

## Drosophila m. - where normal males tend to be female, and the supermachos are fruitless

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**Abstract**—The principles according to which genes are expressed in behavior are still unknown, but of great interest for neurogenetics. We are interested in how genetic information is translated into behavior. To study this, we investigate the courtship behavior of the fruit fly (*Drosophila*). We use nonlinear methods to extract salient behavior from observed courtship activity between males, females, and a fruitless male mutant. As a novelty, we describe behavior in terms of repeated periodic orbits in a chaotic system, an approach that goes beyond previous approaches. Our findings are that the different behaviors of *Drosophila* are characterized by unique distributions of orbits. Comparing them shows that courtship behavior is not a mere expression of the (sex) genes, but is contextually adaptive. In particular, in the presence of normal males, mutant males express strong male courtship behavior, whereas the normal males shift to expressing female-like behavior. Obviously, genetics provides the fly with the blueprint for situation-adapted behavior, which fills in the gap between invariably genetically imprinted, and learned, behaviors.

### 1. Methods and materials: Males, females, male mutants and observation

The courtship behavior of the *Drosophila* fruit fly is an interesting object for the study of the expression of genes into behavior [1-4]. This is because the *Drosophila* is easily bred, kept and observed, and its genome is completely sequenced [5-6]. Moreover, large varieties of mutants of *Drosophila* are easily available, which allows the easy collection of behavioral data from animals with distinctions in their gene sets. In our study, female normal *Drosophila* in the virgin, mature, and mated, states were brought together with normal males and with fruitless mutant males in an observation chamber. The latter group was only made to face mature females or normal males. The behaviors of the protagonists during courtship were visually recorded. From the recordings, the behaviors were dissected into actions, these were read out and encoded using the transcription shown in Fig. 1. This resulted in ten possible combinations of the form (recorded animal, partner). For each of these combinations we had six files of observations of about equal length, each from a different individual.

Drosophila actions

1	AbdoBend
2	AbdoWist
3	Attem_cop
4	Circling
5	Copulation
6	Decamp
7	Fencing
8	Following
9	Grooming forelegs
10	Grooming hindlegs
11	Headpos
12	Kick hindlegs
13	Licking
14	Orientation
15	OvipExt
16	Run
17	Standing
18	Still
19	Tapping
20	Walk left
21	Walk right
22	WingExt left
23	WingExt right
24	WingFlicks left
25	WingFlicks right
26	WingFlutter
27	WingSpread
28	WingWave
29	Wingflicks (no specification left/right)
30	Grooming midlegs
31	Tapping forelegs
32	Kick midlegs
34	Walk
35	Kick
36	Wingflicks
37	Run right

Figure 1: Encoding scheme: different actions are encoded into numbers, some of which are sex-specific.

From the encoded observations in the form of a time series, our task was to extract, characterize and compare the different emergent behaviors.

### 2. Methods of describing behavior

To operationalize the definition of behavior in terms of mathematics is a challenge. One of the earliest approaches to characterize *Drosophila* courtship behavior used the courtship index [6] (the measure of the fraction of the whole time spent for courting during courtship experiments). This, however, presupposes that we already know what the relevant actions are during courtship behavior. Moreover, according to our opinion, this approach seems to fail to ask the fundamental question, that is: What do we expect to measure when observing *Drosophila* courtship

behavior? In our opinion, a reasonable working assumption is that the purpose of courtship behavior is to perform a kind of evaluation of potential mate, which should provide information about the health and genetic state of the potential partner.

As a straightforward consequence of this assumption, we may measure this information by calculating the Shannon entropy [7] of the different time series. The Shannon entropy is defined as

$$h_s = \sum_{i=1}^{n_s} p_i \log p_i, \quad (1)$$

where  $n_s$  is the number of symbols used and  $p_i$  denotes their probability. If the behaviors are similar, we expect to measure similar Shannon entropies. In Fig. 2, the Shannon entropies are listed for the different possible configurations contained in our experimental set-up. The numbers are obtained from averaging the results obtained for the five different files in each subgroup. Concatenation yields essentially the same result, as the size of the files is about equal. The variances among the files of the subgroups are negligible. The plot of the Shannon entropy appears to already give some hints on potential relations among the different behaviors.

An interpretation of these results could be the following:

- 1) Virgin females show a restricted richness of activity, whereas males show a rich repertoire of activity
- 2) Mature females show a richer activity, which is reduced with the males
- 3) In the fruitless/normal male combination, it appears that the fruitless plays the male part, whereas the normal males play the female part (as a consequence, in the graph the normal males are plotted as females, and the fruitless males as males)
- 4) Mated females show still a rich activity; also the activity of the males is rather rich.
- 5) Females show a reduced richness of activity towards fruitless males and vice versa.

In order to provide detailed information on the process, however, this measure is unsuitable. 1) The Shannon entropy depends strongly on the symbols used, which is mostly responsible for the difference between female and male behavior (male behavior is encoded by more symbols than female, see Fig. 1). Therefore, an unbiased comparison of the information contents is restricted to within one group (female, males, fruitless). The measure is treacherous in that it is highest for purely random, or uniformly distributed, symbols, which is not what we go for as a measure for behavior. 2) Identical Shannon entropies can be generated from different dominating symbols. To the symbols themselves, no importance at all is attached. The probabilities are the only relevant quantities. In spite of these shortcomings, in our case the Shannon entropies keep track of essential properties of the

behavior, as we shall see later. On its own, however, the Shannon entropy cannot serve as a sufficient analysis tool.

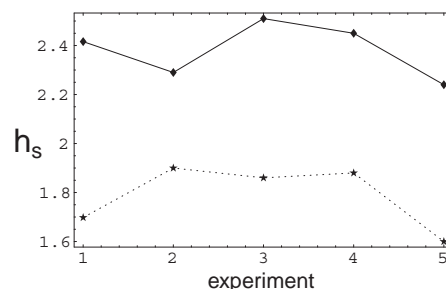


Figure 2: Shannon entropies. From left to right, involving bottom/top partners : 1) mature females/normal males (maturef/nm), 2) virgin females/normal males (virginf/nm), 3) normal males/fruitless males (nm/fruitlm), 4) mated females/normal males (matedf/nm), 5) fruitless males/mature females (fruitlm/maturef).

How then can these shortcomings be removed, the analysis refined and be put in a direct behavioral context? Are the obtained results just coincidences or do they express underlying behavioral structures? And, if the latter case holds, what are these structures? Whereas some results appear to have straightforward explanations, the two male minima of  $h_s$ , e.g., could be generated by two different, in a sense opposite, mechanisms. On one hand, fruitless males may not be too much interested in females because of the *latter's* lack of interest. On the other hand, normal males might show a reduced repertoire of actions vs. mature females only because the testing is non-critical, and they quickly head on towards copulation. In summary, the Shannon entropy certainly gives some clues on what is going on behind the scenes, but certainly cannot give definitive answers.

It appears that in order to make more significant progress, we should concentrate on the question of what kind of information could be conveyed during the courtship behavior. Obviously, *sequences* of actions might have a meaning similar to those of letters in the context of spoken or written language. In such a context, the graph method (see Fig. 3) has traditionally been considered as the appropriate tool of analysis, as it is able to keep track of the transition probabilities among the symbols. Usually, the transition in the next time step is considered, but also longer time horizons could be considered.

Graphs are easily obtained from the data and give a first overview on the dynamical behavior of the system. In dynamical systems theory, this method is suitable and popular in the context strong chaos, where the correlations between the symbols decay exponentially [7]. It is, however, already numerically inefficient in the case of marginal or intermittent chaos, and it is, again, certainly inappropriate

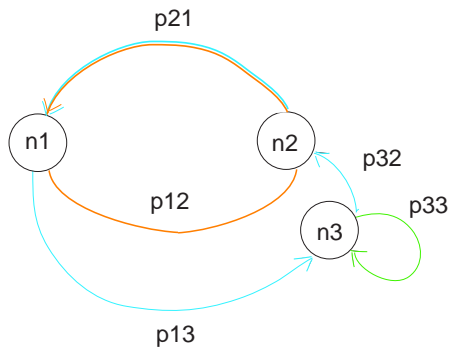


Figure 3: Example of a graph. Indicated are examples of closed orbits. These objects will be the material on which our analysis will be based. The transition probabilities  $p_{i,j}$  are indicated, as well as the nodes  $n_k$  (which correspond in our case to the symbols defined in Fig. 1).

for behavior. Here, we are interested in the orbits that have a particular meaning and are expected to pop out statistically, i.e., we are interested in the occurrence of long-term correlated chains of symbols. The problem with the graph method is that entirely different behaviors can be encoded by identical graphs. As an example, consider dancing the waltz: Statistically, equally many left/right leg movements occur; however, other than from the waltz, the origin of the data could equally well be in a skipping game. A refinement of the graph concept, the thermodynamic analysis of the system [7], is also not able to remedy this situation. The analysis is neither numerically feasible for such a system (the extraction of the eigenvalues of a general  $37 \times 37$  entry matrix is a nontrivial computational problem), nor would it remove the shortcomings previously mentioned.

### 3. Closed orbits of actions

In the present context, we propose to understand behavior as the aggregate of the responses or reactions made by an organism given a specific situation, with respect to this situation. To be able to generate a specific reaction, we believe that it is necessary to generate a well-defined sequential set of simpler actions of the kind as recorded in our time series. Hence, in order to identify behavior, e.g. waltzing, as the underlying behavior, we propose to classify behavior in terms of characteristic sequences of symbols. In the case of the drosophila's courtship behavior, we assume that to trigger final mating behavior, specific behaviors have to be repeated several times, possibly in order to test the partner's health, before the mating will take place. Moreover, these activities are embedded within other activities, which we assume not to interfere too much with the primary mating-inducing behaviors. This implies that we should search for *closed* periodic orbits of actions of unknown (though not too large) lengths. The justification for the required recur-

rence of the first symbol  $s_1$  after completing the cycle is to ensure the completion of the full cycle and that the cycle can be repeated immediately.

As the realization of this concept, we extracted closed orbits  $\{s_1, s_2, \dots, s_n, s_1\}$  of variable lengths from the time series and counted their occurrences. It is on these data that we base our further evaluations and interpretations. As a function of their length, the number of closed orbits can be expected to grow exponentially as  $N(n) \sim e^{n h_{top}}$ , where  $h_{top}$  is the topological entropy of the process. In our case, we went up to a length of 7 symbols. The latter has to be chosen from the context of the behavior and the available date size. We then construct a behavioral vector  $b_j$ , where the vector's length is equal to the number of all detected distinct closed orbits, and its entries equal the number of occurrences (for one orbit, the corresponding vector component has been assigned arbitrarily, but is then fixed). By normalization we obtain a probability vector  $p_j$  of potential behaviors that characterizes the occurrence of orbits in each experiment. In this way, one vector, e.g., characterizes the behavior of the male drosophila, when encountering a (female) virgin, and another when it encounters a mature female.

The different experiments result in distinct probability vectors. To compare these probability vectors emerges to be a difficult job. Only upon scrutiny examination we may believe to see differences in the behaviors, which, however, immediately pose the question of how to assess and quantify the significance of the deviations in the statistical sense. Truncation of low frequencies is questionable and does not solve the problem.

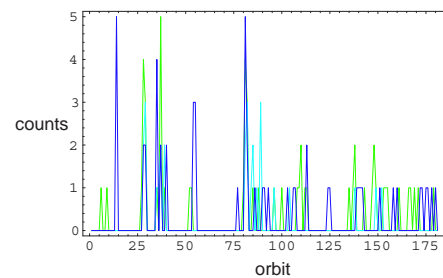


Figure 4: Histograms of observed closed orbits. Orbits are ordered from left (short) to right (long). Superimposed are behavior vectors  $b_j$  from the three stages of female sex life, vs. normal males. Assessment of statistical significance is difficult, which renders the distinction of the different behaviors virtually impossible.

### 4. Results, interpretation and discussion

Fortunately, in the correlation integral between the probability vectors we found an elegant means to evaluate the

overall similarity between the behaviors, that from our point of view also renders the statistical question simple. The correlation matrix

$$M_{cc} : (M_{cc})_{i,j} = \int p_i p_j \quad (2)$$

is able to distinguish the different behaviors encoded in the orbits. Where the result is highest/lowest, the most similar/dissimilar behaviors are measured. Note that this is only an approximate similarity, and that the measure is not necessarily transitive. As we shall see, the correlation method nevertheless not only easily works out the required information similarity/dissimilarity of behaviors of the different species in the different contexts, it also is able to extract additional facts that otherwise can hardly be seen.

Given our particular experimental setting, after extracting from all files the closed orbits up to length 7, we ended up with 10 probability vectors. From these, we evaluated the correlation matrix shown in Fig. 5.

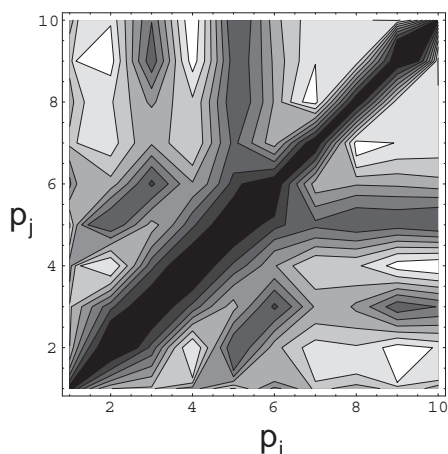


Figure 5: Correlation matrix of the 10 different probability vectors, where the correspondence is as follows (we use obvious abbreviations and indicate the observed protagonist by a capital letter). 1) matedf-nM, 2) matedF-nm, 3) maturef-nM, 4) matureF-nm, 5) virginf-nM, 6) virginF-nm, 7) fruitlM-maturef, 8) fruitlM-nm, 9) fruitlM-matureF, 10) fruitlM-nM. The self-correlation has been subtracted.

Using obvious abbreviations, the results are that the maximal correlations are measured for the combinations (4, 10) (matureF-nm, fruitlM-nM) and (7, 8) (fruitlM-maturef, fruitlM-nm), and (2, 9) (matedF-nm, fruitlM-matureF). Also high, but lesser correlations are found for (2, 4) (matedF-nm, maturef-nM), (2, 7) (matedF-nm, fruitlM-maturef), and (4, 9) (matureF-nm, fruitlM-matureF). The detailed inspection of the correlation integral leads us directly to the following **summary of results**:

1) matureF and matedF behave similarly

- 2) matureF vs. fruitless males behave as if they were mated
- 3) males behave vs. fruitless males as if they themselves were mature females
- 4) fruitless males behave vs. males as towards matureF
- 5) matureM and virginF are orthogonal
- 8) also virginM and virginF fall far apart
- 6) to a lesser degree also matureM, and matureF when together with fruitless males, fall far apart
- 7) virgins fall far apart from the fruitless cases

An explanation for these results could be from evolution. It might be evolutionary advantageous if the fruitless males are absorbed by males presuming to be females, be it to exhaust them, or to just screen out the latter for the good of other healthy males. The sterile fruitless males are taken out of the mating competition by means of other males, without the reduction of female reproductive effort, making the process more efficient. Classically, there is a division between genetically hardwired, and learned, behavior. By showing that even genetic information leaves the potential for situation-dependent behavior, our example fills in the gap between these two situations [8,9].

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