An open-source platform for analyzing and sharing worm-behavior data

To the Editor — Animal behavior is increasingly being recorded in systematic imaging studies that generate large datasets. To maximize the usefulness of these data, there is a need for improved resources for analyzing and sharing behavioral data that will encourage reanalysis and methodological developments¹. However, for behavioral data, unlike genomic or protein structural data, there are no widely used standards. It is therefore desirable to make data available in a relatively raw form to enable flexibility in data analysis. For computational ethology to approach the level of maturity of other areas of

a Databases

bioinformatics, at least three challenges must be addressed: storing and accessing video files; defining flexible data formats to facilitate data sharing; and developing software to read, write, browse, and analyze the data. We have generated an open resource to begin addressing these challenges for *Caenorhabditis elegans* behavioral data.

To store video files and the associated features and metadata, we use a Zenodo. org community (an open-access repository for data) that provides durable storage and citability, and that supports contributions from other groups. We have also developed a web interface that enables filtering of the video files on the basis of feature histograms that can return, for example, fast and curved worms in addition to more standard searches for particular strains or genotypes (Fig. 1 and http://movement.openworm. org/). The database currently consists of 14,874 single-worm tracking experiments representing 386 genotypes (building on 9,203 experiments and 305 genotypes in a previous publication²) and includes data from several larval stages as well as data from aging experiments consisting of more than 2,700 videos of animals tracked daily from the L4 stage to death (Nature Research

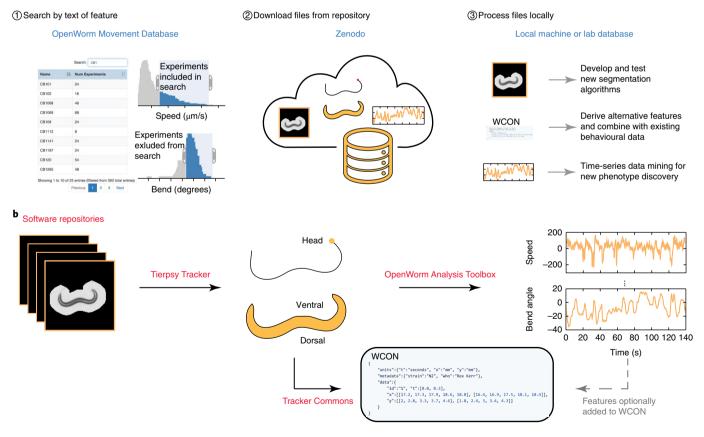


Fig. 1 Schematic of the searchable database and Tierpsy analysis pipeline. a, The OpenWorm Movement Database provides a web interface for searching by genotype, strain, and/or other discrete values, and interactive histograms with sliders to filter results on the basis of feature values. The interface points to data stored on Zenodo. The video and feature data can be further analyzed or combined with data collected by using other worm trackers through the Worm tracker Commons Object Notation (WCON), a human- and machine-readable JSON format. **b**, Tierpsy (short for tierpsychology, the German word for ethology) segments and tracks worms, extracting the outline and skeleton of each animal then determining the head-tail orientation. These data are saved in WCON. The OpenWorm Analysis Toolbox is then used to extract behavioral features.

Reporting Summary). Full-resolution videos are available in HDF5 containers that include gzip-compressed video frames, time stamps, worm outlines and midlines, feature data, and experimental metadata. HDF5 files are compatible with multiple languages including MATLAB, R, Python, and C. We have also developed an HDF5 video reader that allows video playback with adjustable speed and zoom (an important feature for reviewing high-resolution multiworm tracking data), as well as toggling of worm segmentation over the original video to verify segmentation accuracy during playback.

Second, we have defined an interchange format named Worm tracker Commons Object Notation (WCON), to facilitate data sharing and software reuse among groups working on worm behavior. WCON uses the widely supported JSON format to store tracking data as text that is readable by both humans and machines. It is compatible with single and multiworm³ tracking data at any resolution, from a single point representing worm position over time⁴ to many points representing the high-resolution skeleton of a moving worm². It also supports custom feature additions so that individual laboratories can store their own specific datasets alongside the existing set of basic worm data. WCON readers are available for Python, MATLAB, Scala, and C. Detailed documentation for the file formats and software is available on the project page (https://github.com/openworm/ tracker-commons/).

Finally, we have complemented the database and file formats with open-source software written in Python for single and multiworm tracking, feature extraction, review, and analysis (Supplementary Discussion; code and documentation in Supplementary Software or at https://doi.org/10.5281/zenodo.1323782, where compiled versions are also available).

The suite of tools reported here makes quantitative behavioral analysis and reanalysis accessible for both experimentalists and computational scientists. It may also serve as a template for similar efforts in other model-organism communities.

Reporting Summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Code availability

Tierpsy Tracker is available as Supplementary Software and at https:// doi.org/10.5281/zenodo.1323782. Updated versions will be made available at http:// ver228.github.io/tierpsy-tracker/.

Data availability

Videos, skeleton (WCON) files, and feature files are available under a Creative Commons attribution (CC BY) license through the database page http://movement. openworm.org/ and Zenodo community page https://zenodo.org/communities/openworm-movement-database/.

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Author contributions

A.J. wrote Tierpsy Tracker and analyzed data; M.C. wrote WCON viewer, the database, and OpenWorm Analysis Toolbox; C.W.L. wrote the database, web interface, and WCON viewer; J.H. wrote the MATLAB WCON viewer and OpenWorm Analysis Toolbox; K.L. wrote stage-alignment code; C.N.M. collected data; E.Y. wrote the skeletonization algorithm and stagealignment code; L.J.G. collected data; C.L. contributed strains and planned experiments; Q.C. contributed strains and planned experiments; W.R.S. planned the study; E.A.A.N. contributed strains and planned experiments; R.K. designed WCON and wrote several readers; A.E.X.B. planned the study and wrote the manuscript.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41592-018-0112-1.

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Reporting Summary

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\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
\boxtimes		Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

 Policy information about availability of computer code

 Data collection
 The single worm tracking data was collected using WormTracker 2.0 (custom code written in Java) as described previously in Yemini et al. (2013) Nature Methods. The multiworm data (Fig. S5) was collected using Gecko version 2.0.3.1 to capture data from the cameras (http://gecko.visionexperts.co.uk/).

 Data analysis
 All tracking and feature extraction was performed using custom written code available at http://ver228.github.io/tierpsy-tracker/. The classification results reported in Fig. S5 were computed using the open source library PyTorch.

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

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Videos, skeleton (WCON) files, and feature files are available with a Creative Commons attribution (CC BY) license through the database page http://movement.openworm.org/ and Zenodo community page https://zenodo.org/communities/open-worm-movement-database/

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Life sciences study design

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

Sample size	Sample sizes were chosen to be approximately 20 worms per strain. This provides a power of 0.8 to detect a ~1 standard deviation effect (see Yemini et al. (2013) Fig. S3).
Data exclusions	Data were excluded if worms were lost during tracking or if fewer than 100 frames were skeletonized (this corresponds to less than 0.05% of frames being skeletonised) to exclude severely under-sampled worms. Data were also excluded if a filename contained an error (for example, a non-existent gene name) that could not be reliably corrected with reference to lab notebooks since these data cannot be associated with a strain and therefore compared to other strains. Data were also excluded if the pipeline failed to complete for a given video (e.g. due to a corrupted video) since no feature data are available to analyze in this case. These exclusions were not pre-established before analysis.
Replication	No replication was performed.
Randomization	The tracker used for collecting control data from the reference strain (N2) was varied from day-to-day.
Blinding	No blinding was performed because the same features were extracted and the same analysis performed automatically regardless of strain identify. There was thus low risk of experimenter bias affecting the results.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
\boxtimes	Unique biological materials
\ge	Antibodies
\boxtimes	Eukaryotic cell lines
\boxtimes	Palaeontology
	Animals and other organisms

Human research participants

Methods

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

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

This study involved both hermaphrodites and a small number of male C. elegans. Most data were collected from day 0 adults, but other ages are included and noted in the database. Over 300 strains were used. Strain identify is recorded in the database and presented with analysis results in the paper.

	Wild isolates (Fig. S5) were obtained from the C. elegans Natural Diversity Resource (CeNDR). Details on the strains are available from the CeNDR page: https://www.elegansvariation.org/	
Field-collected samples	No field samples were used in this study.	