nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Confocal images were obtained using the Zeiss microscopy ZEN 2.3 SP1 software. Drosophila Activity Monitor data were collected using the DAM System software, version 311X.
Data analysis	All image analysis was performed in Fiji running v2.9.0 of ImageJ. RNA spots were detected using the RS-FISH macro, and Scholl analysis was performed using the neuroanatomy plug-in. Sequences were analyzed and visualised using SnapGene (www.snapgene.com) running MUSCLE (v3.8.1551), and Jalview (v2.11.2), and using the R package Phangorn (v2.11.2). Sequence alignments were perfomed using Samtools (v1.19.2), and tests of neutrality were performed using the DNA sequence polymorphism software (v6). Drosophila Activity Monitor data were analysed using Rethomics in R (v3.6.3). All R code used for data analysis are available at: github.com/mshahandeh/circ_plasticity.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data generated for this study are available in the Dryad digital repository (doi:10.5061/dryad.vq83bk42z).
We additionally used D. melanogaster SNV data from PMID 26547394 (data available at Dryad doi:10.5061/dryad.7440s)
We used sequences of D. sechellia from PMID 29684059 (available from SRA:SRP113415). To align these sequences, we used the D. sechellia reference genome (ASM438219v2).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample size. For behavioural experiments, we aimed for a sample size of 25-30 individuals, as significant differences were easily detected at these sizes. In the case of our hybrid screen, where hybrid flies were harder to make, we aimed for 15 individuals per strain but due to the strong reproductive isolation between species, some genotypes were difficult to cross to D. sechellia, resulting in a lower sample size. For image analyses, we obtained images from 5 brains from each strain per treatment because this allowed for parallel processing of multiple genotypes and time points. Additionally, quantifications of these stainings were largely consistent and significant differences were apparent at this sample size.
Data exclusions	No data were excluded.
Replication	All behavioural experiments were collected over at least two technical replicates with corresponding controls to ensure reproducibility, with the exception of those shown in Fig. 1c, where we replicated only two photoperiod treatments: 12:12 h LD and 16:8 h LD, because we continued the use of these specific photoperiods throughout the work. All brain dissections and stainings for a given experiment were performed in parallel to ensure comparability between time points and fixed microscope settings were used within each experiment. Two time-points of each histological experiments (Pdf smFISH, Pdf immunofluorescence, and Scholl analysis of s-LNv axonal projections) were replicated once in order to ensure the overall patterns of expression were the same.
Randomization	We did not randomise within experiments, and instead ran all genotypes in parallel for all experiments, except for the hybrid screening, where we measured the behaviour of hybrids as and when crosses produced offspring, with at least one replicate run in parallel with corresponding controls (data collection for hybrid screening took >2 years).
Blinding	Quantifications for stainings and collection of behavioural data were performed blind to treatment.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	All antibodies used are described in Supplementary Table 2.
Validation	All antibodies were previously validated: - mouse anti-Pdf, obtained from DSHB with confirmed species reactivity in Drosophila for IHC-IF (https://dshb.biology.uiowa.edu/PDF-C7) - rat anti-Cadherin-N, obtained from DSHB with confirmed species reactivity in Drosophila for IHC-IF (https://dshb.biology.uiowa.edu/DN-Ex-8) - rabbit anti-GFP, Molecular Probes AB_221570; this is a fully-validated commercial antibody

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	All fly strains used are described in Supplementary Table 1. All fly strains were domesticated and have been reared in common laboratory conditions for 20+ years, with the exception of the MD and LZV strains, which were collected and domesticated in ~2014 (PMID 24920013). For all experiments, flies were 3-5 days old at the start of the experiment.
Wild animals	No wild animals were used in this study.
Reporting on sex	As is standard in the Drosophila circadian field, we only used males in most of the experiments of this study because they do not reproduce within the long-term behavioural assay. Males were identified by the presence of the male genital arch under a stereo-microscope. However, we verified that the key species-specific behaviours described in males were also observed in females (Extended Data Fig. 1c-d).
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	No ethical approval or guidance was required for this study, as we used exclusively invertebrate animals.

Note that full information on the approval of the study protocol must also be provided in the manuscript.