop-helix
DPOGS203238	non-stop
DPOGS212889	histone PARylation factor 1
DPOGS203920	solute carrier family 36 (proton/amino acid symporter), member 4
DPOGS207222	roadblock
DPOGS205079	serine hydrolase-like
DPOGS214099	mannose-P-dolichol utilization defect 1
DPOGS215591	THAP domain containing, apoptosis associated protein 2
DPOGS204071	lactase
DPOGS200817	alkaline phosphatase, liver/bone/kidney
DPOGS204091	Salt-inducible kinase 3
DPOGS204153	Neurochondrin
DPOGS214218	glycerophosphocholine phosphodiesterase 1
DPOGS211367	CoRest
DPOGS202815	Excitatory amino acid transporter 2
DPOGS212557	hippocalcin-like 1
DPOGS202145	Dpr-interacting protein gamma
DPOGS202879	Nucleoporin at 44A
DPOGS203017	Ataxin-2
DPOGS213275	chondroadherin
DPOGS215237	Mid1
DPOGS207224	pudgy
DPOGS202306	kon-tiki
DPOGS205911	Vacuolar protein sorting 20
DPOGS204363	kekkon-2
DPOGS212492	CG31324
DPOGS214212	c12.1
DPOGS204463	Matrix metalloproteinase 1
DPOGS212989	POC1 centriolar protein A
DPOGS203710	major facilitator superfamily domain containing 12
DPOGS206978	nessun dorma
DPOGS214045	transmembrane and tetratricopeptide repeat containing 2

DPOGS202609	Acetylcholine esterase
DPOGS205855	solute carrier family 46, member 3
DPOGS207157	Formin homology 2 domain containing
DPOGS200409	Integrin betanu subunit
DPOGS214408	solute carrier family 22 (organic cation transporter), member 21
DPOGS214262	zinc finger protein 27
DPOGS205964	Ubiquitin specific protease 20/33
DPOGS203155	Cullin 5
DPOGS213576	Hexosaminidase 2
DPOGS211174	Cwc25
DPOGS200295	dachshund family transcription factor 2
DPOGS205353	Mystery 45A
DPOGS213527	Zinc/iron regulated transporter-related protein 48C
DPOGS203890	scavenger receptor acting in neural tissue and majority of rhodopsin is absent
DPOGS203868	solute carrier family 36 (proton/amino acid symporter), member 4
DPOGS202827	scavenger receptor acting in neural tissue and majority of rhodopsin is absent
DPOGS200883	CG16791
DPOGS203228	Cln7
DPOGS201881	nervana 1
DPOGS201625	3-hydroxybutyrate dehydrogenase, type 2
DPOGS213552	Ecdysone-induced protein 28/29kD
DPOGS209521	Fumarylacetoacetase
DPOGS215624	Glutaminase
DPOGS200617	Runt related B
DPOGS200157	Rab5
DPOGS215437	Pyrroline-5-carboxylate reductase-like 2
DPOGS214155	DEAH (Asp-Glu-Ala-His) box polypeptide 33
DPOGS207405	Protein 1 of cleavage and polyadenylation factor 1
DPOGS205442	king tubby
DPOGS209063	taxilin alpha
DPOGS205077	neurotrimin
DPOGS207714	astray
DPOGS208868	Membrin
DPOGS200168	Tetraspanin 29Fa
DPOGS206850	pantothenate kinase 3
DPOGS209776	Ugt86Di

DPOGS209371	choline dehydrogenase
DPOGS206851	CG17162
DPOGS205076	kirre like nephrin family adhesion molecule 1
DPOGS212748	scheggia
DPOGS200091	rotatin
DPOGS204998	Ccp84Ae
DPOGS207585	tropomyosin 1, alpha
DPOGS202068	Ras-related protein interacting with calmodulin
DPOGS203923	lethal (1) G0289
DPOGS208959	Vacuolar H[+] ATPase 100kD subunit 2
DPOGS202283	pre-mRNA processing factor 39
DPOGS206412	endoplasmic reticulum metallopeptidase 1
DPOGS201651	Negative Cofactor 2alpha
DPOGS209186	Cadherin 86C
DPOGS201463	defective proventriculus
DPOGS209864	jim
DPOGS207448	Bestrophin 1
DPOGS214570	Melanization Protease 1
DPOGS213793	vermilion
DPOGS208231	kallikrein related-peptidase 13
DPOGS208188	Semaphorin 5c
DPOGS201845	wing blister
DPOGS207077	sperm antigen with calponin homology and coiled-coil domains 1-like
DPOGS205727	Glycoprotein hormone alpha 2
DPOGS209262	peroxidasin
DPOGS212060	Fanconi anemia, complementation group M
DPOGS214879	prune
DPOGS200824	zinc finger CCCH type containing 14
DPOGS200985	Leucine-rich repeat-containing G protein-coupled receptor 1
DPOGS203808	arc
DPOGS213355	lipase, member I
DPOGS213007	Cytochrome P450-18a1
Gene ID	AR-R
DPOGS210620	miranda
DPOGS210559	NA
DPOGS214770	testis expressed gene 2
DPOGS205355	Ucp4A

DPOGS206716	mind-meld
DPOGS215438	cell division cycle 14
DPOGS200105	Purine-rich binding protein-alpha
DPOGS213926	lethal (2) essential for life
DPOGS215242	chico
DPOGS207729	centromere protein I
DPOGS205032	predicted gene 5141
DPOGS212156	lola like
DPOGS207552	Mitogen-activated protein kinase phosphatase 3
DPOGS206443	gamma-glutamyltransferase 1
DPOGS212331	lethal (1) G0469
DPOGS203967	Glucose transporter 1
DPOGS213347	rudimentary-like
DPOGS204413	F-box and leucine-rich repeat protein 6
DPOGS214030	mulet
DPOGS210848	starvin
DPOGS205386	phosphoglucose mutase
DPOGS206473	solute carrier family 2, (facilitated glucose transporter), member 8
DPOGS210324	weary
DPOGS209591	outspread
DPOGS202650	jing
DPOGS211108	BRWD3
DPOGS207955	Tissue inhibitor of metalloproteases
DPOGS211622	purine-nucleoside phosphorylase 2
DPOGS215723	Zinc finger CCHC-type containing 7
DPOGS208796	transmembrane protease, serine 2
DPOGS205218	eukaryotic translation release factor 1
DPOGS200508	zinc finger protein 85
DPOGS209575	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2
DPOGS216018	RIKEN cDNA 5031439G07 gene
DPOGS202658	laminin, alpha 3
DPOGS213604	family with sequence similarity 205, member A2
DPOGS213632	pallbearer
DPOGS203702	naked cuticle
DPOGS205398	rha
DPOGS207508	forked
DPOGS215797	nicotinate phosphoribosyltransferase
DPOGS215758	Transportin

DPOGS205469	G patch domain containing 2 like
DPOGS206880	aldehyde dehydrogenase 1 family, member L1
DPOGS205541	CD68 antigen
DPOGS211133	phosphorylase kinase beta
DPOGS213985	XRCC1
DPOGS204403	solute carrier family 7 (cationic amino acid transporter, y+ system), member 9
DPOGS209508	phosphoglucose mutase
DPOGS206382	lethal (2) essential for life
DPOGS211598	Peroxidase
DPOGS205122	pollux
DPOGS212285	PNN interacting serine/arginine-rich
DPOGS207495	real-time
DPOGS205231	protease, serine 30
DPOGS201368	ER degradation enhancer, mannosidase alpha-like 2
DPOGS205510	CG42304
DPOGS211989	family with sequence similarity 98, member A
DPOGS203197	pasiflora 2
DPOGS212041	heartless
DPOGS210450	glycine-N-acyltransferase-like 3
DPOGS213101	Collagen type IV alpha 1
DPOGS209745	Reversion-inducing-cysteine-rich protein with kazal motifs
DPOGS202664	membrane steroid binding protein
DPOGS215931	Aldehyde dehydrogenase type III
DPOGS207783	Dual oxidase
DPOGS213914	solute carrier family 2, (facilitated glucose transporter), member 8
DPOGS200925	CG13643
DPOGS210130	folliculin interacting protein 2
DPOGS208609	Adh transcription factor 1
DPOGS214409	solute carrier family 22 (organic cation transporter), member 1
DPOGS215544	zinc finger, FYVE domain containing 28
DPOGS206919	lethal (1) G0196
DPOGS201015	Sterol carrier protein X-related thiolase
DPOGS206369	Mediator complex subunit 27
DPOGS214271	Sodium/solute co-transporter-like 5A11
DPOGS207312	Src oncogene at 64B
DPOGS208891	Relish
DPOGS213513	lethal (3) 72Dn

DPOGS204840	ecto-NOX disulfide-thiol exchanger 1
DPOGS212145	Adenosine receptor
DPOGS210680	Kallmann syndrome 1
DPOGS214160	Na ⁺ /H ⁺ hydrogen antiporter 1
DPOGS211339	N(alpha)-acetyltransferase 20 A
DPOGS206184	mitochondrial ribosomal protein L35
DPOGS203649	Integrator 6
DPOGS210429	Rab23
DPOGS202082	lectin, mannose-binding 2-like
DPOGS200944	Required for cell differentiation 1
DPOGS207391	clavesin 2
DPOGS207599	mitochondrial ribosomal protein L55
DPOGS205264	aldehyde dehydrogenase 18 family, member A1
DPOGS209146	solute carrier family 22 (organic cation transporter), member 3
DPOGS207504	Farnesyl pyrophosphate synthase
DPOGS208519	Usf
DPOGS203115	Tyrosyl-tRNA synthetase
DPOGS205880	RIKEN cDNA 1110038F14 gene
DPOGS207273	Histone deacetylase 1
DPOGS207041	BUD23, rRNA methyltransferase and ribosome maturation factor
DPOGS215524	Glutathione Synthetase
DPOGS202243	ribosomal RNA processing 15 homolog (<i>S. cerevisiae</i>)
DPOGS213269	amidohydrolase domain containing 2
DPOGS209232	Replication factor C 38kD subunit
DPOGS201279	clavesin 1
DPOGS205906	gem nuclear organelle associated protein 7
DPOGS206662	N(alpha)-acetyltransferase 38, NatC auxiliary subunit
DPOGS203217	Chaperonin containing TCP1 subunit 8
DPOGS200290	penguin
DPOGS210994	Cellular Repressor of E1A-stimulated Genes
DPOGS209768	Signal recognition particle receptor beta
DPOGS206466	PTC7 protein phosphatase homolog
DPOGS214393	Glia maturation factor
DPOGS204523	eukaryotic translation initiation factor 2 subunit alpha
DPOGS202974	MAP kinase activated protein-kinase-2
DPOGS213401	Origin recognition complex subunit 5
DPOGS215555	Minichromosome maintenance 6

DPOGS201992	x16 splicing factor
DPOGS205993	Malignant T cell amplified sequence 1
DPOGS202915	Signal peptide peptidase
DPOGS206597	Fasciclin 2
DPOGS203773	M-phase phosphoprotein 10 (U3 small nucleolar ribonucleoprotein)
DPOGS212221	NOP2-Sun domain family, member 2
DPOGS201325	mitochondrial translation elongation factor Ts
DPOGS207501	Actin-related protein 3
DPOGS206108	Dodeca-satellite-binding protein 1
DPOGS207351	PIN2/TERF1 interacting, telomerase inhibitor 1
DPOGS207279	NA
DPOGS216037	Rho GTPase activating protein at 68F
DPOGS214375	Dihydropteridine reductase
DPOGS200286	Regulatory particle non-ATPase 10
DPOGS205508	CCAAT/enhancer binding protein zeta
DPOGS211776	rush hour
DPOGS214580	NOP10 ribonucleoprotein
DPOGS204316	Isocitrate dehydrogenase
DPOGS208154	uridine phosphorylase 1
DPOGS203757	Asparagine synthetase
DPOGS211134	Proteasome beta2 subunit
DPOGS200182	Dual oxidase
DPOGS212387	sans fille
DPOGS207188	Glutamate receptor IB
DPOGS215227	Cyclin-dependent kinase subunit 85A
DPOGS213746	Proteasome alpha6 subunit
DPOGS200285	Mesencephalic astrocyte-derived neurotrophic factor
DPOGS214164	Sec61 beta subunit
DPOGS209926	hattifattener
DPOGS211106	chickadee
DPOGS211051	FER tyrosine kinase
DPOGS202316	optic atrophy 3
DPOGS204777	Sec61 gamma subunit
DPOGS212707	potassium channel, subfamily K, member 18
DPOGS209613	tiptop
DPOGS213650	coiled-coil serine rich 2
DPOGS207200	His1:CG33861

DPOGS208768	Jrk-like
DPOGS212261	Kaz1-ORFB
DPOGS204192	Glutamyl-prolyl-tRNA synthetase
DPOGS203404	highwire
DPOGS202523	Dynein heavy chain 64C
DPOGS209960	Vacuolar protein sorting 13
DPOGS209394	protein arginine N-methyltransferase 2
DPOGS213134	HECT and RLD domain containing protein 2
DPOGS212667	CG8740
DPOGS201500	PAS kinase
DPOGS207418	rugose
DPOGS205736	endosomal maturation defective
DPOGS210906	myopic
DPOGS203769	Ca ²⁺ -channel protein alpha[[1]] subunit D
DPOGS204111	sodium channel, voltage-gated, type X, alpha
DPOGS206600	radish
DPOGS204827	unc-13
DPOGS211160	Piezo
DPOGS205809	blue cheese
DPOGS205030	Rap GTPase activating protein 1
DPOGS204823	lymphocyte specific 1
DPOGS208356	DEAH (Asp-Glu-Ala-His) box polypeptide 35
DPOGS210629	APC-like
DPOGS212969	ring finger protein 144A
DPOGS207346	NA
DPOGS209535	meiosis specific with coiled-coil domain
DPOGS203506	CG15894
DPOGS216202	castor
DPOGS206932	RIM-binding protein
DPOGS212524	NA
DPOGS205581	Cullin 1
DPOGS206816	CG4658
DPOGS207452	Rab3 GDP-GTP exchange factor
DPOGS210020	inositol polyphosphate-5-phosphatase F
DPOGS215603	rapamycin-insensitive companion of Tor
DPOGS211143	WD repeat domain 59
DPOGS206164	Brahma associated protein 170kD
DPOGS212332	Adenylyl cyclase 76E

DPOGS206043	ftz transcription factor 1
DPOGS209239	zinc finger and BTB domain containing 40
DPOGS204972	ring finger protein 165
DPOGS201030	Marcal1
DPOGS207938	still life
DPOGS203622	meiosis regulator and mRNA stability 1
DPOGS209769	septin interacting protein 1
DPOGS206403	nicotinic Acetylcholine Receptor alpha6
DPOGS205084	RNA-binding Fox protein 1
DPOGS203803	c-Maf inducing protein
DPOGS200827	Formin-like
DPOGS202986	Gigyf
DPOGS203978	predicted gene 11639
DPOGS209780	family with sequence similarity 160, member B1
DPOGS204256	tramtrack
DPOGS204257	tramtrack
DPOGS214346	wunen
DPOGS215331	Ecdysone-induced protein 74EF
DPOGS201750	grappa
DPOGS202907	centrosomal protein 162
DPOGS203253	Hepatocyte growth factor regulated tyrosine kinase substrate
DPOGS201547	X11Lbeta
DPOGS213525	Shaker cognate b
DPOGS202684	Wnk kinase
DPOGS212980	armadillo repeat containing 8
DPOGS211471	collagen, type XXIII, alpha 1
DPOGS201244	CAP
DPOGS202471	pseudouridylate synthase 7
DPOGS211197	SET domain containing 1A
DPOGS203274	Rho GTPase activating protein at 100F
DPOGS214481	Wnk kinase
DPOGS204391	female sterile (1) homeotic
DPOGS207677	R3H domain and coiled-coil containing 1 like
DPOGS211802	skywalker
DPOGS206516	proteoglycan 4 (megakaryocyte stimulating factor, articular superficial zone protein)
DPOGS201245	CAP
DPOGS208933	Nna1 carboxypeptidase

DPOGS211301	Synaptosomal-associated protein 25kDa
DPOGS205950	lethal (3) malignant brain tumor
DPOGS212207	pinstripe
DPOGS202457	PDGF- and VEGF-receptor related

Table S3. List of genes with similar temporal expression patterns in the brains of monarchs raised in LP and in summer-like monarchs and/or in the brains of monarchs raised in SP and in wild-caught migrants. R-R: Genes rhythmic in all four conditions; R-AR: Genes rhythmic in LP and summer-like monarchs, and arrhythmic in SP monarchs and migrants ; AR-R: Genes arrhythmic in LP and summer-like monarchs, and rhythmic in SP monarchs and migrants. The annotation is based on *Drosophila* (no shading) or mouse (colored shading) orthologues. NA, monarch genes without orthologues in *Drosophila* or the mouse.

Gene ID	R-R
DPOGS200190	similar
DPOGS201012	Leucine-rich pentatricopeptide repeat containing 2
DPOGS201013	high mobility group box transcription factor 1
DPOGS202237	DnaJ heat shock protein family (Hsp40) member B4
DPOGS203797	Heat shock factor
DPOGS203908	period
DPOGS204253	calsyntenin 2
DPOGS204552	solute carrier family 2, (facilitated glucose transporter), member 8
DPOGS204644	phospholipid phosphatase 1
DPOGS205105	ATP binding cassette subfamily G member 4
DPOGS205549	G protein-coupled receptor 158
DPOGS207058	choline dehydrogenase
DPOGS207730	CG10082
DPOGS208606	vriille
DPOGS209175	phosphatidylinositol-specific phospholipase C, X domain containing 3
DPOGS209797	Vacuolar H ⁺ ATPase 100kD subunit 2
DPOGS209874	solute carrier family 2, (facilitated glucose transporter), member 8
DPOGS209925	clockwork orange
DPOGS210128	PAPS synthetase
DPOGS211121	ubiquitin-conjugating enzyme E2Q family-like 1
DPOGS212590	neither inactivation nor afterpotential B
DPOGS213114	Choline transporter-like 2
DPOGS213900	Heat shock protein 68
DPOGS213901	Heat shock protein 68
DPOGS213925	Heat shock protein 68
DPOGS214070	stumps
DPOGS214162	Na ⁺ /H ⁺ hydrogen antiporter 1
DPOGS214179	timeless

DPOGS214215	Na ⁺ /H ⁺ hydrogen antiporter 1
DPOGS215159	Trehalose transporter 1-1
DPOGS215160	Trehalose transporter 1-2
DPOGS215419	dumpy
DPOGS215738	eiger
Gene ID	R-AR
DPOGS201241	CAP
DPOGS201544	Organic anion transporting polypeptide 74D
DPOGS201894	Maltase A4
DPOGS202126	very low density lipoprotein receptor
DPOGS202609	Acetylcholine esterase
DPOGS202827	scavenger receptor acting in neural tissue and majority of rhodopsin is absent
DPOGS203228	Cln7
DPOGS203808	arc
DPOGS203890	scavenger receptor acting in neural tissue and majority of rhodopsin is absent
DPOGS205077	neurotrimin
DPOGS205727	Glycoprotein hormone alpha 2
DPOGS207157	Formin homology 2 domain containing
DPOGS207651	UDP glucuronosyltransferase 2 family, polypeptide B1
DPOGS207671	branchless
DPOGS208868	Membrin
DPOGS208999	retinol dehydrogenase 13 (all-trans and 9-cis)
DPOGS210627	carbonic anhydrase 3
DPOGS212492	CG31324
DPOGS213594	Glycogen binding subunit 76A
DPOGS214045	transmembrane and tetratricopeptide repeat containing 2
DPOGS214921	Multicopper oxidase-1
DPOGS215237	Mid1
Gene ID	AR-R
DPOGS200182	Dual oxidase
DPOGS202243	ribosomal RNA processing 15 homolog (<i>S. cerevisiae</i>)
DPOGS203217	Chaperonin containing TCP1 subunit 8
DPOGS204316	Isocitrate dehydrogenase
DPOGS205508	CCAAT/enhancer binding protein zeta
DPOGS209768	Signal recognition particle receptor beta
DPOGS210429	Rab23
DPOGS210994	Cellular Repressor of E1A-stimulated Genes

DPOGS211622	purine-nucleoside phosphorylase 2
DPOGS212331	lethal (1) G0469
DPOGS213101	Collagen type IV alpha 1
DPOGS213604	family with sequence similarity 205, member A2

Table S4. RNA-seq mapping summary for LP, SP, summer-like (summer), and migrant monarchs. R: replicate.

Samples	Number of reads	Mapped reads	Mapping percentage
LP ZT01 R1	13,522,929	12,569,145	92.9
LP ZT01 R2	13,678,471	12,641,190	92.4
LP ZT04 R1	15,821,730	14,679,157	92.8
LP ZT04 R2	16,370,285	15,082,230	92.1
LP ZT07 R1	18,287,614	16,960,023	92.7
LP ZT07 R2	18,547,268	17,043,095	91.9
LP ZT10 R1	13,373,316	12,350,949	92.4
LP ZT10 R2	16,462,573	15,232,242	92.5
LP ZT13 R1	15,378,742	14,253,836	92.7
LP ZT13 R2	18,124,188	16,640,088	91.8
LP ZT16 R1	16,141,542	14,943,063	92.6
LP ZT16 R2	17,448,980	15,981,062	91.6
LP ZT19 R1	14,581,672	13,501,921	92.6
LP ZT19 R2	15,306,522	14,013,930	91.6
LP ZT22 R1	15,207,486	14,104,449	92.7
LP ZT22 R2	16,168,931	14,936,831	92.4
SP ZT01 R1	10,939,912	10,068,478	92
SP ZT01 R2	11,026,339	10,288,670	93.3
SP ZT04 R1	11,957,374	11,060,333	92.5
SP ZT04 R2	11,984,004	11,214,567	93.6
SP ZT07 R1	14,661,160	13,598,461	92.8
SP ZT07 R2	11,503,579	10,754,899	93.5
SP ZT10 R1	11,591,287	10,850,911	93.6
SP ZT10 R2	11,747,485	10,928,317	93
SP ZT13 R1	13,794,223	12,895,039	93.5
SP ZT13 R2	11,163,015	10,402,241	93.2
SP ZT16 R1	12,703,894	11,832,060	93.1
SP ZT16 R2	12,174,241	11,372,549	93.4
SP ZT19 R1	11,931,961	11,165,608	93.6
SP ZT19 R2	11,276,364	10,498,869	93.1
SP ZT22 R1	9,356,277	8,741,841	93.4
SP ZT22 R2	9,315,476	8,698,696	93.4
Summer ZT01 R1	8,329,736	7,474,898	89.7
Summer ZT01 R2	11,307,582	9,768,700	86.4

Summer ZT04 R1	8,308,713	7,353,942	88.5
Summer ZT04 R2	9,752,315	8,477,008	86.9
Summer ZT07 R1	7,489,482	6,551,179	87.5
Summer ZT07 R2	9,802,883	8,436,946	86.1
Summer ZT10 R1	8,292,323	7,183,739	86.6
Summer ZT10 R2	10,067,093	8,634,538	85.8
Summer ZT13 R1	7,414,155	6,488,395	87.5
Summer ZT13 R2	9,848,313	8,840,377	89.8
Summer ZT16 R1	8,373,408	7,151,758	85.4
Summer ZT16 R2	10,074,990	9,041,720	89.7
Summer ZT19 R1	8,023,590	6,893,871	85.9
Summer ZT19 R2	8,636,414	7,766,293	89.9
Summer ZT22 R1	8,279,696	7,136,863	86.2
Summer ZT22 R2	10,286,399	9,071,636	88.2
Migrant ZT01 R1	16,093,489	15,043,013	93.5
Migrant ZT01 R2	15,823,203	14,866,974	94
Migrant ZT04 R1	14,592,336	13,293,343	91.1
Migrant ZT04 R2	17,808,260	16,668,099	93.6
Migrant ZT07 R1	17,429,487	16,324,273	93.7
Migrant ZT07 R2	17,125,519	16,034,231	93.6
Migrant ZT10 R1	15,979,866	14,920,559	93.4
Migrant ZT10 R2	14,289,759	12,589,285	88.1
Migrant ZT13 R1	17,412,821	16,261,315	93.4
Migrant ZT13 R2	18,173,157	17,048,724	93.8
Migrant ZT16 R1	19,273,821	18,000,493	93.4
Migrant ZT16 R2	15,910,647	14,849,245	93.3
Migrant ZT19 R1	18,186,048	16,980,976	93.4
Migrant ZT19 R2	17,892,892	16,129,214	90.1
Migrant ZT22 R1	15,167,938	14,404,139	95
Migrant ZT22 R2	15,017,821	13,750,935	91.6
Average	13,312,703	12,230,335	91.5

Table S5. Expressed and cycling genes in the brains of LP, SP, summer-like and migrant monarchs.

Samples	Genes expressed	Cycling genes	% of cycling genes
LP	10,333	272	2.7
SP	10,113	268	2.66
Summer-like	10,079	282	2.8
Migrant	10,353	306	3.04

Dataset S1. RPKM values of genes with similar or differential temporal expression patterns in the brain of monarchs raised in LP and SP. Legends as in Table S1.

Dataset S2. RPKM values of genes with similar or differential temporal expression patterns in the brain of summer-like monarchs and wild-caught migrants. Legends as in Table S2.

References

1. Zhu H, Gegear RJ, Casselman A, Kanginakudru S, & Reppert SM (2009) Defining behavioral and molecular differences between summer and migratory monarch butterflies. *BMC Biol* 7:14.
2. Merlin C, Gegear RJ, & Reppert SM (2009) Antennal circadian clocks coordinate sun compass orientation in migratory monarch butterflies. *Science* 325(5948):1700-1704.
3. Lugena AB, Zhang Y, Menet JS, & Merlin C (2019) Genome-wide discovery of the daily transcriptome, DNA regulatory elements and transcription factor occupancy in the monarch butterfly brain. *PLoS Genetics* 15(7):e1008265.
4. Zhan S & Reppert SM (2013) MonarchBase: the monarch butterfly genome database. *Nucleic Acids Res* 41(Database issue):D758-763.
5. Kim D, *et al.* (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol* 14(4).
6. Trapnell C, *et al.* (2012) Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc* 7(3):562-578.
7. Trapnell C, *et al.* (2010) Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat Biotechnol* 28(5):511-U174.
8. Thaben PF & Westermark PO (2014) Detecting Rhythms in Time Series with RAIN. *Journal of Biological Rhythms* 29(6):391-400.
9. Wu G, Anafi RC, Hughes ME, Kornacker K, & Hogenesch JB (2016) MetaCycle: an integrated R package to evaluate periodicity in large scale data. *Bioinformatics* 32(21):3351-3353.

10. Altschul SF, Gish W, Miller W, Myers EW, & Lipman DJ (1990) Basic Local Alignment Search Tool. *Journal of Molecular Biology* 215(3):403-410.
11. Labun K, Montague TG, Gagnon JA, Thyme SB, & Valen E (2016) CHOPCHOP v2: a web tool for the next generation of CRISPR genome engineering. *Nucleic Acids Research* 44(W1):W272-W276.
12. Montague TG, Cruz JM, Gagnon JA, Church GM, & Valen E (2014) CHOPCHOP: a CRISPR/Cas9 and TALEN web tool for genome editing. *Nucleic Acids Research* 42(W1):W401-W407.
13. Hwang WY, *et al.* (2013) Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat Biotechnol* 31(3):227-229.
14. Jao LE, Wente SR, & Chen WB (2013) Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system. *P Natl Acad Sci USA* 110(34):13904-13909.
15. Zhang Y, Markert MJ, Groves SC, Hardin PE, & Merlin C (2017) Vertebrate-like CRYPTOCHROME 2 from monarch regulates circadian transcription via independent repression of CLOCK and BMAL1 activity. *Proc Natl Acad Sci U S A* 114(36):E7516-E7525.
16. Markert MJ, *et al.* (2016) Genomic Access to Monarch Migration Using TALEN and CRISPR/Cas9-Mediated Targeted Mutagenesis. *G3-Genes Genom Genet* 6(4):905-915.
17. Rohland N & Reich D (2012) Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Res* 22(5):939-946.
18. von Lintig J, Dreher A, Kiefer C, Wernet MF, & Vogt K (2001) Analysis of the blind *Drosophila* mutant *ninaB* identifies the gene encoding the key enzyme for vitamin A formation *in vivo*. *Proc Natl Acad Sci U S A* 98(3):1130-1135.