Supplementary Methods (Hardaway and Wang *et al.*)

C. elegans **Strains**

The *dat-1(ok157)III* strain was obtained from J. Duerr and J. Rand (Oklahoma Medical Research Foundation, Oklahoma City), and is a complete loss of function mutation that eliminates the majority of the DAT-1 coding sequence. Rescue of this deletion via 6-hydroxydopamine toxicity and for swimming behavior was performed in McDonald *et al.* and Hardaway and Hardie *et al* respectively[1,2]. A strain producing a loss of function disruption of DOP-3 *(dop-3(vs106)X)* was obtained from the Caenorhabditis Genetics Center, which is funded by the NIH Office of Research Infrastructure Programs (P40 OD010440). The specific impact of this deletion on *dop-3* signaling was demonstrated by rescue of exogenous DA-induced paralysis by introduction of a wild type *dop-3* fragment in Chase *et al*[3]. We obtained *cat-2(tm2261)* from Shohei Mitani at the National Bioresource Project at Tokyo Women's Medical University and backcrossed it three times to the N2 strain before use. The *cat-2(tm2261)* deletion exhibits altered 2-nonanone avoidance, which was rescued by introduction of a wild type *cat-2* transgene[4], and exhibits significantly reduced tissue DA levels relative to the N2 strain[2].

Assessment of Swip Behavior

In both batch and automated analyses, we generated synchronous populations of these strains by hypochlorite treatment and harvesting arrested L1 animals. L1s were plated at a moderate density on fresh NGM/OP50 plates and incubated at temperatures ranging from 12 to 20 degrees. We observe no differences in Swip for N2 and *dat-1* animals reared between these temperatures. On test days, middle stage L4 animals were identified by characteristic morphological features and used for behavior as N2 animals show some stochastic Swip and quiescence bouts prior to and during the last larval molt. At this stage, the crescent structure should be fully formed and visible and the worms should be actively crawling. Young adults should not be used for Swip as we observe a reduction in penetrance following the L4 stage. We placed worm test plates at room temperature for 15 min to acclimate to room temperature (22°C) before testing. A stock of IMI (Sigma – Cat No. I7379) was dissolved in water to 100 mM on test days and serially diluted in water to the desired concentrations. For batch analyses, 100 µL of water or drug were dispensed in multiple wells of a Pyrex Spot Plate (Fisher catalog number 13-748B) and 10-15 L4 animals were gently transferred using an eyelash pick. After 10 min., we recorded the number of paralyzed worms/total worms. Two independent observers performed assays over several weeks using multiple worm preparations, blind to genotype or drug manipulation. For automated video analysis, single L4 hermaphrodites were placed in 20 µL of water or drug in a single well of a Pyrex Spot Plate, and 10 min. movies (uncompressed AVI format) of their swimming behavior were captured as described in the

supplementary materials. Every worm line was recorded sequentially and in parallel over several days to ensure that strains were identical in age.

Tracker2.0 Validation
Following the analysis of individual tracker files generated by Tracker2.0, we compared the individual worm frequency plots against the raw video to validate the fidelity of tracking. Short bouts of paralysis or slow movement provided convenient frequency markers for this process. In cases of bad tracking, the text files were omitted from further analysis or the raw video was retracked using Pause/Reseed to ensure proper placement of the spine.

SwimR Tools
We used validated tracker files (see above) to generate a frequency matrix using SwimR. This matrix was analyzed using the SwimR command (see SwimR manual) according to the default parameters except for outlier analysis. We used a high MAD score (15.4667) for each group that we analyzed, which prevented SwimR from excluding any outliers from subsequent analysis. We reasoned that drug application would produce bimodal effects and that exclusion based on the median of a sample group would exclude genuine drug responsive animals. Therefore all genotypes, including no drug, were analyzed in this way.

Statistical Analysis
SwimR generates several output images and text files of the raw data used to generate them (see Supplementary Materials). Raw data from SwimR was input into Prism 6.0a (Graphpad, La Jolla,CA) and subsequent analyses performed as described in the text or figure legends. IMI dose response curves in Figure 1B were generated by nonlinear regression using a fixed slope and normalized response condition. Values for *dat-1* are connected by a simple line and do not represent a non-linear regression.

References

1. Mcdonald, P. W. *et al.* Vigorous motor activity in Caenorhabditis elegans requires efficient clearance of dopamine mediated by synaptic localization of the dopamine transporter DAT-1. *Journal of Neuroscience* **27,** 14216–14227 (2007).
2. Hardaway, J. A. *et al.* Forward Genetic Analysis to Identify Determinants of Dopamine Signaling in Caenorhabditis elegans Using Swimming-Induced Paralysis. *G3: Genes| Genomes| …* (2012).
3. Chase, D. L., Pepper, J. S. & Koelle, M. R. Mechanism of extrasynaptic dopamine signaling in Caenorhabditis elegans. *Nat Neurosci* **7,** 1096–1103 (2004).
4. Kimura, K. D., Fujita, K. & Katsura, I. Enhancement of Odor Avoidance Regulated by Dopamine Signaling in Caenorhabditis elegans. *Journal of*

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Acquisition of Worm Swimming Videos

Summary

There are many free and low-cost video acquisition programs that can be used to record video from an USB-driven camera that is mounted to a dissecting scope via a C-mount port. Our system consists of either a Zeiss Stemi SV11 or Zeiss Stemi 2000 with traditional fiber optic illumination (Fostec or Kramer Scientific) connected to a ThorLabs DCC1545M High Resolution USB 2.0 CCD Camera linked to a PC workstation running either Windows XP(32-bit) or Windows 7(64-bit). The software described below interface with both operating systems. In both cases, these dissecting scopes have been outfitted with a 0.4X stereo lens to allow for additional wide-field applications in the lab. For these recordings, however, a conventional 0.65X or 1.0X stereo lens in combination with the zoom built into the dissecting scope should allow the user the flexibility necessary to acquire videos at the appropriate magnification.

Video Quality Guidelines

As with additional worm tracking programs used for solid substrate, the quality of the videos ultimately determines the fidelity of the tracking program outputs. Care must be taken to ensure that several variables are eliminated that interfere with subsequent tracking that include: poor contrast (see examples), gradient shading(proper positioning of mirror), bubble contours, and debris.

To Record Swimming Videos

1. Make sure camera is attached to USB port.
2. Open VirtualDub.
3. Click on File->Capture AVI.
4. Select the capture device. Click on Device -> uc480 Capture Device 1(DirectShow)
5. Turn off the audio. Click on Audio -> Click off “Enable Audio Capture”. Click on Audio -> Audio input -> No input
6. Turn off Compression. Click on Video -> Compression. Select “Uncompressed RGB” and click OK.
7. Add the resize filter. Click on Video -> Filter Chain -> filter list. Click on Add. Click on resize. In the first open box, type in 320(X). Maintain aspect ratio. Y will automatically be set to 256.
8. Set the capture file. Click on File -> Set capture file. Type in the name of the file. Suggested naming conventions are “genotype_date_video #”. See examples below. Do not use parentheses or spaces.

9. Add worm to water using an eyelash pick. We have determined that this is the gentlest method of transferring a single animal. This must be done quickly to avoid worm dessication. If some OP50 bacteria have been transferred with the eyelash, swirl the bubble to dissolve any clumps. The amount transferred is minimal and has no impact on swimming behavior at this low concentration.
10. Capture Video. Click on Capture -> Capture Video.
11. Be sure to set a new destination file before clicking on capture again or you will overwrite the existing file.

SwimR File Naming Conventions

Do not use spaces or parentheses. Use underscores to separate pertinent information. You may use dashes to denote genotypes and separate the date.

Genotype_Drug(if used)_Dose(if used)_Date_#

Examples:

N2_AMPH_100uM_2-11-14_1
dat-1ok157_IMI_10uM_12-12-12_7
cat-2e1112dat-1ok157_2-20-09_2
dat-1ok157dop-3vs106_5-17-06_4

Processing Videos

Now that you have recorded your videos, they must be prepared for tracking in Tracker2.0. If you have acquired short videos(<3-4 min.), no preparation is necessary. We routinely acquire 10 min. videos, so these videos must be split so that Tracker2.0 can analyze files of the appropriate size(<800 Mb). Tracker2.0 is built to recognize videos that have been split into several segments. For example, a 10 min. video of N2 animals in water(N2_4-5-12_1) would be split into 3 segments[N2_4-5-12_1-(1-3)]. Tracker2.0 will recognize these 3 split segments when the first segment is selected for analysis. Raw videos can be split using either a free video splitter(http://easy-video-splitter.en.softonic.com/) or a commercial video splitter that allows for batch capability(http://www.solveigmm.com/en/products/video-splitter/).

Tracker2.0 Manual

Computer Environment

Dell OptiPlex 990
Intel Core i7-2600 CPU @ 3.4 GHz
16.0 GB RAM
Windows 7 64-bit OS

A Windows machine with lower RAM and running XP should also work fine.

Installation

1. Unpack and copy the TrackerV2 folder into the directory of your choice.
2. To open Tracker2.0, double click Tracker.exe. Create a shortcut to this icon if you wish.

Using Tracker2.0

Tracker2.0 can analyze individual video files or in batch. Using Windows 7 or XP, multiple copies of Tracker2.0 can be run at one time(the most we have used is 8.) Tracker2.0 can only load video files of 700 Mb or less, therefor prior to loading your video files, you must split them into pieces using a Video splitter as previously mentioned. The program is designed to identify contiguous video files within the same directory that have been appended with "- split #". For example, a large video named N2_5-10-13_1 is broken into three splits: N2_5-10-13_1-1, N2_5-10-13_1-2, and N2_5-10-13_1-3. After tracking, Tracker2.0 creates a text file of the positional coordinates that merges all three of the splits that can be used for analysis in SwimR.

Load Video

For a single video:

1. Click on File -> Load Video. Select the video or video split that you would like to analyze.
2. Modify the tracking parameters. See below. Click Process.
3. Click on the center of the worm.

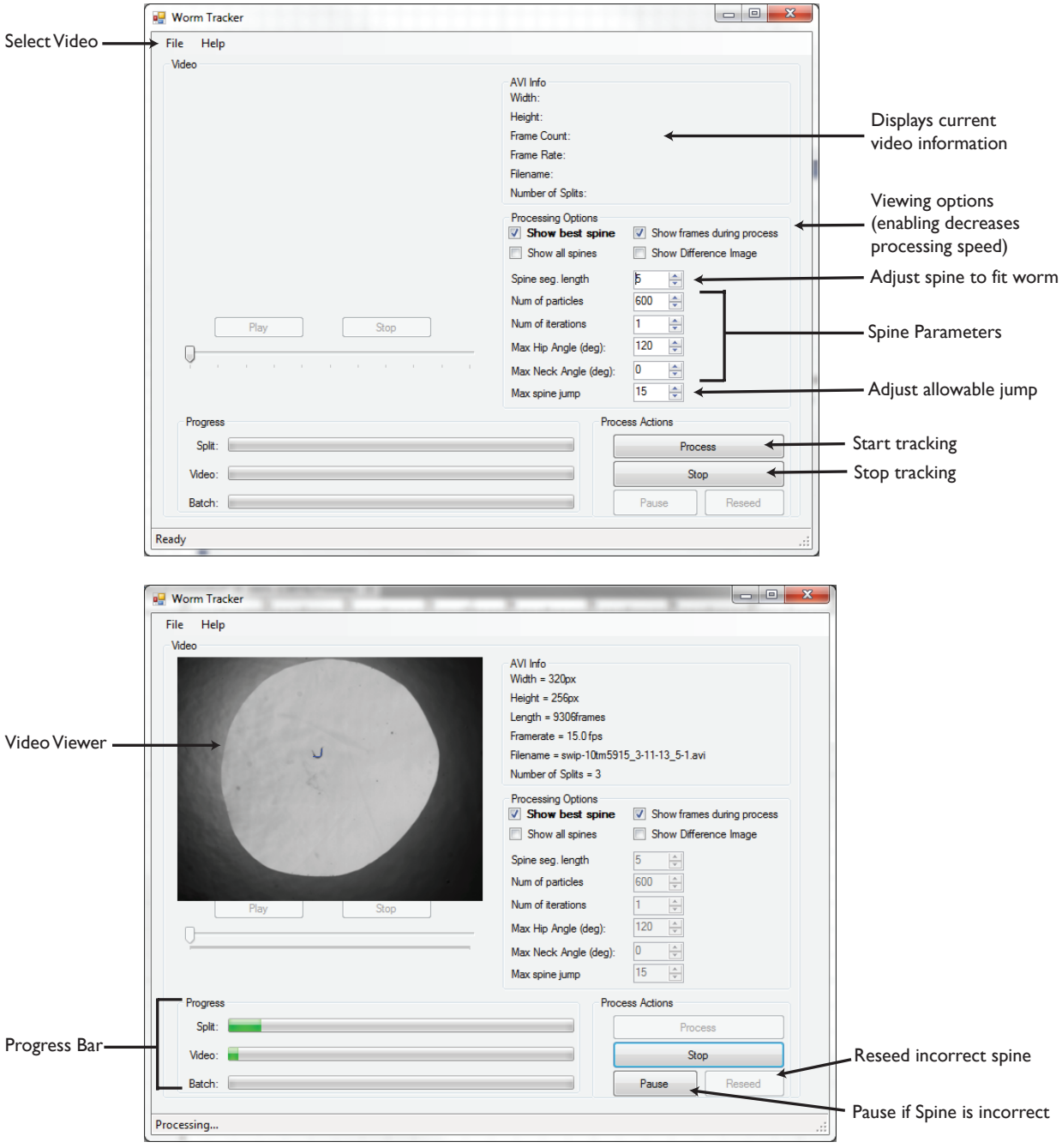
For Batch Process(<8 videos for each Tracker2.0 Window)

1. Modify the tracking parameters.
2. Click on File -> Batch Process. Select the videos that you want to analyze. For videos with multiple splits, one only need select the first split in the series.
3. For each video, click on the center of the worm.

Pause/Reseed

If you notice that the assigned spine does not correspond to the worm, you can press the “Pause” button, navigate backward using the video progress bar below the video viewer, and then press Reseed. You will be asked to click on the center of the worm again at this frame.

Tracker2.0 Parameters and Screenshots



Description of Tracker2.0 Parameters

Number of particles: Number of spines used in algorithm (these are displayed in red when show all spines is selected). In general, more spines equals more possible guess so the typical trade off is more spines gives better accuracy but lengthens processing time.

Number of iterations: Number of times particle filter algorithm is run on each frame. The cost of each iteration time-wise is substantial but can greatly improve accuracy of spine fitting.

Segment length: The length in pixels of each segment. On a worm spine there are 5 segments and 3 angles. The angles are the knee, hip, and neck while the segments run from the neck out to the head, from the neck to the hip and so on. When setting the segment length, ensure that the spine is equal in length, or preferably slightly shorter, than the worm itself.

Maximum hip angle: This is the maximum allowable hip angle the spine is allowed to take on. If this is set too large the spine my double-up on itself and assume and incorrect posture, however, too small might lead to the hip angle under-estimating the worm's actual range of motion.

Maximum neck (or knee) angle: This angle limits the angles on the non-hip angles. In thrashing, it is often useful to limit these to 0, so that the spine only bends at the hip. However, it may be worthwhile to enable these by increasing the hip angle, and then tuning the algorithm to show that the spines nicely fit the underlying worm.

Manual of SwimR

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1 Introduction

The nematode Caenhorhabditis elegans offers great power for the identification and characterization of genes that regulate many behaviors, from locomotion to learning and memory. To more precisely quantify these behaviors, analytical methods are required that provide dimensional analysis of subcomponents of behavior. Thus, we developed the package SwimR, to illustrate and quantify C. elegans Swip, which can reveal novel kinetic alterations in swimming produced by these manipulations that can be of use in dissecting the differential control of swimming behavior by converging signaling pathways.

2 Environment

The R version is at least 2.13.0, which can be downloaded in the website http://www.r-project.org/. Before applying SwimR to analyse data, the users should install the following packages: gplots (>=2.10.1), heatmap.plus (>=1.3), signal (>=0.7) and bimodalitytest (>=1.0). The installation process of first three packages is as follows.

>install.packages("gplots")

>install.packages("heatmap.plus")

>install.packages("signal")

3 Directory Navigation

The use of the SwimR tools assume a working knowledge of how to navigate to and from different directories. For simplicity, it is recommended that you create a folder on the desktop that contains the SwimR package. You can then copy and paste tracker files into a SwimR subdirectory for analysis. If you perform the analysis this way you will only need to navigate directories once upon opening R. Following Frequency Analysis(see below) commands and SwimR commands you can then move these files to the desired directory for long term storage.

To set the working directory use one of two methods:

Drop Down Menu Click on File -> Change dir. A browser window will pop up asking you to select a folder(i.e. C:\SwimR). Select the folder.

Command Line Using the command line, type "setwd(directory path)"

To check that the working directory is correct: Using the command line type "getwd()". This should return the current working directory path. Make sure that the files to be analyzed are in this directory.

4 Creation of frequency matrix

After building up the basic environment mentioned above, the users can install SwimR package and create frequency matrix and annotation file for the example files returned by Tracker prgram.

```
> library("SwimR")

****************************************
*                                      *
*             Welcome to SwimR!        *
*                                      *
****************************************

> inputPath <- system.file("extdata","trackerFiles",package="SwimR")
> outputPath <- getwd()
> createFrequencyMatrix(inputPath, outputPath, method = "Extrema", Threshold = 0.6,
+ DeltaPeakDt = 1.6, MinFrameBtwnMax = 4, MinDelta = 2.5, longPeriod = 5, AvWindowSize = 10,
+ fps = 15, ZP_Length = 100, WindowSize = 30, MaxCompWin = 2, minTime = 0, maxTime = 600)

Processing...

File:  N2_M9_10-14-11_1-1.txt is in process...

File:  N2_M9_10-14-11_2-1.txt is in process...

File:  N2_M9_10-14-11_3-1.txt is in process...

File:  N2_M9_10-14-11_4-1.txt is in process...

File:  N2_M9_10-14-11_5-1.txt is in process...

File:  dat-1_(ok157)_1-10-11_1-1.txt is in process...

File:  dat-1_(ok157)_1-10-11_2-1.txt is in process...

File:  dat-1_(ok157)_1-11-11_1-1.txt is in process...

File:  dat-1_(ok157)_1-11-11_4-1.txt is in process...

File:  dat-1_(ok157)_1-12-11_3-1.txt is in process...
Processing completed!

Please see the frequency matrix
and detailed information for each animal in the outputPath directory!
```

4.1 Input

Here is a description of all the arguments needed to get the frequency matrix and annotation file:

1. *inputPath* is a directory which contains the files returned by the Tracker program. If you are using a common directory as described above, you may type in inputPath <-"folder name" where folder name is a subfolder containing the tracker files to be analyzed. Because annotation file is generated by extracting the genotype information from tracker file names, the user should use dashes to denote genotypes and separate the date in the file name like Genotype_Drug(if used)_Dose(if used)_Date_#. The following are some examples of tracker file names: N2_AMPH_100uM_2-11-14_1 (genotype is N2), dat-1ok157_IMI_10uM_12-12-12_7 (genotype is dat-1ok157), cat-2e1112dat-1ok157_2-20-09_2 (genotype is cat-2e1112dat-1ok157) and dat-1ok157dop-3vs106_5-17-06_4 (genotype is dat-1ok157dop-3vs106).

2. *outputPath* is a directory which saves the plots and files returned by the function.

3. The function provides four different counting methods: "FFT" (Fast Fourier Transform), "Extrema", "PeakDet" (peak delta) and "RT+GP" (Get Peaks plus Racetrack Filter) and the users can select one of them to output the corresponding frequency matrix. The default outputted *method* is "Extrema".

4. *Threshold* is the amount of degrees (in radians) required to count at as thrash and the default is 0.6.

5. *DeltaPeakDt* is the threshold for "peak delta" algorithm, similar to *Threshold* and the default is 1.6.

6. *MinFrameBtwnMax* is the minimum number of frames between maxima and the default is 4.

7. *MinDelta* is similar to *DeltaPeakDet* and the default is 2.5.

8. *longPeriod* is the longest period cycle which is not zerod and the default is 5.

9. *AvWindowSize* is the length of the average window in seconds and the default is 10 seconds.

10. *fps* is the frame per second and the default is 15.

11. *ZP_Length* is the Zero-padding length and the default is 100.

12. *WindowSize* is the size of window for computing the Fast Fourier Transform and the default is 30.

13. *MaxCompWin* is the window size on deciding if the current angle is a maxima and the default is 2.

14. *minTime* is the minimum threshold of time points for the following analysis and the default is 0 second.

15. *maxTime* is the maximum threshold of time points for the following analysis and the default is 600 seconds.

4.2 Output

The createFrequencyMatrix function outputs four files:

1. *XFig.jpg* is the image of scatter plot of one animal plotted as "Frequency vs Time(min)" with all four counting methods overlaid (see Figure 1 for file dat-1_(ok157)_1-10-11_2-1.txt). "X" of "XFig.jpg" represents the input file names.

2. *XFigSub.jpg* is the same as *XFig.jpg* except counting methods are broken up into four different plots. This is very helpful in checking through a video to make sure that Tracker tracked the worm properly. Bad contrast can be a problem with Tracker missing the worm and these files help to identify troublesome videos for retracking or discarding.

3. *XFreq.csv* is the CSV file of raw data organized by column, where column one represents frequency as counted by FFT, column two represents frequency calculated by Extrema, column three represents frequency calculated by PeakDt , column four represents frequency as counted by RT+GP and column five represents time in seconds.

4. *frequencyMatrix.txt* is a TXT file which combines the analysis results of the frequency of worm thrashing over time for all Tracker files in the *inputPath*.

5. *annotationfile.txt* is a TXT file which contains all genotype information extracted from file names of all Tracker files in the *inputPath*.

5 SwimR

SwimR can analyze and visualize worm swimming data returned by the above function. It places a particular emphasis on identifying paralysis and quantifying the kinetic elements of paralysis during swimming.

```
> expfile <- system.file("extdata","SwimExample","SwimR_Matrix.txt",package="SwimR")
> annfile <- system.file("extdata","SwimExample","SwimR_anno.txt",package="SwimR")
> projectname <- "SwimR"
> outputPath <- getwd()
> SwimR(expfile, annfile, projectname, outputPath, color = "red/green",
+ data.collection.interval = 0.067, window.size = 150, mads = 4.4478,
+ quantile = 0.95, interval = 20, degree = 0.2, paralysis.interval = 20,
+ paralysis.degree = 0.2, rev.degree = 0.5)

Processing...
Processing completed!
Please see the detailed information in the outputPath directory!
```

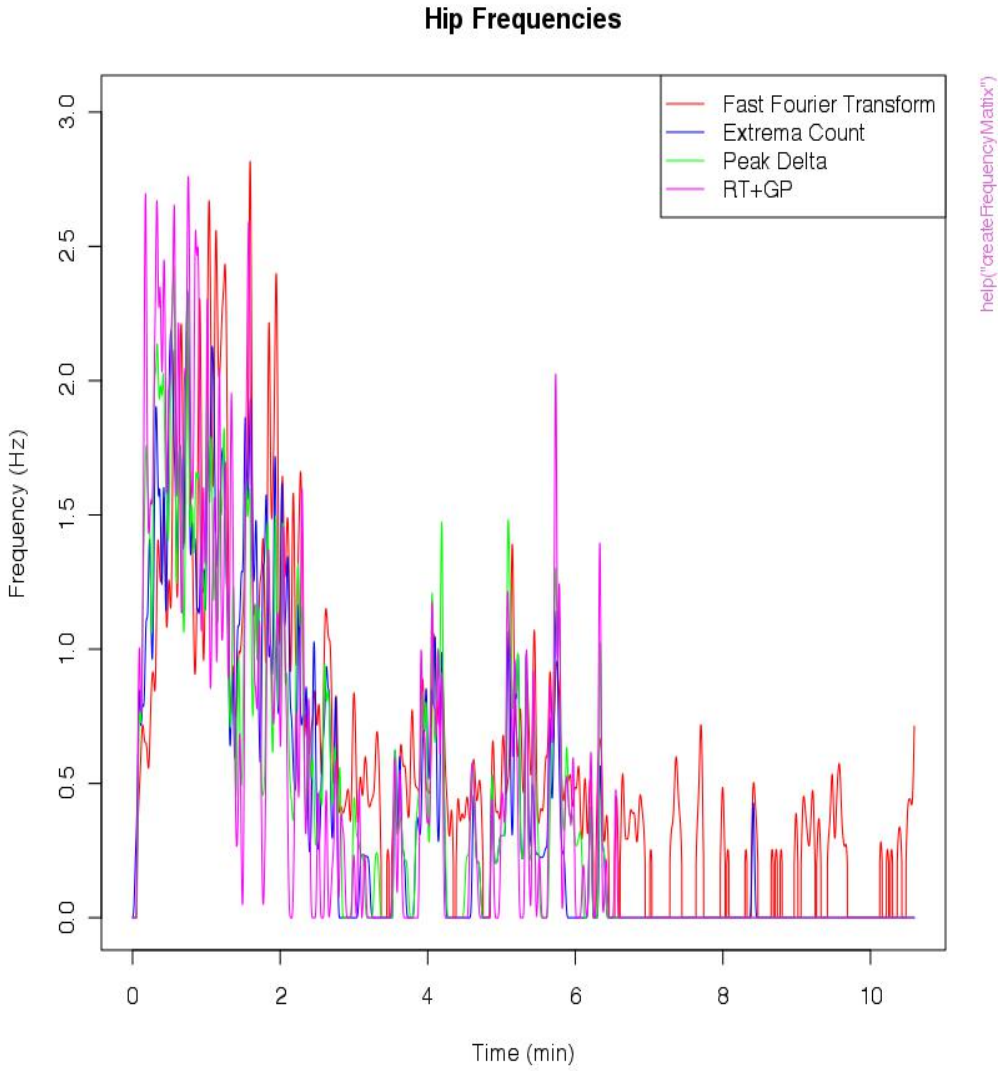

Figure 1: *A scatter plot of three methods for file dat-1_(ok157)_1-10-11_2-1.txt*

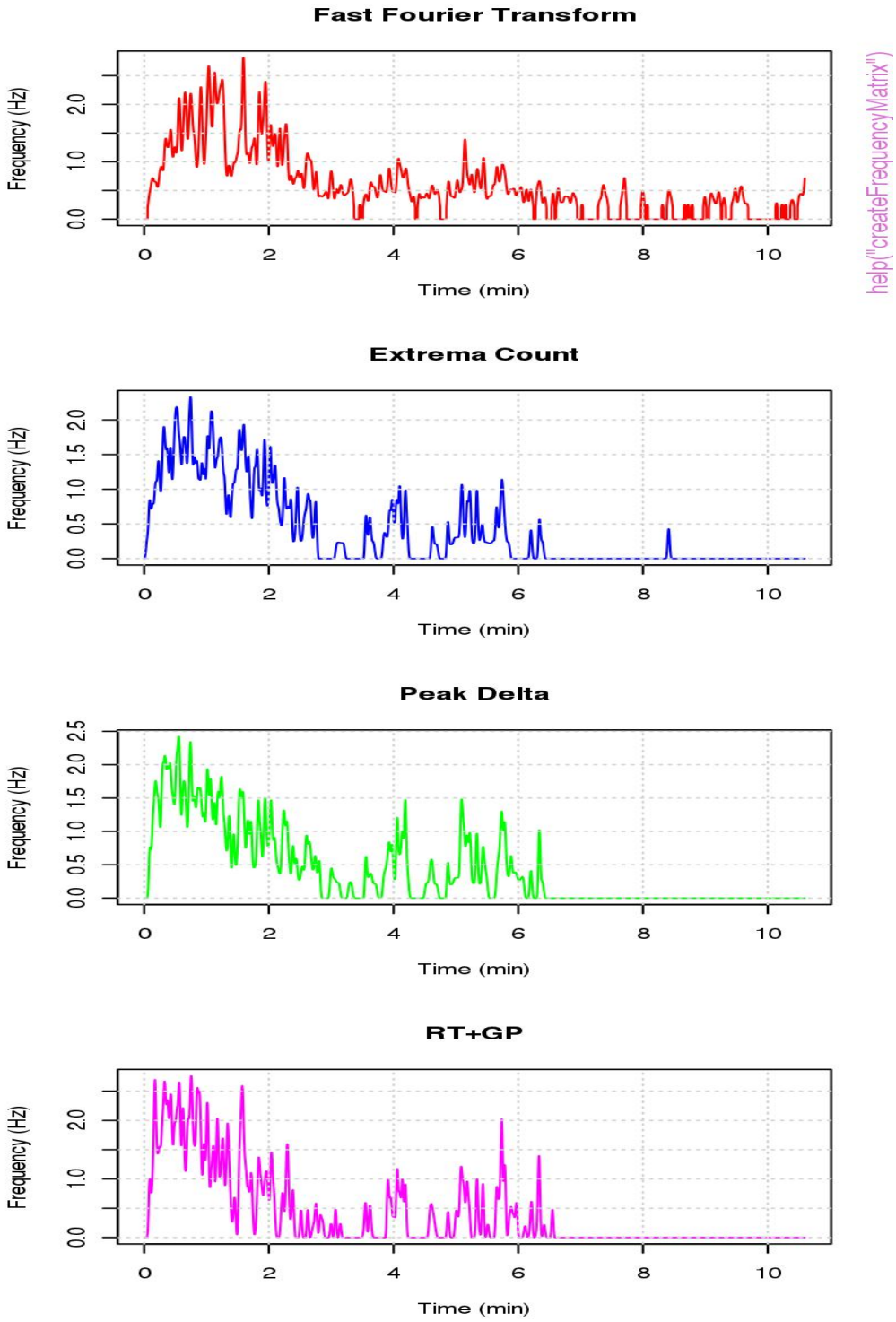

Figure 2: *Three separate scatter plots of four methods for file dat-1_(ok157)_1-10-11_2-1.txt*

5.1 Input

Here is a description of all the arguments for SwimR:

1. *expfile* is the path of the frequency matrix returned by the *createFrequencyMatrix* function.

2. *annfile* is the path of annotation file returned by the *createFrequencyMatrix* function.

3. *projectname* is the name of the project.

4. *outputPath* is a directory which saves the plots and files returned by the function.

5. The function provides three colors to plot the heatmap plot:"red/green", "red/blue", "yellow/blue" and "white/black". The default *color* is "red/green".

6. *data.collection.interval* is the time interval between two points and the default is 0.067.

7. *window.size* is the size of the window for the running average that is calculated to smooth the data. The default is 150.

8. *mads* is the number of median absolute deviations that a given animal must deviate from the median sum of frequencies to be called an outlier. The default is 4.4478.

9. *quantile* is the proportion of data points that are used in calculating the color scheme for the heat map and the default is 0.95.

10. *interval* is the minimum time that a given animal must lie below a threshold to be called a paralyzed worm for the first calculation and the default is 20.

11. *degree* is the paralytic degree for the first calculation and the default is 0.2.

12. *paralysis.interval* is the same as *interval* but for the second calculation and the default is 20.

13. *paralysis.degree* is the paralytic degree for the second calculation and the default is 0.2.

14. *rev.degree* is the threshold that an animal must cross to be called a revertant and the default is 0.5.

5.2 Output

The SwimR function outputs 13 files:

1. *P_sample_t_half.txt* is the TXT file of each animal and their corresponding latency to paralyze. For non-paralyzers, N/A will be listed. "P" of "P_sample_t_half.txt" is the *projectname* inputted by users.

2. For animals that paralyze at the threshold by the users in the parameters (default is below their 20% Max for at least 20 seconds), *P_group_t_half.txt* contains the average latency and standard deviation for those animals to paralyze. It also returns the raw number of animals in the entire samples that are paralyzers and non-paralyzers.

3. The columns of *P_group_data.txt* is defined as follow. "freq_max_mean": Mean maximal swimming frequency; "freq_max_sd": Standard deviation of Mean maximal swimming frequency; "freq_min_mean": Mean minimum swimming frequency; "freq_min_sd": Standard deviation of Mean minimum swimming frequency; "freq_range_mean": Mean range between maximum and minimum; "freq_range_sd": Standard deviation of Mean range between maximum and minimum; "paralytic_count": The number of paralyzed animals amongst the samples; "non-paralytic_count": The number of non-paralyzed animals amongst the samples; "t_half_-mean": Mean latency to cross the paralytic threshold set by the users (default is 20% of Frequency range) and stay below it for the interval specified interval (default is 20 seconds); "t_half_sd": Standard deviation of *t_half_mean*; "t_p_start_mean": The mean time point (in seconds) at which each animal crosses a frequency that is min+paralytic threshold and stays below that threshold for the paralytic interval; "t_p_start_sd": Standard deviation of *t_p_start_mean*; "t_p2end_mean": The average range of time after paralysis; "t_p2end_-sd": Standard deviation of *t_p2end_mean*; "rev_count": The number of revertants amongst the samples as defined by the threshold set by the user (default is animals have to recross 50% of their frequency range for any length of time; "rev_percent": The number of revertants; "rev_frequency_mean": The number of reversion events; "t_p2r_mean": Mean time between 1st reversion and *t_p_start_mean*; "t_p2r_sd": Standard deviation of *t_p2r_mean*; "t_r_total_mean": Mean of total time spent in reversion for all revertants; "t_r_to-tal_sd": Standard deviation of *t_r_total_mean*; "t_r_average_mean": Mean length of an individual reversion

event; "t_r_average_sd": Standard deviation of *t_r_average_mean*; "r_amp_mean": Mean of total amplitude of reversion for all revertants, where amplitude is defined by the area beyond the reversion threshold set by user (default is 50% Freq range) during reversion, calculated by summing up discrete values for each measurement (same unit as frequency); "r_amp_sd": Standard deviation of *r_amp_mean*.

4. *P_heatmap_withingroup_ordered_globalcentering.pdf* is a PDF of the heat map of all of the samples included in the data matrix after outlier exclusion, smoothing, ordering based on the latency to paralyse, and centering the color based on the quantile percent that can be set by the user in the parameters section of SwimR.

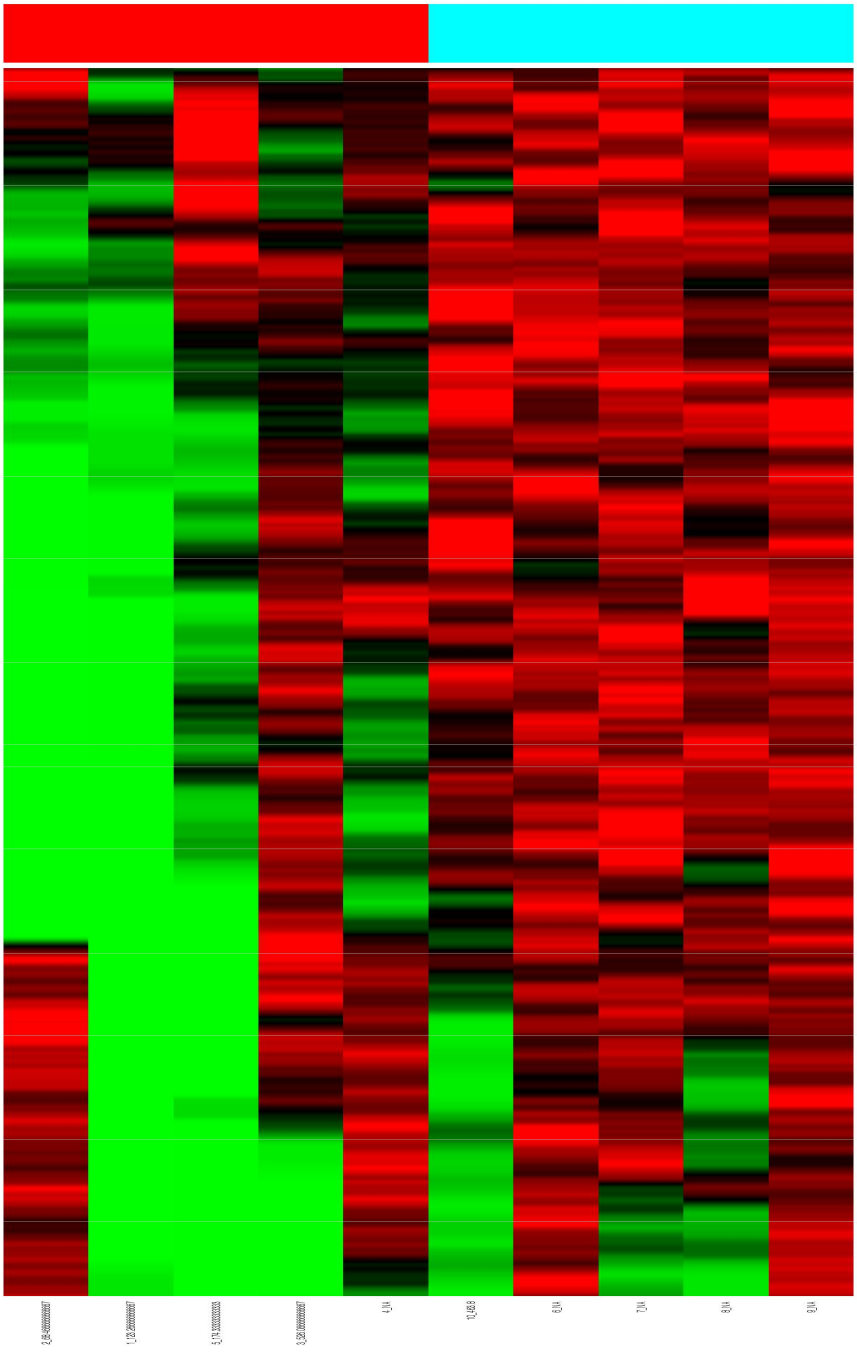

Figure 3: *Heat map of all samples after outlier exclusion, smoothing, ordering based on the latency to paralyse*

5. *P_heatmap_withingroup_ordered.txt* is a TXT file of the raw data used to plot the heat map after outlier exclusion, smoothing and ordering based on the latency to paralyze.

6. After exclusion and smoothing, *P_histogram.nooutliers.smoothed.pdf* is a PDF file of all frequency data points broken up into increasing 0.1 Hz bins and then plotted as the fraction of the total as a histogram (see Figure 4 as an example).

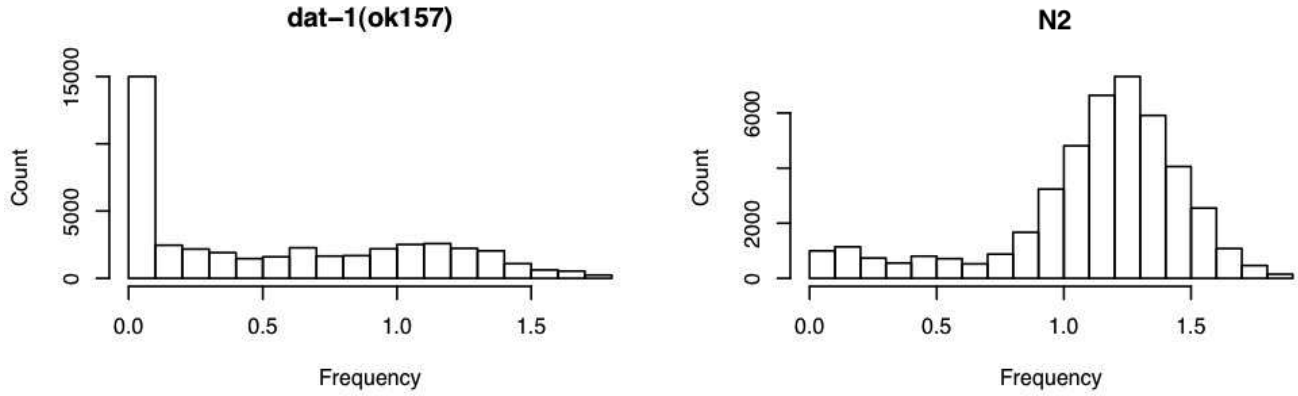

Figure 4: *Histogram of all frequency data points broken up into increasing 0.1 Hz bins*

7. *P_histogram.nooutliers.smoothed.data.G.txt* is a TXT file of the raw data used to plot the histogram. "G" in the "P_histogram.nooutliers.smoothed.data.G.txt" is the genotype in the annotation file.

8. *P_individual_data.txt* is a TXT file that returns reversion information for individual animals. The definitions are identical to the P_group_data.txt file, but "R_count" is the number of reversion events for that animal. If there is no paralyzed animal, this file will not be outputted.

9. *P_individual_data1.txt* is a TXT file. For animals that paralyzed: The R_instances row tells the user exactly when the animal reverted. For animals that did not revert, N/A will be listed. If there is no paralyzed animal, this file will not be outputted.

10. *P_intermediate.results.txt* describes some key features of your samples after running SwimR, and is a great way to get a quick look at the incidence of paralysis amongst your samples. At the top of the file, it lists the parameters used in the subsequent calculations. Below that, it lists the summed frequency values for each of the animals included in the sample. And then the p value of the bimodal test for each genotype was listed. Below that, it lists each of the animals included and excluded after outlier detection. After that, it lists which animals were considered paralyzed and which not. For paralyzed animals, it then lists which of them were called revertants.

11. *P_scatter.pdf* is a PDF image of the average frequency plotted against time after outlier exclusion, but w/o smoothing (see Figure 5 as an example).

12. *P_nooutliers_smoothed_scatter.pdf* is a PDF image of the average frequency plotted against time after outlier exclusion and smoothing (see Figure 6 as an example).

13. *P_nooutliers_smoothed_scatter_data.txt* is a TXT file of the raw data used to plot the smoothed scatter.pdf. Column one is time, Column two is average frequency and Column three is standard deviation.

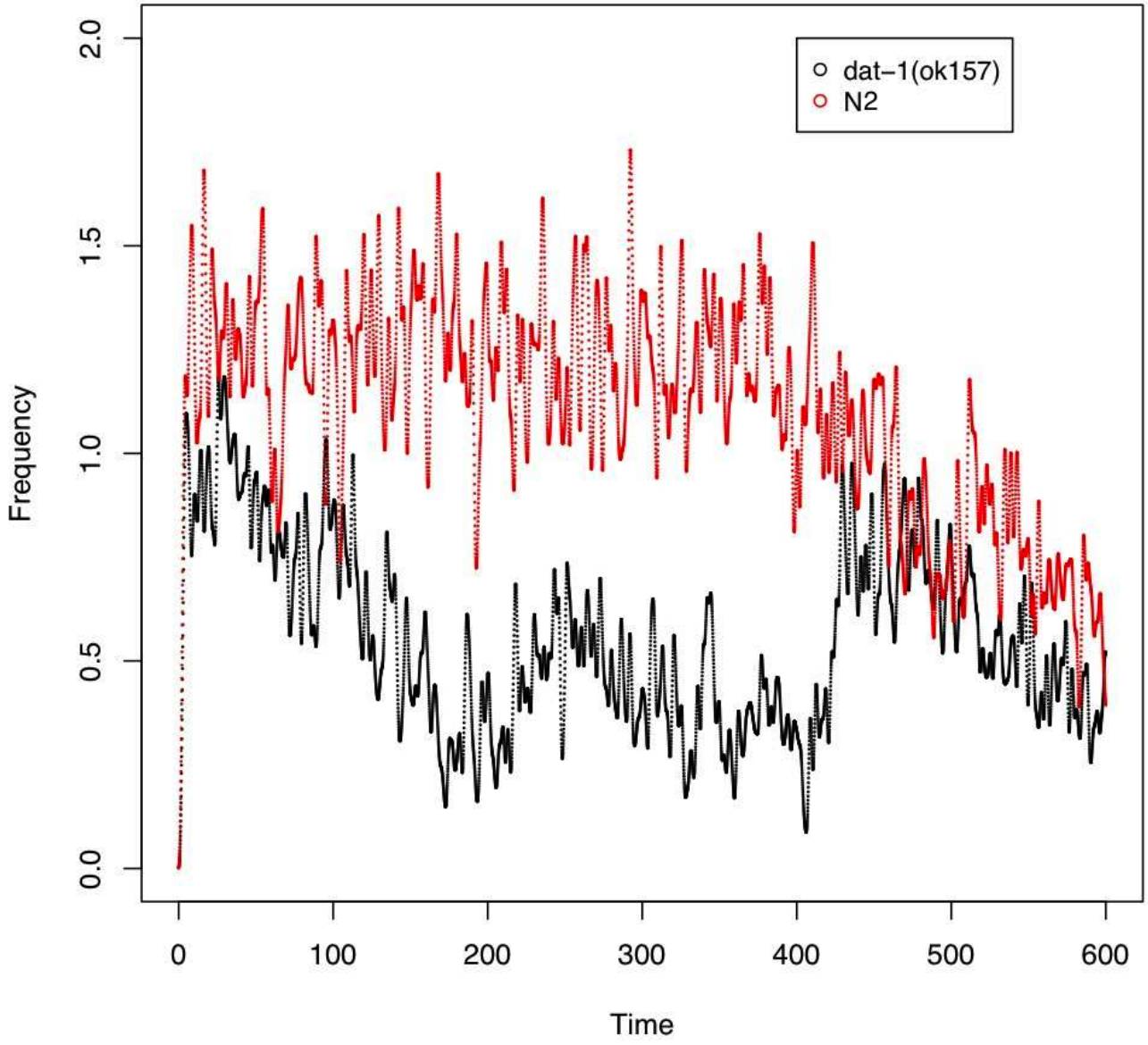

Figure 5: *Scatter plot of the average frequency plotted against time after outlier exclusion, but w/o smoothing*

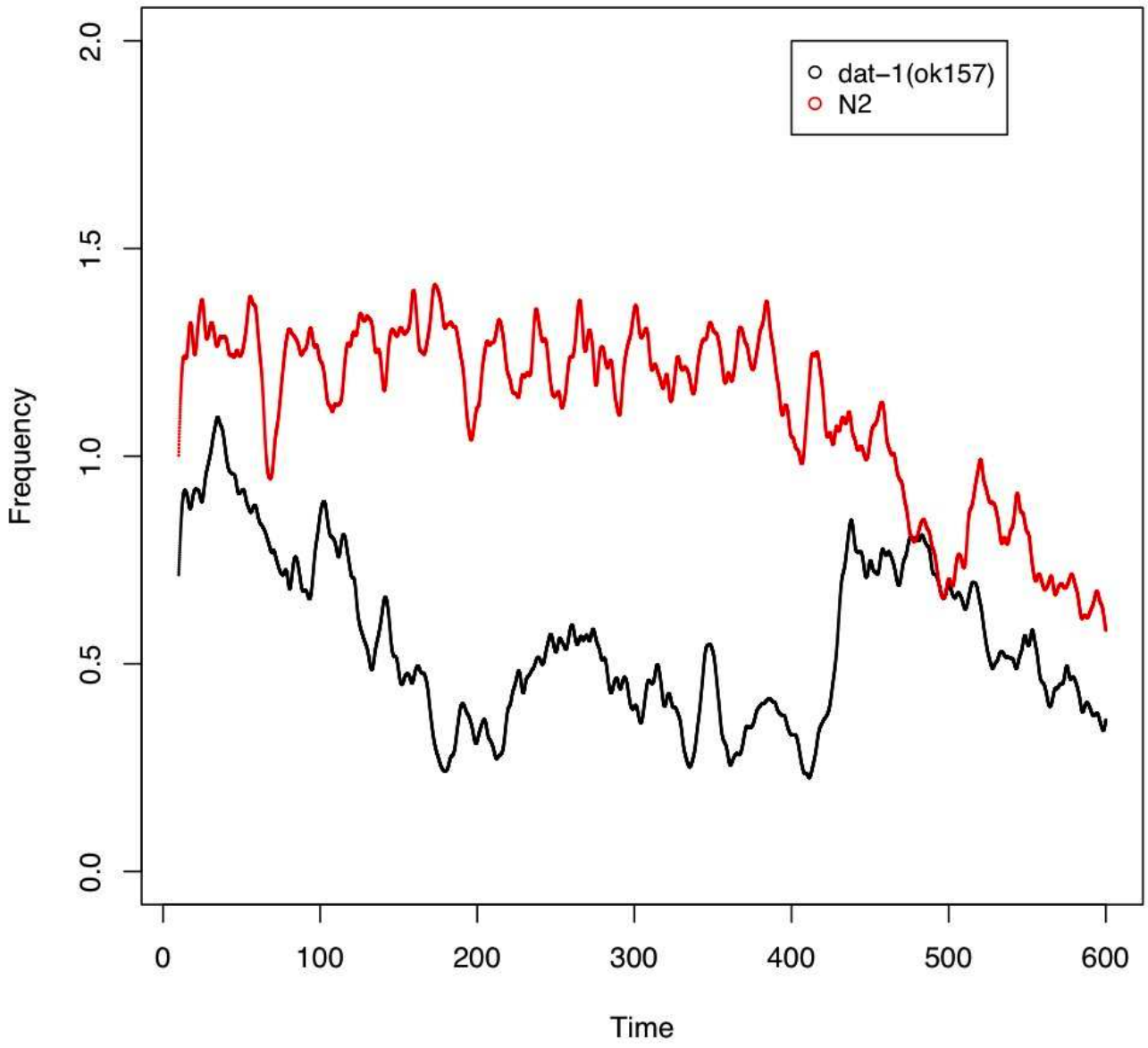

Figure 6: *Scatter plot of the average frequency plotted against time after outlier exclusion and smoothing*