Enter the matrix: How to analyze the structure of behavior

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Several methods are available for analyzing different aspects of behavioral transition matrices, but a comprehensive framework for their use is lacking. We analyzed parasitoid foraging behavior in environments with different plant species compositions. The resulting complex data sets were analyzed using the following stepwise procedure. We detected abrupt changes in the event log files of parasitoids, using a maximum likelihood method. This served as a criterion for splitting the event log files into two parts. For both parts, Mantel's test was used to detect differences between first-order transition matrices, whereas an iterative proportional fitting method was used to find behavioral flows that deviated from random transitions. In addition, hidden repetitive sequences were detected in the transition matrices on the basis of their relative timing, using Theme. We discuss the results for the example from a biological context and the comprehensive use of the different methods. We stress the importance of such a combined stepwise analysis for detecting differences in some parts of event log files.

Behavioral data show great variation at different levels. When data on experimental animals have been collected in different environments, an important question is, in what parts of the displayed behavior are the differences most pronounced? In the literature, several methods are available for analyzing and comparing the structure of behavioral flows in complex data sets (De Vries, Netto, & Hanegraaf, 1993; Goodman, 1968; Haccou & Meelis, 1992; Magnusson, 2000). Some methods use event log files (Haccou & Meelis, 1992; Magnusson, 2000) as the basis of the analysis, whereas others use a summary of event log files—for example, the first-order behavioral transition matrices (De Vries et al., 1993; Goodman, 1968). In these matrices, event log files are summarized as the total frequencies at which a preceding behavioral element is followed by one of the other behavioral elements that have been distinguished. All these analytical methods detect and compare different properties of the behavioral data sets.

Here, we approach the problem of analyzing event log files and the associated transition matrices by using and comparing the above-mentioned analytical tools. These tools are complementary and, thus, give answers to questions at different levels. The breakpoint analysis is used to detect one breakpoint, an abrupt change over time, in an event log file (Haccou & Meelis, 1992). The correlation *between* behavioral transition matrices is investigated with MatMan 1.1 (De Vries et al., 1993; Noldus Information Technology, 2003a). The proportional fitting method of Goodman (1968) is used to detect differences in the suite of behaviors displayed by the animal in varying environments. This method is a *within*-matrix method. The higher order transitions based on the relative timing of different acts are studied with Theme 5.0 (Patternvision Ltd. & Noldus Information Technology, 2005).

To complete their development, the larvae of parasitoids feed on the bodies of other arthropods, their so-called *hosts*, eventually and inevitably killing them (Godfray, 1994). A single host is required for a parasitoid to finish its development. Because of their lifestyle, parasitoids are important biological control agents in crop production systems. The efficiency in finding hosts can be affected by the structural and infochemical complexity of the plants on which they search for hosts.

In this study, we analyzed whether the host-searching behavior of a parasitoid insect is influenced by changes in the plant species composition of the vegetation wherein they search. We carried out an analysis on the behavioral data set of Gols, Bukovinszky, et al. (2005). In a flight chamber experiment, the target plant—that is, the plant

containing the hosts—was surrounded by plants of the same species or by a combination of this plant species and another plant species, the so-called *companion* plant species. This companion plant species was either a close or a distant relative of the target plant species. We hypothesized (1) that a breakpoint in behavior should coincide with the first landing on the target plant and (2) that the companion plant species would have a different effect on the parasitoid's behavior until the first host was found.

Material and Methods

Experimental setup. The details of the rearing and the experiment can be found in Gols et al. (2005). The basic setup consisted of 9 Brussels sprout plants (three rows of 3 plants; see the circles in Figures 1A and 1B). The plant in the center is the target plant, with 15 host larvae: caterpillars of the diamond back moth, *Plutella xylostella* (L.). This is the low-density monotypic setup (A). In between these 9 plants, 12 Brussels sprout, barley, or mustard plants were placed in two different configurations (mixed or row; see Figures 1A and 1B). These different situations will be called (B) the high-density monotypic setup (21 Brussels sprout plants), (C) the mixed mustard setup, (D) the row mustard setup, (E) the mixed barley setup, and (F) the row barley setup. At the start of an observation, a vial with the female parasitoid of the species *Diadegma semiclausum* (Hellén) was placed in a release station, a glass cylinder (30 cm long, diameter of 15 cm) placed on

Figure 1. The experimental setup: (A) mixed and (B) row configurations. The circles represent Brussels sprout plants, and the squares companion plants. The Brussels sprout plant in the center is the target plant—that is, the only plant with hosts.

a 10-cm-high socket, which was placed at one end of the table at a distance of about 90 cm from the target plant. For each of the six defined experimental setups, at least 25 trials were performed.

Recorded behavior. The behaviors or acts distinguished during continuous observations were (1) walking, in which the parasitoid walked over a leaf surface; (2) grooming, in which the parasitoid cleaned itself by grooming or scraping its body parts while stationary; (3) standing still, in which the parasitoid was motionless; (4) flying, in which the parasitoid was airborne; and (5) ovipositing, in which the parasitoid inserted its ovipositor into the host. In addition, the place where the behavior took place was denoted. After the fifth oviposition, the observation stopped. We used a handheld computer (Psion Workabout) with The Observer 3.0 (Noldus Information Technology, 1993) to record the foraging behavior of the parasitoids in the setup; further data handling was carried out by The Observer 5.0 (Noldus Information Technology, 2003b).

In the sequel, we will use event log file for the list of recorded events or acts, from the start to the end of the observation. Each line in such a file is a behavioral record.

Detection of a breakpoint. During an observation period, the behavior of animals can show systematic changes. Sometimes, this is referred to as time *inhomogeneity*. For instance, changes in the duration of a behavior or the appearance or disappearance of a certain act can happen. Here, we were interested in whether the characteristics of sequences of acts changed abruptly during an observation recorded in an event log file, because we hypothesized that the behavior of the parasitoid before and after the first landing on the target plant would be different and we wanted to compare only the parts of the event log files with a time-homogeneous behavioral structure. Each event log file for the three different plant combinations was first analyzed with a test, in order to detect one breakpoint (Haccou & Meelis, 1992), and subsequently, the occurrence of the breakpoint was plotted against the occurrence of the first landing. The test used is called the *maximum likelihood estimate* of a breakpoint in a transition matrix. On the basis of the event log file for each parasitoid, the following log-likelihood is defined:

$$L = \sum_{i=1}^{5} \left(\sum_{\substack{j=1; \\ j \neq i}}^{5} n_{ij} \log n_{ij} \right) - \sum_{i=1}^{5} n_i \log n_i, \qquad (1)$$

with n_i being the number of occurrences of Act i ($i = 1, 2, 3, 4, 5$), as defined above. The number of transitions from Act i to Act j is n_{ij}. The event log file with a total of N acts is split into the first T acts and the last $N - T$ acts. For both these parts of the record and the total event log file, $L(T)$, $L(N - T)$, and $L(N)$ have been computed with Formula 1. By definition, the breakpoint occurs at the value for which the likelihood ratio test statistic $\Lambda(T)$ is maximized:

$$\Lambda(T) = 2\left[L(T) + L(N - T) - L(N) \right]. \qquad (2)$$

Analysis with MatMan. After having determined the breakpoint in each event log file, we constructed two
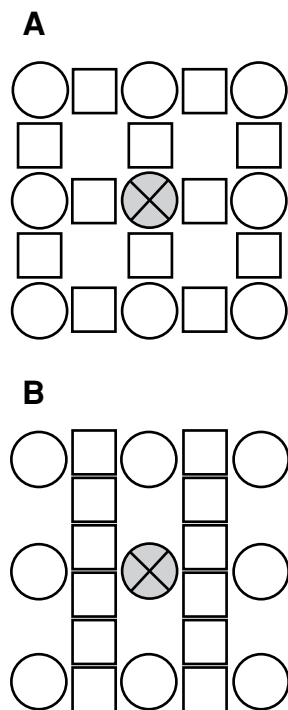
different first-order behavioral transition matrices (one before and one after the first landing; see the Results section) for each event log file within the spreadsheet program Excel. For the analysis, we assumed that the behavioral sequences manifested by the individual parasitoids did not differ significantly within a given treatment, and the data were pooled for all the individuals in that treatment. Because the behavioral transition matrices were based on different total numbers of transitions, the matrices could be compared only after accounting for these different numbers. This is called *normalization*. The pooled first-order behavioral transition matrices were first normalized with MatMan 1.1 (Freeman, Freeman, & Romney, 1992; van Dierendonck, De Vries, & Schilder, 1995) and subsequently compared with each other with the same program. The pairwise correlations between every possible combination of two matrices from Setups A, B, C, D, E, and F were calculated using Mantel's Z and Pearson's r tests. The significance of the correlations was based on 2,000 permutations in order to asses how extreme the actual Mantel's Z value was, relative to the Z values generated under the null hypothesis of no association between the compared matrices (Dietz, 1983).

Proportional fitting. At this stage, we know which matrices are highly correlated, because we have performed an analysis for correlation between matrices. However, within a matrix, the balance between the occurrences of behavioral elements can also differ. This kind of analysis calls for a within-matrix approach.

In each transition matrix, the values on the diagonal are logical zeros, since a particular behavior cannot logically follow itself in such a record. The iterative proportional fitting method of Goodman (1968) is used to find the expected values for the nonzero matrix elements. A log-likelihood ratio test (G test) is used to evaluate whether the overall matrix differs significantly from a matrix with random transitions. Throughout the procedure, Yates's correction for continuity is applied. If the deviations in the overall matrix table are significant, significant transitions can be found by collapsing the full matrix table into a 2 × 2 matrix around each transition. The significance of these individual tests is adjusted to a table-wide level of 5% with the sequential Bonferroni method (Rice, 1989). The goal of this analysis is to provide a clear picture of how the observed suite of behaviors differs when parasitoids forage in different environments.

This method identifies which transitions occur more frequently or less frequently than they would by chance alone, conditional on the observed column and row totals. To gain insight into how the behavior of searching parasitoids is organized, behavioral kinetograms (Field & Keller, 1993; Wang & Keller, 2002) are constructed (see Figure 4). This allowed a visualization of the differences in the behavioral sequences between parasitoids foraging in different environments.

Analysis with Theme. Thus far, only a breakpoint has been detected in each event log file, and first-order transitions from the original event log files have been explored. The time structure of the data has been ignored during the analysis of the first-order transition matrices. Within the event log files, however, repeating structures and higher order transitions might occur frequently. Therefore, we used Theme Version 5.0 (PatternVision Ltd & Noldus Information Technology, 2005) to detect so-called T-patterns (Magnusson, 2000). These are sequences of two or more acts that are found on the basis of their relative timing in an event log file that can be interrupted by other events that do not belong to the pattern and that occur repeatedly. Theme performs an intensive structural analysis of the behavioral data, and it takes into consideration not only the order of events, their relative and real timing, but also the hierarchical organization of the events. Among the other variables that Theme detects in an event log file are (1) the number of different patterns, (2) the total number of pattern occurrences, and (3) the mean number of behavioral elements in these patterns. We analyzed these results from Theme with nonparametric tests: the Kruskal–Wallis ANOVA, followed by pairwise comparisons with the Mann–Whitney U test in SPSS 11.0. Because we performed multiple tests on the same data, the Bonferroni correction was used (Sokal & Rohlf, 1995).

Results

Detection of a breakpoint. The maximum likelihood estimate of a breakpoint in all the event log files is calculated, and the number of behavioral acts that preceded the detected breakpoint is denoted. All these act numbers are plotted against the act numbers of the first landing on the target plant for all the setups.

In all cases (not shown), the regression line with intercept set to zero explained a large part of the variation ($p < .001$). The r^2s for these regression lines in Setups A, B, C, D, E, and F were .35, .42, .80, .96, .53, and .62, respectively. Figure 2 shows the results for the mustard (C and D) and barley (E and F) setups, pooled over the mixed and row configurations because of similarities (see also the description of the Matman analysis, below). The plot for the monotypic setups (A and B) is not given but looks similar to that in Figure 2B.

It should be noted that the method for detecting a breakpoint in a event log file by maximization of $\Lambda(T)$ (Formula 2) is not very good at detecting these points near the beginning or the end of an event log file—that is, when the total number of acts in one part of the likelihood is small (Haccou & Meelis, 1992). In the barley (E and F) and high-density monotypic (B) setups, the first landing on the target plant was near the beginning of the event log file.

On the basis of the high correlation between the act numbers of the first landing and the detected breakpoint, we continued our analysis with separate first-order behavioral transition matrices before and after the first landing.

Analysis with MatMan. For the analysis with MatMan, we thus started with a total of 12 first-order transition matrices. When all 6 normalized matrices for the event log files after the landing on the target plant were compared, the null hypothesis of no association between the matrices was never rejected when row and mixed configurations,
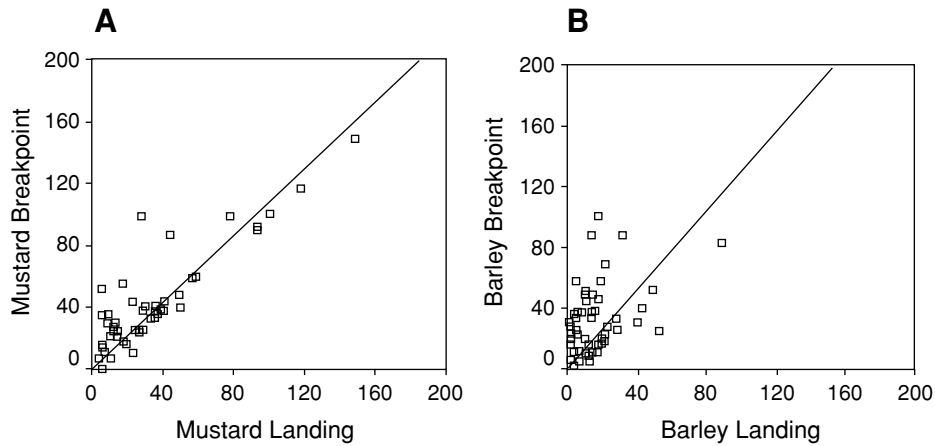
Figure 2. The act number of the first landing on the target plant is plotted against the breakpoint detected with the maximum likelihood method. The regression lines and points are given for (A) the combined C and D setups of the mustard ($r^2 = .89$) and (B) the combined E and F setups of the barley ($r^2 = .42$).

low- and high-density monotypic setups, or the species of the companion plants were compared in pairs. The Pearson correlation was .81 or higher at a significance level for the permutation test of $p = .014$ or lower.

For the parts of the event log files before the first landing on the target plant, we determined the correlation between the high- and the low-density monotypic setups and between the mixed and row configurations for the two companion plant species. Figure 3 shows that the three squares with a white background located around the diagonal indicate that the alternative hypothesis of association is accepted. This suggests that the mixed and row configurations do not differ. In the off-diagonal square matrices with gray shades, the null hypothesis of no association could not be rejected in all cases, suggesting an effect of the plant species in the environment on the first-order behavioral transitions.

This meant that plant species composition influenced the first-order behavioral transitions, where configuration did not. This allowed us to pool all the individuals from the different configurations and compare the transition matrices between different plant species. After pooling these first parts of individual event log files into three classes—(AB), (CD), and (EF)—representing the species composition, the resulting transition matrices for the monotypic (AB) and mustard (CD) setups were correlated significantly (Pearson's $r = .92$, $p < .001$). Between the barley (EF) setup and the other two setups, no association could be shown [for (AB) and (CD), p values of .133 and .086, respectively]. Thus, the behavior of the parasitoids is similar in the monotypic setups (AB) and the composition with mustard as the companion plant (CD), but different when barley is used as the companion plant species (EF).

Proportional fitting. We used the iterative proportional fitting method of Goodman (1968) to find the expected values for the nonzero matrix elements. The observed behavioral transitions within the three pooled

setups were significantly different from the expected transitions (G test; $p < .05$). Thus, the females did not display different behavioral elements by chance only. When the transitions were tested around single behaviors, certain behavioral transitions significantly deviated in a positive or negative way with respect to the expected number of transitions. The structure of the transitions between the behavioral elements is presented in the kinetograms (Figures 4A, 4B, and 4C), where black (missing) arrows represent behavioral elements that occurred more (less) often than would be expected by chance. The white arrows show transitions that were not significantly different from the expected values. The size of the circles is proportional to the overall frequency of the behavioral elements. The thickness of an arrow is proportional to the likelihood of occurrence of each behavioral transition. The numbers on the arrows are percentages of transitions to succeeding behaviors.

In the monotypic setup (AB), flight behavior was often followed by walking or, on a few occasions, by grooming (Figure 4A). Walking was more than proportion-

	(A)	(B)	(C)	(D)	(E)	(F)
(A)	–	.90	**.62**	.97	**.81**	**.76**
(B)	*	–	.74	.86	**.61**	**.56**
(C)	**.08**	.04	–	.68	**.35**	**.63**
(D)	.04	*	.04	–	.89	**.85**
(E)	*	.12	**.27**	.04	–	.81
(F)	**.11**	**.17**	**.09**	**.05**	*	–

Figure 3. The upper triangular matrix contains the estimated values of Pearson's r, and the lower triangular matrix the significance level. An asterisk is given for a significance level of $p < .001$. The codes for the setups (A etc.) are explained in the main text.
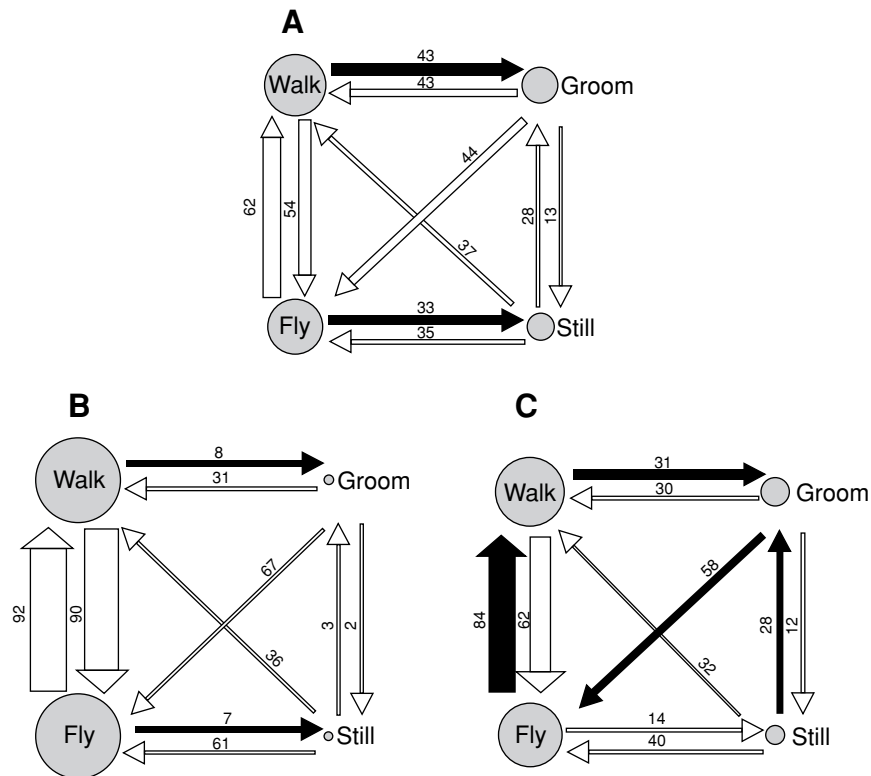
Figure 4. Behavioral kinetograms of *D. semiclausum* females before the target plant was found. They were searching in (A) monotypic, (B) mustard, or (C) barley setups. The size of the circles is proportional to the overall frequency of the act. The thickness of the arrows is proportional to the likelihood of occurrence of each behavioral transition.

ally followed by grooming. The behavior of the females searching in the mustard setup (CD) was similar to that in the monotypic setups (AB): The same transitions were significantly absent or present (Figure 4B). In the barley setups (EF), the behavior of the females was unlike that in the monotypic (AB) and in the mustard (CD) setups (Figure 4C). After landing on a plant, the females started walking. Walking and standing still often occurred before grooming, which was often followed by flight behavior.

Analysis with Theme. Theme was developed to find patterns that repeat themselves throughout an event log file, on the basis of their relative timing. With Theme, we found differences between (1) the number of different patterns, (2) the total number of occurrences of patterns in event log files, and (3) the mean number of events in the detected patterns. A Kruskal–Wallis ANOVA showed a significant effect of the experimental setup for all three characteristics ($p < .001$).

We analyzed the parts of the event log file before the first landing on the target plant. Figure 5A shows that the numbers of different patterns in the mustard (CD) and barley (EF) setups were not significantly different from each other, whereas they were different from the monotypic (AB) setup. The mean length of the patterns was not different between the barley (EF) and the monotypic (AB) setups, but this variable was significantly higher in

the mustard (CD) setup (Figure 5B). It should be noted that sometimes, no patterns could be detected because the parasitoid landed after some acts on the target plant. These occasions are represented by patterns of zero length in Figure 5B. This happened in (AB), (CD), and (EF) in 54%, 4%, and 34% of the event log files, respectively. The total number of occurrences in the mustard (CD) setup (not shown) is higher than that in the barley (EF) setup ($p = .001$) or the monotypic (AB) setup ($p < .001$). Event log files from the barley (EF) setup contained significantly more patterns than did the monotypic (AB) setup ($p = .003$). Parasitoids in the monotypic stands needed less landing on the nontarget plants, and landing on the target plant often followed flight behavior. Parasitoids in the mustard and barley setups often landed on the noninfested Brussels sprout or companion plants, and walking or grooming more frequently occurred until the target plant was found. In summary, Theme reveals that the behavior of the parasitoid *D. semiclausum* is simpler in a monotypic environment than in environments containing mustard or barley plants.

Conclusion and Discussion

The analysis of event log files as performed in this study uncovers a lot of information that was hidden in the behavioral data.
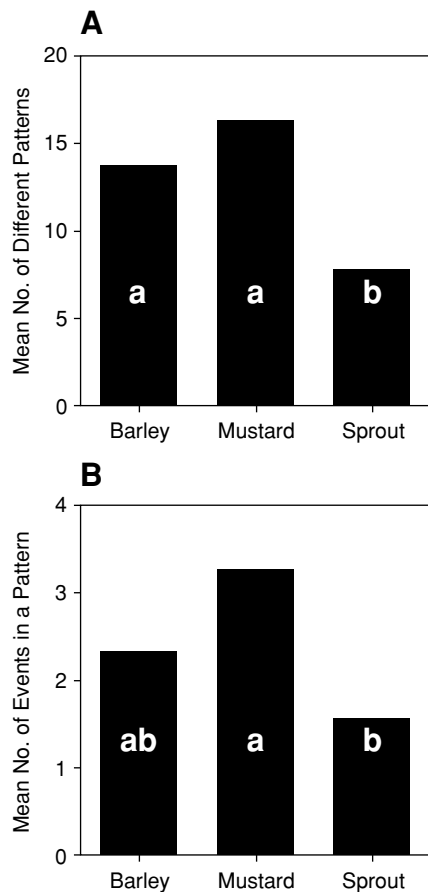
A

B

Figure 5. Bar plots for (A) the number of different patterns and (B) the mean number of events in a pattern, as detected by Theme. Bars with different letters differ significantly ($3p < .05$), according to a pairwise comparison with the Mann–Whitney U test with Bonferroni correction.

It is known that the method for detecting a breakpoint in an event log file by maximization of $\Lambda(T)$ is less suitable for detecting such points near the beginning or the end of an event log file (Haccou & Meelis, 1992). Our data supported this finding: The first landing on the target plant in the mustard setup occurred after more behaviors and, thus, correlated well with the breakpoint. In the other two setups, the majority of the first landings on the target plant occurred right near the beginning of the event log file (e.g., Figure 2B), and the test could not always locate this as a breakpoint.

We used the results of the analysis with MatMan only for pooling our event log files for acts occurring before the first landing in low- and high-density monotypic setups and for pooling the mixed and row configurations for both the barley and the mustard setups. The behavioral transition matrix before the first landing on the target plant in the barley setup was different from those for both of the other setups. This may be explained by the fact that both mustard and Brussels sprout plants belong to the same plant family. Both of these closely related plants can be

host plants for the herbivore *P. xylostella* and have different attractivity for the parasitoids (Bukovinszky, Gols, Posthumus, Vet, & van Lenteren, 2005). However, barley is from an unrelated plant family and never contains caterpillars of *P. xylostella*.

The behavioral kinetograms constructed after performing Goodman's (1968) proportional fitting analysis give support to the fact that the parasitoid is searching differently when potential host plants (Brussels sprouts) are interspersed with nonhost plants (barley). Detected treatment differences in within- and between-matrices comparisons were similar, showing complementary results with MatMan and the proportional fitting method.

The analysis with Theme reveals mostly details about the different structural characteristics of parasitoid behavior in the mustard setup, when compared with the other setups, which is probably the consequence of combining two related host plants with different attractivity for the parasitoids (Bukovinszky et al., 2005).

With these different analyses, we are able to compose an overall picture of the behavioral elements displayed in the different environments. When a complete quantitative description with the timing of the behavioral elements is wanted, transition rates between the behavioral elements should also be estimated.

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