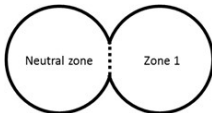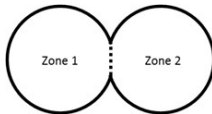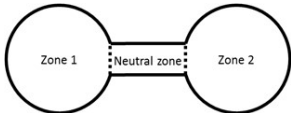# Noldus

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.
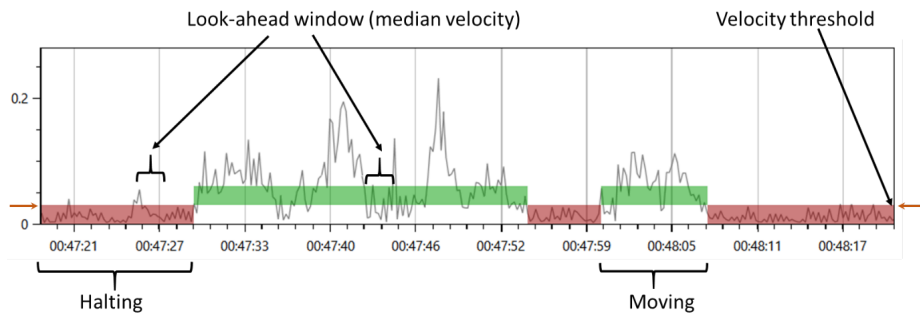
ARENA DESIGNS

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	Overal 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.

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.

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.

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).

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.

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)

Simple correlation analysis of all selected variables with each other.

EthoAnalysis

Project | Input data | Data filter | Data selection | Behaviour statistics | Analysis | Output
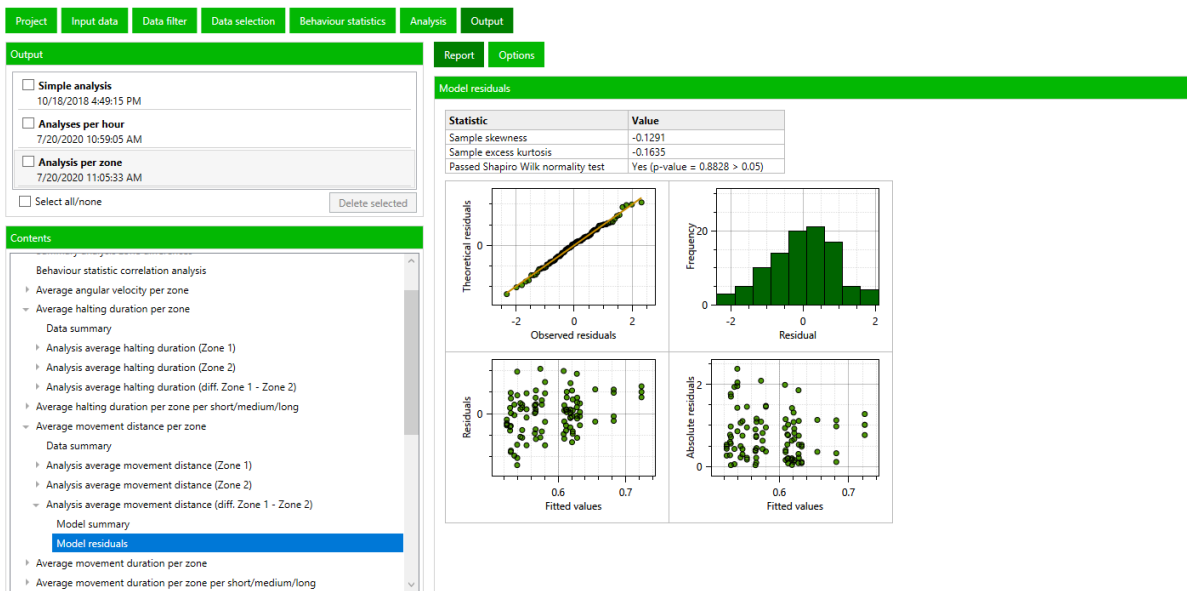
Output

Report | Options

- [ ] **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

Model residuals

Statistic	Value
Sample skewness	-0.1291
Sample excess kurtosis	-0.1635
Passed Shapiro Wilk normality test	Yes (p-value = 0.8828 > 0.05)

Contents

- 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 - Zone 2)
  - ▸ Average halting duration per zone per short/medium/long
- ▾ 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 - Zone 2)
    - Model summary
    - **Model residuals**
- ▸ Average movement duration per zone
- ▸ Average movement duration per zone per short/medium/long

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

We are also represented by a worldwide network of distributors and regional offices. Visit our website for contact information.

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