



EntoLab data analysis


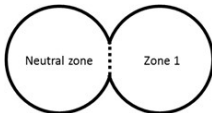
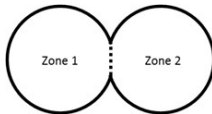
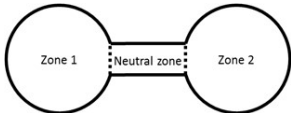
COMPUTING INSECT BEHAVIOR STATISTICS WITH ETHOANALYSIS

EthoAnalysis is the tool designed for the analysis of video tracking data obtained in EntoLab experiments. It can be used to combine data of multiple experiments, extract statistics per experiment, report summary statistics per analysis level (i.e., specific genotype/treatment combinations), and perform comparative analysis on different analysis levels to aid identifying differences in insect behavior that may be due to differences in plant resistance or treatment.

Track files generated by EthoVision XT are imported into EthoAnalysis. Based on various user settings, which can be specific for an insect species, EthoAnalysis translates the raw track data into series of zone-specific behavior events of three types: halting, moving, and not-detected (events that do not contribute to the calculation of behavior statistics). From these series of events, various behavior statistics are computed.

ARENA DESIGNS

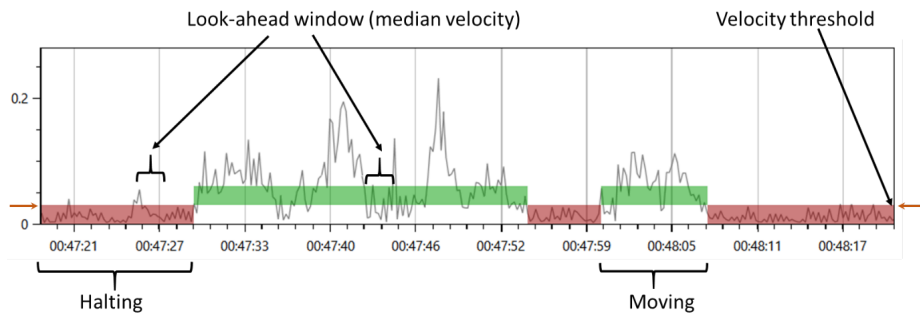
EthoAnalysis is designed to support variations of a fixed experimental format shown in the table below. Experiments are set up in one or more trials. Each trial consists of multiple arenas that form the experimental units. In each arena, one insect is placed together with one (no-choice) or two (two-choice) leaf parts of the genotype(s)/treatment(s) of interest.

Arena design	Layout	Multiple zones	Statistics of interest
Single zone No-choice		No	Overall statistics Statistics per time bin
Two zones No-choice		Yes	Overall statistics Zone-specific statistics Statistics per time bin Statistics per zone and time bin
Two zones Two-choice		Yes	Zone-specific statistics Statistics per time bin Statistics per zone and time bin
Three zones Two-choice		Yes	Overall statistics Zone-specific statistics Statistics per time bin Statistics per zone and time bin

FROM TRACKING DATA TO BEHAVIOR EVENTS

EthoAnalysis translates the track data into series of zone-specific events of three types: halting, moving, and not-detected. Each event has a starting time and a duration, and from these series of events, the various behavior statistics are extracted. The series of behavior events are constructed by iterating over the track samples and determining for each sample whether the current state is moving, halting, or unknown. Each consecutive sequence of track samples with the same movement state and occurring in the same zone forms a behavior event of the movement state of that series.

For this purpose, EthoAnalysis applies a user-defined 'look-ahead window' and a velocity threshold. The look-ahead window exists to ignore minor drops below or spikes above the velocity threshold within a movement or halting event, and is an important tool to accurately follow the unique behavior of specific insects, for instance feeding behavior by sucking insects such as aphids or whiteflies.



IMPORTING DATA

Tracking data are imported into EthoAnalysis based on certain threshold settings. It is possible to inspect the correctness of the assignment of the velocity threshold using the velocity histograms of selected records. This can also serve to guide modifications to the velocity threshold setting that are rapidly recalculated.

EthoAnalysis

Project Input data Data filter Data selection Behaviour statistics Analysis Output

Add/remove data files

Select

Filename	Tracking frame rate (f/s)	IsLoaded
..\Track-Frankliniella-Arabidopsis-Trial 1-1-Subject 1.txt	3.33	True
..\Track-Frankliniella-Arabidopsis-Trial 1-10-Subject 1.txt	3.33	True
..\Track-Frankliniella-Arabidopsis-Trial 1-11-Subject 1.txt	3.33	True
..\Track-Frankliniella-Arabidopsis-Trial 1-12-Subject 1.txt	3.33	True
..\Track-Frankliniella-Arabidopsis-Trial 1-13-Subject 1.txt	3.33	True
..\Track-Frankliniella-Arabidopsis-Trial 1-14-Subject 1.txt	3.33	True
..\Track-Frankliniella-Arabidopsis-Trial 1-15-Subject 1.txt	3.33	True
..\Track-Frankliniella-Arabidopsis-Trial 1-16-Subject 1.txt	3.33	True
..\Track-Frankliniella-Arabidopsis-Trial 1-17-Subject 1.txt	3.33	True
..\Track-Frankliniella-Arabidopsis-Trial 1-18-Subject 1.txt	3.33	True
..\Track-Frankliniella-Arabidopsis-Trial 1-19-Subject 1.txt	3.33	True
..\Track-Frankliniella-Arabidopsis-Trial 1-2-Subject 1.txt	3.33	True

Import settings

Look-ahead window: Seconds

Look-ahead rule start method: Median velocity greater

Velocity threshold:

Recover halting from non-detect events

Multiple zones

Include arena / neutral zone

Zones

Zone 1

Zone 2

Zone 3

Velocity distribution raw track samples

Trial	Arena	Genotype	Zone 1	Plant Zone 1	Leaf Zone 1	Genotype Zone 2	Plant Zone 2	Leaf Zone 2	Genotype Zone 3	Data file
Trial 1 0	Cur3		1	4	RMX180	10	4	Neutral	..\Track-Frankliniella-A	
Trial 1 1	Cur3		4	4	RMX180	2	4	Neutral	..\Track-Frankliniella-A	
Trial 1 2	Cur3		2	4	RMX180	9	4	Neutral	..\Track-Frankliniella-A	
Trial 1 3	Cur3		9	4	RMX180	5	4	Neutral	..\Track-Frankliniella-A	
Trial 1 4	Cur3		8	4	RMX180	1	4	Neutral	..\Track-Frankliniella-A	
Trial 1 5	Cur3		3	4	RMX180	8	4	Neutral	..\Track-Frankliniella-A	
Trial 1 6	Cur3		6	4	RMX180	7	4	Neutral	..\Track-Frankliniella-A	
Trial 1 7	Cur3		7	4	RMX180	4	4	Neutral	..\Track-Frankliniella-A	
Trial 1 8	Cur3		5	4	RMX180	3	4	Neutral	..\Track-Frankliniella-A	
Trial 1 9	Cur3		10	4	RMX180	6	4	Neutral	..\Track-Frankliniella-A	
Trial 1 10	Cur3		7	3	RMX180	8	3	Neutral	..\Track-Frankliniella-A	
Trial 1 11	Cur3		5	3	RMX180	5	3	Neutral	..\Track-Frankliniella-A	
Trial 1 12	Cur3		6	2	RMX180	6	2	Neutral	..\Track-Frankliniella-A	

DATA FILTERING

For various reasons, it may be necessary to exclude tracks (arenas) or certain types of events from the analysis. EthoAnalysis contains a number of filters with which you can eliminate an entire arena track or change such events to non-detect events and thus exclude them from analysis. This is important to eliminate artefacts, e.g. when insects are killed during inoculation showing little movement or poor detection. Similarly, ultrafast movements can be caused by a tracking artefact that bears no relation to the biology. The velocity trace shows in color the accepted halting (red) and moving (green) events, and the zone information shows where those events were detected. The trace provides feedback on the suitability of the applied filters. You can interactively modify the filters, see the effect and optimize the settings.

EthoAnalysis

The screenshot shows the 'Data filter' tab in EthoAnalysis. On the left, there are several filter sections: 'Trials' (Trial 3 is selected), 'Record filters' (Filter by detection percentage is checked), 'Event filters' (Filter extreme velocity events is checked), and 'Filter settings' (Velocity threshold is set to 3). The main area features a table with the following data:

Exclude	Trial	Arena	Genotype Zone 1	Plant Zone 1	Leaf Zone 1	Genotype Zone 2	Plant Zone 2	Leaf Zone 2	Genotype
<input type="checkbox"/>	Trial 3	0	Cur3	22	1	RMX180	24	1	Neu
<input type="checkbox"/>	Trial 3	9	Cur3	30	2	RMX180	29	2	Neu
<input type="checkbox"/>	Trial 3	10	Cur3	27	2	RMX180	26	2	Neu
<input type="checkbox"/>	Trial 3	11	Cur3	29	2	RMX180	25	2	Neu
<input type="checkbox"/>	Trial 3	12	Cur3	26	2	RMX180	21	2	Neu
<input type="checkbox"/>	Trial 3	13	Cur3	22	2	RMX180	24	2	Neu
<input type="checkbox"/>	Trial 3	14	Cur3	25	2	RMX180	29	2	Neu
<input type="checkbox"/>	Trial 3	15	Cur3	23	2	RMX180	23	2	Neu

Below the table, a spatial map shows the arena layout, and a velocity trace plot shows movement events over time from 04:48:11 to 04:50:28. The plot indicates 39 of 112 records are selected.

DATA SELECTION

You can select the data for which the statistics are of interest. Here one can exclude, for example, the neutral zones without sample as irrelevant.

EthoAnalysis

The screenshot shows the 'Data selection' tab in EthoAnalysis. On the left, the 'Genotypes' section shows 'Selected 2 of 3 genotypes' with Cur3 and RMX180 checked. The main area features a table with the following data:

Genotype Zone 1	Genotype Zone 2	Genotype Zone 3	Total experiments	Selected experiment
Cur3	RMX180	Neutral	112	39

At the bottom right, it indicates 'Selected 39 of 112 experiments'.

CALCULATION OF BEHAVIOR STATISTICS

The behavior statistics are extracted from the series of behavior events. The software contains a number of behavior statistics that can be extracted for all events of the complete trial, but some of these statistics are also of interest when looking at the statistics per zone, per hour, per zone/hour, or per type of behavior event, e.g., event duration category (short/medium/long) or movement velocity (slow/medium/fast).

EthoAnalysis

Project
Input data
Data filter
Data selection
Behaviour statistics
Analysis
Output

Behaviour statistics
Chart
Chart per category
Summary statistics
Statistics per record
Settings
General settings

Behaviour statistics

- ▾ Average angular velocity
- ▾ Average halting duration
- ▾ Average movement distance
 - Average movement distance (robust)
 - Average movement distance per hour (robust)
 - Average movement distance per zone (robust)
 - Average movement distance per zone per hour (robust)
- ▾ Average movement duration
 - Average movement duration (robust)
 - Average movement duration per short/medium/long (robust)
 - Average movement duration per hour (robust)
 - Average movement duration per zone (robust)
 - Average movement duration per zone per hour (robust)
 - Average movement duration per zone per short/medium/long (robust)
- ▾ Average velocity
- ▾ Detection percentage
- ▾ Distance moved
- ▾ Duration detection
- ▾ Duration halting
- ▾ Duration moving
- ▾ Estimated distance moved
- ▾ Estimated duration moving
- ▾ Estimated duration moving
- ▾ Halt frequency
- ▾ Movement frequency
- ▾ Number of halts
- ▾ Number of movements
- ▾ Ratio detection/trial duration
- ▾ Ratio halting/detection duration
- ▾ Ratio halting/trial duration
- ▾ Ratio movement/detection duration
- ▾ Ratio movement/halting duration
- ▾ Time to first halt
- ▾ Zone entries

Select all
Select robust
Select robust per zone
Include per hour statistics

Chart settings

Chart type: Line chart means and standard errors

Display order: None of Average movement distance (H0 - Zor

Display chart on transformed scale

average movement distance per zone per hour

Hour	Cur3 vs RMX180 vs Neutral Zone 1 (mm)	Cur3 vs RMX180 vs Neutral Zone 2 (mm)
0	4.7	2.5
1	4.5	2.4
2	4.3	1.9
3	4.2	2.1
4	4.0	1.6
5	3.8	1.5
6	3.7	1.4
7	3.9	1.5

STATISTICAL ANALYSIS

EthoAnalysis provides standard statistical models for assessing whether the differences in insect behavior can be attributed to differences in genotype and/or treatment and, if so, evaluating the magnitude of these differences. Different analyses can be performed for no-choice and two-choice assays, since these assays focus on different research questions. In the former, the focus lies on evaluation of the differences between arenas with different genotypes/treatments, whereas in the latter, the focus lies on evaluation of the differences between zones within arenas. If needed, the analyses can include corrections for trial, plant, and leaf.

EthoAnalysis

Project
Input data
Data filter
Data selection
Behaviour statistics
Analysis
Output

Run analysis
Analysis settings
Analysis data

Current activity:

Average movement distance (H0 - Zone 2)

Zone comparison

Zone comparison analysis

Comparison between Zone 1 and Zone 2

Analyse zone differences using generalized linear (mixed) models

Analysis settings

Factor type trial: Fixed

Factor type plant: Random

Factor type leaf: Random

Additional analysis factors

- Arena settings: Exclude
- Detection settings: Exclude
- Start time: Exclude
- Trial duration: Exclude
- Recording duration: Exclude
- Track: Exclude
- Video file: Exclude

Analysis model

1 + (1)GenotypeZone_1:Plant_Zone_1 + (1)Genotype_Zone_2:Plant_Zone_2 + (1)Genotype_Zone_1:Plant_Zone_1:Leaf_Zone_1 + (1)Genotype_Zone_2:Plant_Zone_2:Leaf_Zone_2

REVIEWING OR EXPORTING THE ANALYSIS OUTPUT

The results of analysis runs can be viewed within EthoAnalysis, but you can also download the full output report as a PDF document or export the results as a CSV file for analysis with another software package relevant to your study (e.g. genetic association software)

EthoAnalysis

Project Input data Data filter Data selection Behaviour statistics Analysis Output

Output

- F3R
10/31/2018 10:54:25 AM
- Output Frontiers paper revised
10/6/2020 8:43:07 PM
- Output Frontiers paper revised
10/8/2020 8:48:47 PM
- Select all/none Delete selected

Contents

- › Average halting duration per zone per short/medium/long
- › Average movement distance per zone
- › Average movement distance per zone per hour
- › Average movement duration per zone
- › Average movement duration per zone per hour
- › Average movement duration per zone per short/medium/long
- › Average velocity per zone
- › Average velocity per zone per hour
- › Estimated distance moved per zone
- › Estimated distance moved per zone per hour
- › Estimated duration halting per zone
- › Estimated duration halting per zone per hour
- › Estimated duration halting per zone per short/medium/long
- › Estimated duration moving per zone
- › Estimated duration moving per zone per hour
- › Estimated duration moving per zone per short/medium/long
- › Estimated duration moving per zone per slow/medium/fast
- › Halt frequency per zone
- › Halt frequency per zone per hour
- › Halt frequency per zone per short/medium/long
- › Movement frequency per zone
- › Movement frequency per zone per hour

Report Options

Estimated duration halting per zone per short/medium/long

Selected zones	Zone 1, Zone 2
Event duration categories	duration < 2, 2 <= duration < 10, duration >= 10
Data transformation	Natural logarithm
Analysis	Zone difference analysis

Model result estimated duration halting per zone per short/medium/long

Results difference tests Zone 1 - Zone 2: p values and 95% confidence intervals of the difference on the transformed scale for each statistic.

Behaviour statistic	Cur3-RMX180	Remark
Estimated duration halting duration < 2 (diff. Zone 1 - Zone 2)	p=8.08E-09**** [-1.46, -0.718]	CR
Estimated duration halting 2 <= duration < 10 (diff. Zone 1 - Zone 2)	p=9.97E-12**** [-1.77, -0.977]	
Estimated duration halting duration >= 10 (diff. Zone 1 - Zone 2)	p=3.15E-06**** [-1.47, -0.601]	

CR = Check residuals

EthoAnalysis

Project Input data Data filter Data selection Behaviour statistics Analysis Output

Output

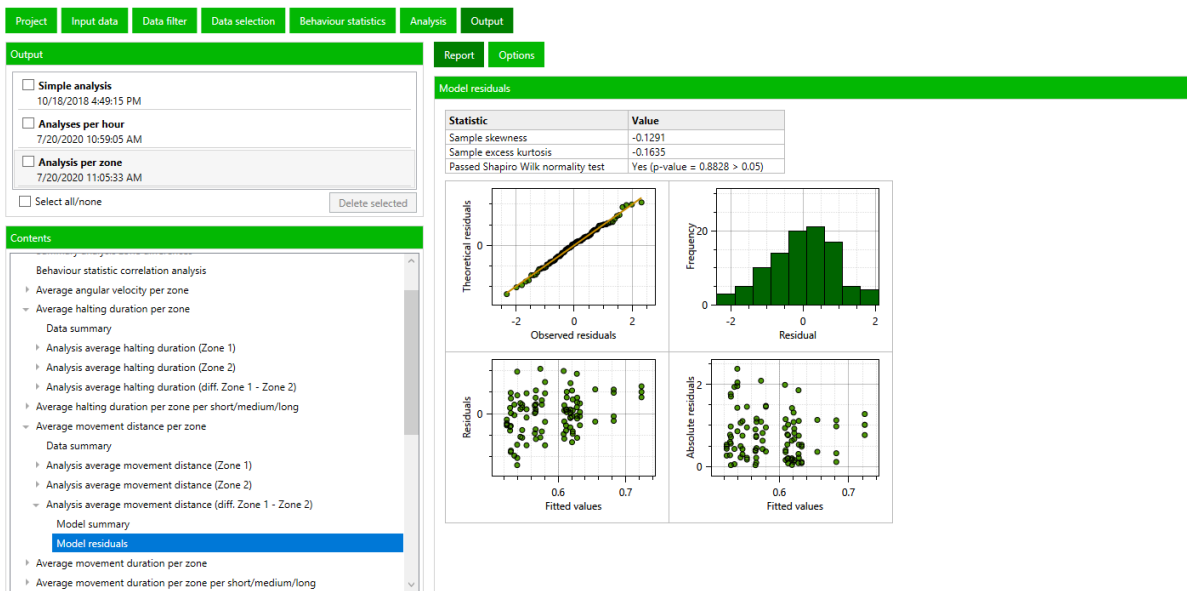
- Simple analysis
10/18/2018 4:49:15 PM
- Analyses per hour
7/20/2020 10:59:05 AM
- Analysis per zone
7/20/2020 11:05:33 AM
- Select all/none Delete selected

Contents

- › Assay info
- › Analysis results
 - Summary analysis zone differences
 - Behaviour statistic correlation analysis
 - › Average angular velocity per zone
 - › Average halting duration per zone
 - Data summary
 - › Analysis average halting duration (Zone 1)
 - › Analysis average halting duration (Zone 2)
 - › Analysis average halting duration (diff. Zone 1 - Zo
 - › Average movement distance per zone
 - Data summary
 - › Analysis average movement distance (Zone 1)
 - › Analysis average movement distance (Zone 2)
 - › Analysis average movement distance (diff. Zone 1 -

Behaviour statistic correlation analysis

Simple correlation analysis of all selected variables with each other.



The automatically generated output report contains extensive information on the fit of the applied statistical model to the results.

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