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Periodic orbit analysis demonstrates genetic constraints, variability, and switching in *Drosophila* courtship behavior

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We use symbolic dynamics to describe *Drosophila* courtship communication. We posit that behavior should be defined in terms of irreducible periodic orbits of fundamental acts. This leads to a first operational definition of behavior, which allows for a fine grained quantitative analysis of behavior. We obtain evidence that during *Drosophila* courtship, individual characteristics of the protagonists are exchanged (predominantly from the male to the female) and that males in the presence of fruitless males perform a behavioral switch from male to female behavior. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2918912]

Understanding animal courtship is of central importance for relating brain dynamics to behavior and to separate the latter into innate, environmentally determined, and learned components. A novel characterization of behavior based on periodic orbits of fundamental behavioral acts, enables us now to analyze courtship behavior in a fine-grained manner. The characterization captures all commonly observed facets of courtship among male, fruitless male, and female *Drosophila melanogaster* in different developmental stages, as well as the fact that normal males have a vast behavioral expression space, which complies with the idea that during courtship they convey individual characteristics to the female. We also corroborate that when males are courted by *fruitless* mutant males, they switch to the behavior profile of mature females. Thus normal males harbor a bisexual role that is context-dependent, which adds a novel aspect to the present discussion on the neuronal origins of courtship behavior. Our behavioral analysis framework can be applied to a large field of comparative behavioral studies, where it provides a bridge between displayed behaviors and their neurogenetical and neurophysiological origins.

I. INTRODUCTION

Courtship activities play a prominent role in sexual selection and reproductive decision making; how this is achieved, however, is hardly known. Rituals prepare the sexes towards mating, allow for recognition and help a female to choose a mate. When it comes to mating, females are generally thought of as being more selective than male animals, who produce sperm in greater quantities and therefore need not be so selective. Courtship behavior, in particular that of the fruit fly *Drosophila*, has long been the standard example of genetically hardwired behaviors. Normal male *Drosophila melanogaster* court females only. Using advanced genetic techniques applied to the *fru* gene, it is now possible to generate males that court only males or court both genders, and females that court males or court only females. This provides hard evidence that gene information in addition to determining how living beings are built also defines to a "considerable extent how they behave."[1,2]

Here, we investigate whether, despite this hardwiring, precopulation courtship could serve as a platform for exchanging information about a potential partner's suitability for passing on genes. Such an ability would imply behavioral variability of genetically normal animals, whereas *fruitless* mutants, for example, should be characterized by a completely changed courtship behavior, potentially not only in a reduced courtship fitness, but also in a lack of being able to variably express individuality by means of behavior.

For this investigation, the traditional tools are unsuitable. The courtship index, which is the fraction of the whole time spent by an animal for obvious courtship,[1,3,4] and the mating success, i.e., the fraction of successful versus total attempts of copulation, are unable to resolve more specific aspects of behavior. Precursors in our attempt to refine the description of courtship are behavioral transition graphs.[5–8] One would expect to see genetic variations be reflected in topological and metric modifications of these graphs,[5,6] as well as in changed salient individual behaviors. Unfortunately, the usual first-order Markov transition graph approach (by this we understand a directed graph equipped with the corresponding transition probabilities) allows only few behavioral states to be considered and no precise information about longer *successions* of states are conveyed. Although the better suited higher order models could be converted into first order, this quickly leads beyond tractability.

We have worked out an alternative way of characterizing behavior. Animal (and obviously also human) courtship is characterized by well-defined *sequences* of acts.[9] From this, we deduce that (variable length) sequences of acts could provide a powerful description of behavior,[10] comparable to the role of letters with respect to words in spoken language. More technically, we propose to "*characterize behavior in terms of their irreducible orbits of fundamental acts*" (undecomposable orbits of no further dissectable acts of behavior).

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As redundancy is an important element of biological information exchange, we expect that a potential reiteration of the orbit is a highlight of this characterization; hence, for the characterization of behavior, we concentrate on closed orbits. This idea is in close analogy to decomposing complex dynamical networks into strongly connected subnetworks.[11] On a more general mathematical level, courtship behavior can be seen as a prototype of complex symbolic dynamics. The description of behavior in terms of closed orbits is therefore also closely related to the dissection of complex chaotic motion into its periodic orbits.[12–14]

II. EXPERIMENTS

In our behavioral experiments, normal females in the immature, mature, and mated states were paired with normal males in an observation chamber (yielding a set of six distinct protagonist roles). In extension of these experiments, *fruitless*[15] mutant males were paired with either mature females or mature normal males (resulting in four more protagonist roles). The emergent courtship behavior has tactile, gustatory, olfactory, acoustic, and visual sensory dimensions,[3,16] with the olfactory and the visual components being most salient,[5,17–19] where the latter is easy to access, which is why our analysis will focus on that component. The sensory reduction is not as problematic as one may surmise as the multidimensional system behavior can, in principle, be faithfully reconstructed from the single-component measurement, by using embedding techniques (see, e.g., Ref. 20). Although our analysis is based on time series of one protagonist at a time, we will obtain also considerable insight into the interplay between partners. In order to eliminate ambiguities in the definition of the behavioral states, we used visual recordings of the almost neuronal temporal resolution of 30 frames per second. The starting points of every act were detected using frame-by-frame inspection, and indexed using the symbols of Table I. As a further increase of this temporal resolution provides no further information and because the acts are motorically independent, we refer to them as *fundamental*. For each possible experimental constellation, five trials of the experiment were performed, using different individuals. The individuals were not previously screened for whether they would court or not. In this way, out of 50 protagonists two were lost, from a fruitless/mature female experiment that produced no usable results.

TABLE I. Encoding scheme: 37 fundamental acts found in the time series are encoded into numbers, some of which are sex specific. For illustrations, see Ref. 17.

	Drosophila acts	Gender
1	Abdobend	Female
2	Abdotwist	Female
3	Attemptcop	Male
4	Circling	Male
5	Copulation	Male
6	Decamp	Male/female
7	Fencing	Male
8	Following	Male
9	Grooming forelegs	Male/female
10	Grooming hindlegs	Male/female
11	Headpos	Male/female
12	Kick hindlegs	Female
13	Licking	Male
14	Orientation	Male
15	Ovipext	Female
16	Run	Male/female
17	Standing	Male/female
18	Still	Male/female
19	Tapping	Male
20	Walk left	Male/female
21	Walk right	Male/female
22	Wingext left	Male
23	Wingext right	Male
24	Wingflicks left	Male/female
25	Wingflicks right	Male/female
26	Wingflutter	Female
27	Wingspread	Male/female
28	Wingwave	Male
29	Wingflicks unspec.	Male/female
30	Grooming midlegs	Male/female
31	Tapping forelegs	Male
32	Kick midlegs	Female
33	Walk unspec.	Male/female
34	Kick unspec.	Male/female
35	Wingflap	Male/female
36	Run right	Male/female
37	Run left	Male/female

III. EVALUATED BEHAVIORAL SIMILARITY

Our definition of behavior leads directly to the following computational implementation: From the experimental data, we extract the set of irreducible closed orbits s_i, $i=1\cdots n$. This is achieved in the following way. Let $\mathbf{a}$ be a symbol file associated with a given protagonist. We proceed sequentially through the data file and compare the scanned symbol a_i with symbol a_{i+d} at distance d. If $a_i=a_{i+d}$, we have discovered a closed orbit of length d. In order to count irreducible orbits only, we start the search with $d=1$, which will give us the periodic orbits of length 1. In the next search, we increase the search horizon by one, up to a given maximal length l. Closed orbits that can be transformed by a rotation into a closed orbit already found, are identified (i.e., we consider equivalence classes). In order to arrive at a set of irreducible orbits, it is checked whether a newly discovered closed orbit can be obtained by a suitable composition of shorter detected orbits, in which case the orbit is excluded from the set. The set of irreducible closed orbits found in this way is unique, for a given protagonist as well as for the whole behavioral space, obtained by the union over all protagonists. For given maximal horizon l smaller than the length of the symbol series, not all data elements need to belong to a closed orbit. Our way of extracting periodic orbits is equivalent to the one used in chaotic orbits expansion,[12,13] but, in contrast to the latter, no attempt is made to also take combinations of fundamental orbits into account, which would be necessary for the classical orbit expansion. That is because for noisy systems with a small sampling base that we deal with here, the notion of adjacent orbits needs to be abandoned.

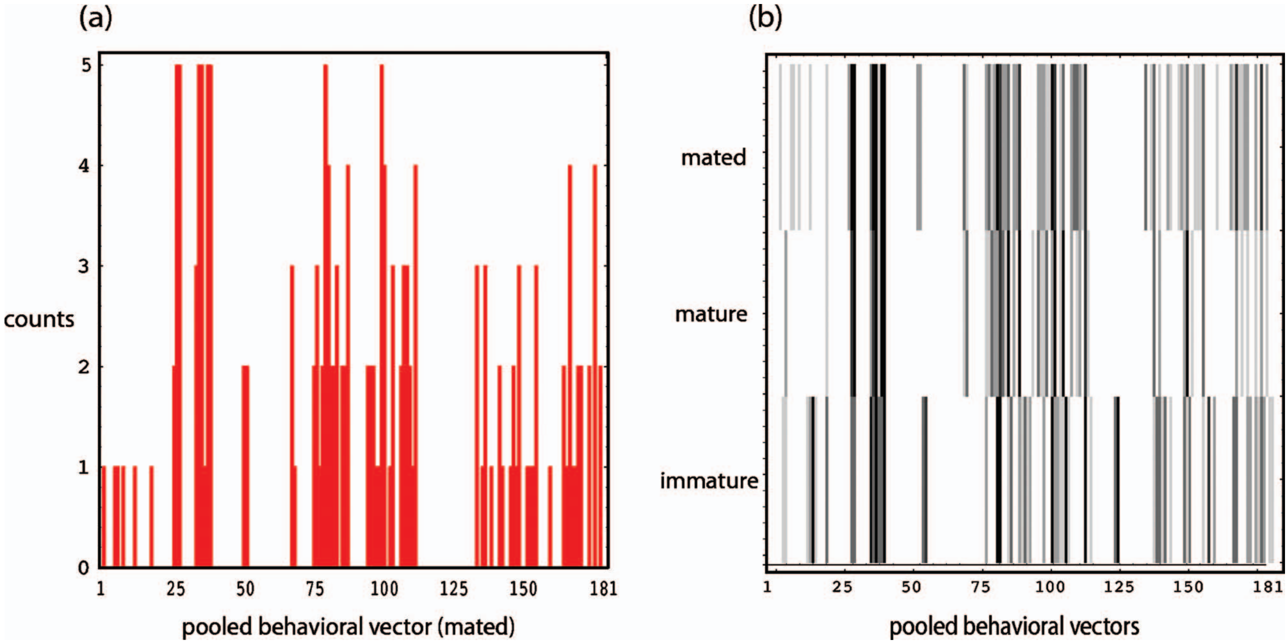

FIG. 1. (Color) Behavioral vectors, pooled over protagonists of the same experiment. Ordering of orbits is as in the template vector (see text): from short orbits (left) towards long orbits (right). Only occurrence/nonoccurrence of orbits was considered (maximal five entries, corresponding to five experimental protagonists). (a) Mated female in the experiment with normal males (histogram display). (b) Females in immature, mature, mated states. Counts are coded from white (0) to black (5 counts). Bars corresponding to particular vector components/orbits have equal widths.

As a function of their length l, the number of closed orbits can be expected to grow no more than $N(l) \sim e^{l\, h_{\text{top}}}$, where h_{top} is the topological entropy of the process.[20] Generally, l has to be chosen taking into account the size of the data and the behavioral context. For our data, we considered orbits up to a length of $l=7$ symbols, for which we obtained totally $n=181$ irreducible closed orbits. From the union of all detected irreducible closed orbits, a template vector of dimension n is then formed, where to each vector component a particular closed orbit is assigned; e.g., by starting with period-1 orbits for the lowest entries and proceeding towards higher indices for longer orbits. The template vector can be interpreted as defining *Drosophila's* behavioral space with respect to all of the experiments considered. For each protagonist j, a behavioral vector b_j can then be constructed, by filling the entries of the template vector with the numbers of occurrences of the corresponding orbits. How much importance is attached to the probability with which a particular orbit occurs (i.e., the metric information) can be monitored by weighting the behavioral vector component-wise by an exponent $\beta \in \mathbb{R}$. This results in going from the normalized vectors $\tilde{b}_j$, where the entries c_i are proportional to the probabilities of occurrence of the ith orbit, to the "generalized" vectors $\tilde{b}_j^{\beta}$, where the components are proportional to the probabilities of occurrence raised to the power of β:

$$\tilde{b}_j = \begin{pmatrix} c_1 \\ \vdots \\ \vdots \\ c_n \end{pmatrix} \rightarrow \tilde{b}_j^{\beta} = \begin{pmatrix} c_1(\beta) \\ \vdots \\ \vdots \\ c_n(\beta) \end{pmatrix}. \tag{1}$$

This method is motivated by statistical physics, where β' s role is that of an inverse temperature (e.g., Ref. 20). This procedure offers the possibility of changing one's focus from a situation where the multiply displayed orbits are most significant (and therefore must be conveyed with certitude), to a situation where the most important orbit is most quickly recognized and therefore does not require repetition at all.

A natural way of how to proceed is to use clustering algorithms in order to group the individual vectors, hoping that the obtained classes coincide with the experimental classes the protagonists originate from. Even when using the most elaborate clustering algorithms,[21] we, however, failed to achieve this goal. We suspect that the high variability of the behavioral vectors built upon the orbit occurrences may be the reason for this (see Fig. 1).

An alternative way of accessing the similarity between vectors is by taking their scalar products, after the vectors have been normalized. The scalar product projects upon the shared behavioral subspaces: The more similar the behavioral vectors are, the larger is therefore their scalar product $\langle \cdot , \cdot \rangle \in [0,1]$. Note, however, that our notion of similarity is only an approximate one that does not include transitivity: If behavior b_1 is similar to behavior b_2 and b_2 is similar to b_3, the behaviors b_1 and b_3 can be already quite dissimilar). In this way, we characterize the behavioral similarity between two protagonists by a one-parameter similarity family,

$$m_{i,j}^{\beta} := \langle \tilde{b}_i^{\beta}, \tilde{b}_j^{\beta} \rangle, \beta \in \mathbb{R}, \tag{2}$$

where $\langle \cdot,\cdot \rangle$ again denotes the scalar product. For $\beta=1$, the natural measure is obtained (i.e., the probabilities for observing a particular cycle are part of the information), whereas for $\beta=0$, this information is reduced to the topological aspect (occurrence or absence of a particular cycle[20]). For $\beta \geqslant 0$, large/small matrix entries indicate similar/dissimilar behaviors.

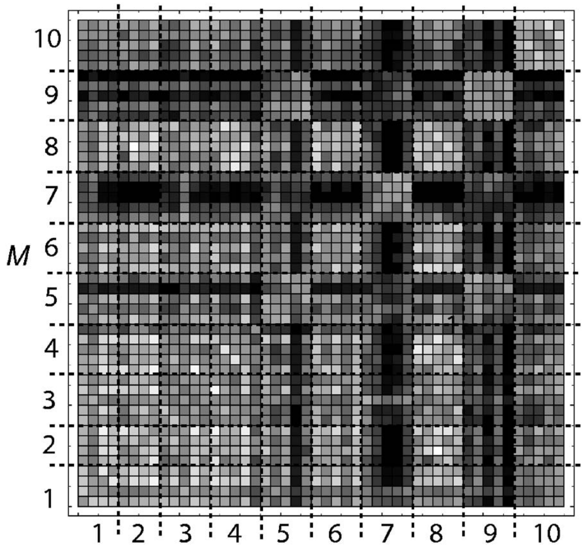

FIG. 2. Similarity matrix $\mathcal{M}(\beta=0)$ evaluated for all individuals involved (light shadings indicate highly similar behaviors; diagonal values were set to 0.5). The plot shows strongly individual normal male behavior, and lack of individual behavior in all experiments involving fruitless mutations. Emerging patterns strongly correlate with group boundaries indicated by thin lines. They demonstrate strong group coherence of individual behaviors. Dark subdiagonal group-squares indicate enhanced behavioral distance between male/female protagonists of the same experiment.

Bearing in mind that aberrant behavior of one single fly could completely dominate the average behavior, we will concentrate in this application on the topological characterization $\beta=0$, because of its robustness against such perturbations. For other applications, other choices, or the whole β-family, could be appropriate. Therefore, in our application, each protagonist j is described by a (below as yet unnormalized) vector

$$b_j = (\ldots,0, \ldots,1, \ldots,0, \ldots,1, \ldots)^T, \tag{3}$$

where a nonzero entry indicates that the irreducible orbit that corresponds in the template vector to this index, has been found in the protagonist's behavior (for consistency, the convention $0^0=0$ has to be adopted). If the behavioral vectors are then normalized, the similarity between two protagonists is mapped on values from the unit interval [0,1], where 1 corresponds to maximal similarity.

Once the individual protagonists are listed according to the experimental groups they belong to, a well-distinguishable class behavior emerges, on top of which widely differing individual behaviors can be identified (see Fig. 2). In Table II, the correspondence ("≙") between the matrix indices and the experiments is given.

It can be shown (see Appendix, Sec. 2) that pooling into the experimental classes is justified. The behavioral similarities among the ten different classes of behaviors is captured in a density plot of the pooled similarity matrix

$$\tilde{\mathcal{M}}(\beta)_{i,j} := \langle \tilde{b}_i^\beta, \tilde{b}_j^\beta \rangle, \tag{4}$$

where $\tilde{b}_j^\beta$, $\tilde{b}_i^\beta$, i, $j \in \{1, \ldots, 10\}$, are the pooled behavioral class vectors, obtained by vector addition. General observations from the similarity matrix $\tilde{\mathcal{M}}(\beta=0)$ displayed in Fig. 3 are (see, for example, Ref. 22) that male *Drosophila* behavior varies strongly with their partners (no peaks of similarity within the columns indexed by 5, 7, 9), highlighting that normal male behavior in the presence of mature and immature females (columns 7 and 9) stands out against all other behaviors. Females, in contrast, vary their behaviors to a much lesser extent. In particular, the behaviors of mature and mated females are quite similar, with that of the immature being only slightly different. The columns with the highest contrast among them are 7 and 8. They indicate the special role of the courtship between mature females and males, which is dominated by the variability of the male's and a certain indifference—until partner selection—by the females. Focusing more on details, *maximal similarities* emerge as follows. (4,2): normal males in the presence of fruitless males behave similarly to mature females in the presence of fruitless males (value: 0.90); (8,4): mature females in the presence of normal males behave similarly to normal males in the presence of fruitless males (value: 0.88); (3,1): fruitless males in the presence of normal males behave similarly to fruitless males in the presence of mature females (value: 0.87). This proves that normal males in contact with *fruitless* males perform a switch to female behavior, and that female *Drosophila* does not particularly discriminate between *fruit-*

TABLE II. Correspondence between indices and *Drosophila* behavioral vectors (see text).

1≙fruitless males in the presence of mature females
2≙mature females in the presence of fruitless males
3≙fruitless males in the presence of normal males
4≙normal males in the presence of fruitless males
5≙normal males in the presence of mated females
6≙mated females in the presence of normal males
7≙normal males in the presence of mature females
8≙mature females in the presence of normal males
9≙immature females in the presence of normal males
10≙immature females in the presence of normal males

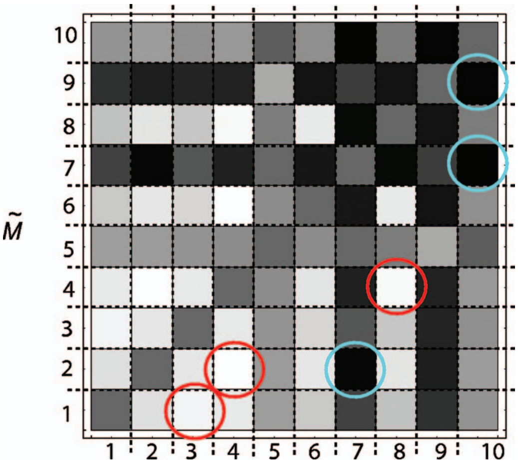

FIG. 3. (Color) Similarity matrix $\tilde{\mathcal{M}}(\beta=0)$ (density plot over $[0,\text{maxcorr}]=[0,1]$, diagonal elements set to 0.5). Lighter shading indicates higher similarities; the self-similarities were set to zero. Circles indicate similarity maxima (red) and minima (blue). For the meaning of the indices, see Table II.

less and normal males. The high similarities displayed at the lower left corner of $\tilde{\mathcal{M}}(\beta=0)$ (indices $[1,\ldots,4]$) express the lack of behavior adaptation of, or triggered by, the *fruitless* mutants.

The *lowest similarities* express the large behavioral distance between male and female, immature and mature, and normal and fruitless *Drosophila* behaviors. They are obtained for (10,7): immature females in the presence of normal males behave nearly orthogonally to normal males in the presence of mature females (value: 0.22); (10,9): immature females in the presence of normal males behave nearly orthogonally to normal males in the presence of immature females (value: 0.23); (7,2): normal males in the presence of mature females behave nearly orthogonally to mature females in the presence of *fruitless* males (value: 0.25).

IV. DISCUSSION

The result that normal males in the presence of *fruitless* males, display a behavior similar to that of females being courted, is of interest for several reasons. It implies that normal males show a context-triggered male-to-female behavioral switch. Consequently, the corresponding, simple-to-observe behavior of the Shannon entropies (see Appendix) indeed indicates a switch of behaviors, a statement that could not be made on the basis of the courtship or the mating index, or the Shannon entropy. Our analysis also shows that the *fruitless* males are incapable of the contextual switch exhibited by normal males. That this might hold generally, is corroborated by the fact that *fruitless* males also court each other.[23] Also in the behavior by females, this switch is absent. Our method objectively confirms earlier observations[22] that their behavior depends mainly on their reproductive status (immature/mature/mated). To underscore the complexity of behavior, *D. melanogaster* courtship behavior has several facets: A genetically hardwired one that endures for lifetime, but can be genetically manipulated.[24] In addition, there is learned behavior, generally lasting from a few hours to days.[19] These two facets involve no sex role switch. In contrast, the newly discovered behavioral phenomenon involves an actual switch, between sexual roles that endure as long as the triggering contexts are present. Our results suggest that (normal) male and female *Drosophila* harbor both sexual behavioral roles. While in females, the male role is inaccessible, in males this role is dominant and the female role is also accessible. Whether this is just a relic from evolution, or whether it plays an active evolutionary role, remains open to investigation. In the context of recent insight into the neurogenetic basis of behavior,[1,2,25] our finding's primary interpretation would be that sexual orientation in male *Drosophila* may be implemented in the form of a bistable neurodynamical system, possessing two major basins of attraction. By means of external contextual input, the male behavior is reversibly pushed towards male or female behavior, whereas the sensory input to the females, although their system is based essentially on the same neuronal circuitry[1] (see also Refs. 25 and 26), would not offer this possibility. This view, which challenges the picture of essentially structurally different male/female neuronal systems, would be in agreement with the major findings of Refs. 1, 25, and 26. Observing the neuronal correlates that correspond to the characteristic behavioral courtship elements, will be an efficient method for studying how courtship behavior is implemented on the neuronal circuitry level.

By providing a quantitative comparison of behavior, we have obtained a detailed insight into the similarity/dissimilarity of individual and class behaviors in *Drosophila* courtship. Our concept of behavioral similarity focuses on salient sequences of fundamental acts, rather than on long-time averages or on isolated acts. A detailed analysis incorporating surrogate data corroborates the significance of our results, in particular the large bandwidth underlying precopulation courtship communication. Our method is general enough to be applicable to a wide variety of comparative behavioral, or behavioral neurogenetics, studies that critically depend upon a sufficiently detailed quantitative analysis. Although these questions are here considered in the context of *Drosophila* courtship, insight into how this works could also be of interest to humans. Estimates by experts using different methodological approaches all seem to agree that even in ordinary communication among humans, a preponderant percentage is nonverbal and is largely genetically predetermined [where the estimates range from above 50% to as high as above 90% (see Ref. 27)].

APPENDIX: SHANNON ENTROPY AND METHOD DETAILS

1. Evaluated Shannon entropies

The evaluation of the Shannon entropy

$$h_s = -\sum_{i=1}^{n_s} p_i \log_{10} p_i, \tag{A1}$$

associated with the symbolic time series, corroborates the switch of normal males from male to female behavior in the presence of fruitless. In the formula, n_s is the number of symbols used, i indexes the fundamental acts (or their corresponding symbols), and p_i their probability of occurrence.[20] For similar behavior, we expect to measure similar values of h_s. The results shown in Fig. 4. confirm observations that are well-know for *Drosophila* male-female courtship:[22] Overall, male *Drosophila* (full line) use a richer repertoire than female (dotted line). Males also use a richer repertoire to court an already mated female (compare experiments 1 and 4). In the mature female/*fruitless* male experiment 5, a decreased activity is accompanied by a reduced repertoire by both protagonists. When normal males are paired with *fruitless* mutant males (experiment 3), from the richness of the response (this is basically what h_s encodes), it appears that the normal males adopt the female role, while the *fruitless* males express a very pure male role ("supermacho behavior"). For a different interpretation, a crossing of the lines would be obtained.

As a measure of behavior, the Shannon entropy, however, has two main deficits. Firstly, h_s depends strongly on the number of symbols used. The latter fact may be partially responsible for the overall difference between female and male behavior [male behavior is encoded by more symbols than female (see Table I)]. As a consequence, an unbiased

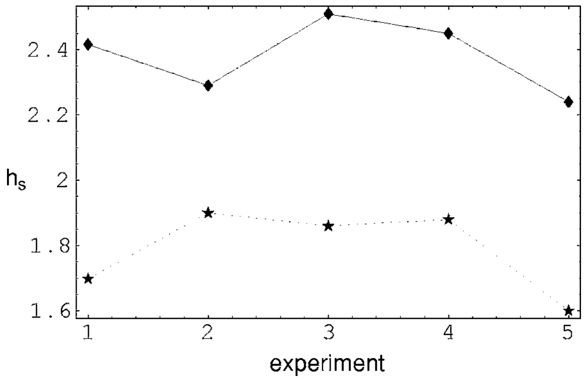

FIG. 4. Shannon entropies h_s calculated for the five courtship experiments. Each data point corresponds to the average obtained from five different *Drosophila* individuals. Involved protagonists, from left to right, bottom/top: (1) mature females/normal males; (2) immature females/normal males; (3) normal males/*fruitless* males; (4) mated females/normal males; (5) mature females/*fruitless* males. In experiment 3, the value obtained for normal males has been connected with the female data points, and that of *fruitless* males with those of the normal males. This avoids crossing of the male/female behavioral categories.

comparison of information is restricted to within the female group (i.e., immature, mature, mated), or the male group (i.e., normal and *fruitless* males). Secondly, identical values of h_s could be obtained from entirely different processes based on of different dominant symbols. For a fine-grained description of behavior, this is not acceptable. The fact that h_s is maximal for a uniformly distributed random process (that would correspond to a fortuitous testing of all available symbols), is a further indicator of the difficulty of relating it to an intrinsic behavioral meaning.

A traditional description of behavior in terms of a transition probabilities matrix of a Markov chain would suffer from similar problems. Again, comparability would be restricted to within each male/female class, because some of the fundamental acts of Table I only apply to females, others to males only.

2. Methods

a. Fly stocks

The *D. melanogaster* flies used in this study are of the wild-type Canton S (CS) strain and mutants carrying an allele of the *fruitless* mutation (fru^1).[28,29] The flies were raised on a cornmeal/agar/molasses/yeast medium at 25 °C on a 12:12 L:D cycle. The flies were sexed and the naïve males and immature females were isolated within two hours after eclosion under cold anesthesia (4 °C). The males were kept individually in test tubes until the recording. The mature male and female flies used for the recording were 4–5 days old. Mutant males used in the assays were homozygous for fru^1.

b. Data acquisition

A pair of flies was transferred, by aspiration, into a cylindrical mating chamber of dimensions 0.8 cm diameter and 0.5 cm height. Environmental conditions were fixed at 25 °C and 75% humidity. A 5-min episode (or until copulation in the case of the mature females) was recorded with a Sony Hi8 video/audio camera, using 29.97 frames per second. The videotape recordings were converted from Hi8 to digital video, compressed, and converted into MPEG 1 format, using Cleaner 6 software (Discreet, New York, NY). The files were loaded onto the THEME coder (Patternvision Inc., Iceland) and analyzed frame by frame. The beginning and ending of every act was systematically registered, for both protagonists of the courtship.

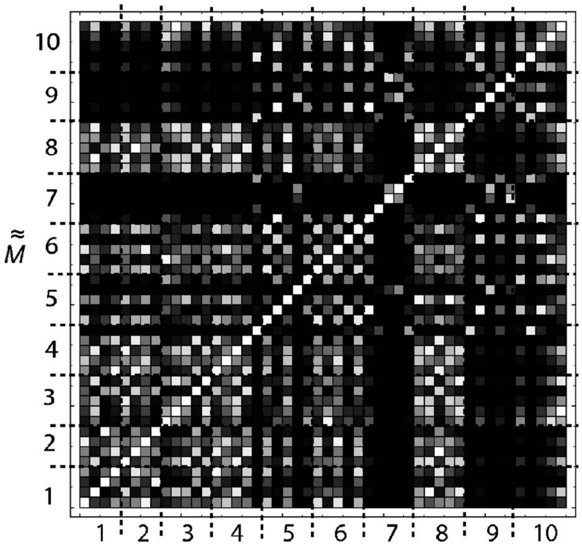

FIG. 5. Behavioral discrimination potential determined by a mean-difference test (light shading indicates low discriminability), showing again largely individual behavioral variability on top of clear group coherence. Majority voting can be used to determine whether one class should be distinguishable from another.

c. Significance estimation

We assessed the statistical significance of the results by calculating the mean-difference significance levels for the hypothesis that the set of similarities characteristic for one particular protagonist and that of another protagonist would originate from the same distribution, which clearly underestimates the behavioral discrimination potential. For highly consistent group behavior, largely identical distributions emerge, up to modifications by nonsalient individuals. By using surrogate data, we were able to check how well individual and group behaviors distinguish from random behavior.

Overall, the tests showed a clear emergence of the underlying class structures at a p-value of 0.88. At a p-value of 0.95, male versus female versus fruitless behavior, and male behavior in dependence on the female reproductory state behavior, could be distinguished. Moreover, a clear identification of individuals sharing, or showing unusual, behavior within one class is possible (see Fig. 5). These findings were very stable with respect to minor modifications of our method that one might arguably opt for.

Individual females obviously use rather similar behavioral patterns. In particular, for mature females in the presence of normal males, only few behavioral alternatives seem to be accessible. In contrast, the individuality of males in the presence of mature females is extremal. This we take as an indication that it is the individuum-specific male information in response to well-specified female patterns that defines the salient information pathway in *Drosophila* courtship.

At a confidence level of $p=0.9$, majority voting distinguishes among almost all classes. At $p=0.95$, experiments involving the fruitless mutation, female behavior and male behavior can still be distinguished. At confidence level $p=0.99$, the male behavior towards mature and immature females still distinguish against the rest. At even higher confidence, only the male behavior towards mature females distinguishes from the rest. The tests also show that the experimental groups are significantly different from surrogate data. Because the tests vastly underestimate the behavioral discrimination potential, they reliably confirm our methods's ability to discriminate in a quantitative and fine-grained manner, among the different behaviors involved in *Drosophila* courtship.

[1] P. Stockinger, D. Kvitsiani, S. Rotkopf, L. Tirian, and B. J. Dickson, Cell **121**, 795 (2005).

[2] E. Demir and B. J. Dickson, Cell **121**, 785 (2005).

[3] H. T. Spieth, Annu. Rev. Entomol. **19**, 5 (1974).

[4] H. B. Dowse, J. M. Ringo, and K. M. Barton, J. Theor. Biol. **121**, 173 (1986).

[5] T. A. Markow, Proc. Natl. Acad. Sci. U.S.A. **84**, 6200 (1987).

[6] T. A. Markow and S. J. Hanson, Proc. Natl. Acad. Sci. U.S.A. **78**, 430 (1981).

[7] A. Hoikkala and S. Crossley, J. Insect Behav. **13**, 71 (2000).

[8] A. Hoikkala, S. Crossley, and C. Castillo-Melenedez, J. Insect Behav. **13**, 361 (2000).

[9] E. M. Banks, J. Anim. Sci. (Savoy, Ill.) **54**, 434 (1982).

[10] S. Chen, A. Y. Lee, N. M. Bowens, R. Huber, and E. A. Kravitz, Proc. Natl. Acad. Sci. U.S.A. **99**, 5664 (2002).

[11] V. S. Afraimovich and L. A. Bunimovich, Nonlinearity **20**, 1761 (2007).

[12] P. Cvitanovic, Physica D **51**, 138 (1991).

[13] P. Cvitanovic, Physica D **83**, 109 (1995).

[14] R. Stoop and C. Wagner, Phys. Rev. Lett. **90**, 154101 (2003).

[15] D. A. Gailey and J. C. Hall, Genetics **121**, 773 (1989).

[16] L. Giarratani and L. B. Vosshall, Neuron **39**, 881 (2003).

[17] J. Billeter, E. Rideout, A. Dornan, and S. Goodwin, Curr. Biol. **16**, R766 (2006).

[18] G.-G. Lee, L.-C. Kim, J. S. Dunning, and K.-A. Han, PLoS ONE **3**, e1391 (2008).

[19] H. V. Hirsch and L. Tompkins, J. Exp. Biol. **195**, 1 (1994).

[20] J. Peinke, J. Parisi, O. E. Roessler, and R. Stoop, *Encounter with Chaos* (Springer, Berlin, 1992).

[21] T. Ott, A. Kern, A. Schuffenhauer, M. Popov, P. Acklin, E. Jacoby, and R. Stoop, J. Chem. Inf. Comput. Sci. **44**, 1358 (2004).

[22] R. J. Greenspan and J. F. Ferveur, Annu. Rev. Genet. **34**, 205 (2000).

[23] A. Villella, D. A. Gailey, B. Berwald, S. Oshima, P. T. Barnes, and J. C. Hall, Genetics **147**, 1107 (1997).

[24] B. I. Arthur, J.-M. Jallon, B. Caflisch, Y. Choffat, and R. Noethiger, Curr. Biol. **8**, 187 (1998).

[25] D. S. Manoli, M. Foss, A. Villela, B. J. Taylor, J. C. Hall, and B. S. Baker, Nature **436**, 395 (2005).

[26] K.-I. Kimura, M. Ote, T. Tazawa, and D. Yamamoto, Nature **438**, 229 (2005).

[27] M. Koneya and A. Barbour, *Louder Than Words: Nonverbal Communication* (Merrill, Columbus, Ohio, 1976).

[28] J. C. Hall, Science **264**, 1702 (1994).

[29] M. D. Adams *et al.*, Science **24**, 2185 (2000).