

Courtship Behavior: Hearing New Notes in Classic Songs

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Courtship depends on communication between partners; for example, male flies sing to entice females. New research, deploying modern statistical techniques, has identified a previously unrecognized note in the song repertoire, expanding the richness of this model system of communication and sexual reproduction.

A boombox held aloft, a courtier's strummed lute, a songbird's lilting trills — the right skillful notes can land a loving mate. Flies sing during courtship: as part of a complex ritual in which they chase a female, tap her abdomen and lick her genitals, male *Drosophila melanogaster* spread their wings and vibrate the air to produce a patterned, staccato song [1] that increases receptivity of conspecific females [2]. This song comprises two 'notes', belted out by the male fly in a different sequence with each performance: a 'sine' note of fairly pure tone at approximately 160 Hz that sounds to humans like a low hum; and a 'pulse' note made of short (~10 ms) clicks emitted at approximately 35 Hz, which sounds to humans like deep vocal fry.

At least, that was the understanding before a new study, reported in this issue of *Current Biology* by Clemens *et al.* [3], revealed a third courtship song mode, a higher pitch version of the pulse song (Figure 1). This new song mode was discovered using a data-driven statistical approach, unsupervised clustering, which exploits the variation across thousands of recorded songs to discover the natural clusters of song notes. This discovery is a standout demonstration of the potential of unsupervised statistical methods to assist in the discovery of biological features. Finding new song notes is unexpected, as fly courtship is a well-studied phenomenon. The genetic and neural mechanisms that produce fly courtship song have been investigated as models of communication [4], action selection [5], and behavioral evolution [6,7]. With the discovery of a new song note, new questions arise in each of these areas, some of which are addressed by Clemens *et al.* [3] in their new paper.

In the lead-up to copulation, fly courtship predominantly consists of the female running away from the male and sometimes actively rejecting him by pushing him away with her legs. He follows, sings and eventually comes into sustained physical contact with the female for genital licking, and ultimately mounting. His song is thought to facilitate copulation by enticing the female to slow down, increasing the chances of more intimate behaviors [8]. This reciprocal interaction is mediated by many senses beyond hearing, including vision, olfaction, gustation, and mechanosensation [4,8,9]. The communication between courting flies is reciprocally causal — males affect females and females affect males — and each partner is highly tuned to the other's behaviors. In a striking example, female flies with masculinized nervous systems exhibit male behaviors (but smell and look fully female). Instead of being the subjects of male courtship, these masculinized females become the subjects of male aggression — a typical male-male interaction [10]. For the production of courtship song, the female's behavior modulates which song notes the male produces [5,11]. For example, when the female speeds up, increasing her distance from the pursuing male, he is likely to increase his production of the pulse note [11].

The extent to which the behavioral feedback loop between the courting male and female determines the note-by-note composition of courtship song has been made clear by data-intensive analyses [5]. Geometric dimensions of courtship, like the speed of the female or her distance to the male, predict the detailed patterning of his song, down to the level of which

note he will sing and when. (Linear-nonlinear models, inspired by the computations performed by neurons and taking the geometric configuration of the courting partners as an input, predict the times and durations of pulse and sine song notes at a level of $0.6 < r < 0.7$ between predictions and observations.) The predictability of courtship song from the dynamics of courtship social interactions implies that courtship songs are note-by-note responses to the male's sensory experience. This overturned earlier thinking that the note-by-note sequence of courtship song was established by random processes (specifically, Markov-like processes) [12]. Like the originally recognized song notes, the new fast pulse note discovered by Clemens *et al.* [3] appears to be under the dynamic, note-by-note control of the male's sensory experience. The best predictor of when a male would produce a fast pulse was his distance from the female (but other factors were also predictive, including the female's lateral speed or his own forward velocity). Proportionally more fast pulses were sung when the female was farther away, and conversely, more slow pulses were sung when she was close. This might reflect different transmission properties of these notes (maybe fast pulses travel better farther through air or the substrate) or differential effects on the female (maybe fast pulses are a last-ditch effort to get the attention of a disengaging female). The context-dependent adaptive value of each pulse style remains to be determined.

Courtship behavior is sexually dimorphic — males and females perform different actions — and this originates in sexually dimorphic neural circuits. The *fruitless* gene is the master-regulator that



drives sexually dimorphic developmental programs during the wiring of the brain [13]. A circuit composed of *fruitless*-expressing neurons coordinates the production of male courtship song [14] and includes neurons in both the head and the ventral nerve cord (the fly equivalent of the spinal cord, where motor signals, like those moving the wings, are generated). Among the most upstream cells in this circuit are the P1 neurons, whose activity encodes social drives, including mating drive, on relatively long timescales. Activating these neurons experimentally causes flies to produce fewer pulse notes (both the slow and fast varieties) and more sine notes, as if the female is nearby, and the mating drive correspondingly high. But inducing activity in a downstream neuron (pIP10) that projects to the ventral nerve cord biases flies to produce more of the newly discovered fast pulse notes [3]. Thus, the nervous system could potentially select among the pulse note options by modulating the activity of pIP10, perhaps in response to cues that indicate if the female is near or far.

Because courtship and mating are proximate determinants of evolutionary fitness, courtship song is a mechanism by which males might attain a competitive advantage, or closely related fly species might distinguish conspecifics among all the potential mates on a piece of rotting fruit. The mechanisms underlying song have therefore been studied in various evolutionary contexts including within [6] and between [7] species variation. The new fast pulse note exhibits such variation and may be a target of evolution. Clemens *et al.* [3] found it in the songs of *Drosophila melanogaster*'s cousin species *D. simulans* and *D. mauritiana*, but, intriguingly, not *D. sechellia*. Perhaps the stage was not set right for *D. sechellia* to perform the fast pulse — not observing them to sing a fast pulse in these experimental circumstances does not imply they cannot sing it in other circumstances. But this observed difference in *D. sechellia* may reflect a genetic difference that could be a substrate for evolution.

For all the research attention that has been focused on courtship song, how did an entire note go unnoticed? Admittedly, the waveforms of fast pulse and slow pulse notes are fairly similar: both are close to impulses (rapid up and down excursions in

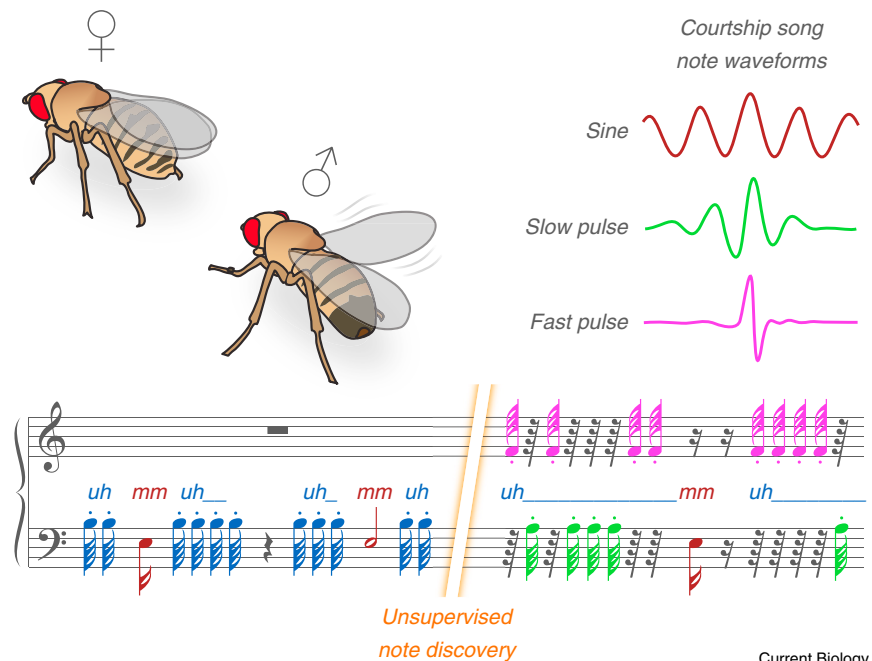


Figure 1. Unsupervised statistical analysis has revealed a new, third note composing *Drosophila* courtship song.

During courtship, a male fly follows a female while singing to her with his extended wing. His song, represented below, is composed of a rapid succession of stereotyped notes, referred to as ‘sine’ and ‘pulse’ elements. Until the recent discoveries of Clemens and colleagues, song notes were divided into just those two types (music at left). But their use of a statistical method called unsupervised clustering, which aims to discover all the natural patterns or clusters in a data set, reveals that there are two distinct subtypes of pulse notes (music at right). Slow pulse notes ring at approximately 200 Hz and have roughly symmetrical waveforms with several lobes. Fast pulse notes ring at 250–400 Hz and have two predominant lobes.

pressure that sound, in isolation, like clicks), but the slow pulse rises and falls more slowly and is symmetrical around its peak (Figure 1). To make their discovery, Clemens *et al.* [3] employed unsupervised clustering. This framework employs relatively unbiased algorithms to identify complete lists of the natural clusters or modes of variation in a data set. Compared to traditional supervised classification methods where experts provide “ground truth” categorical labels as the fodder for training classifiers, unsupervised methods have the potential to be more complete and reproducible by not relying on manual expertise that resides in one or a few experts.

Unsupervised methods have been successfully employed to identify the natural clusters in numerical representations of fruit fly behavior [15,16], including the social behavior of courting dyads [17]. But in these instances the resulting picture of behavior is complicated — the classifiers identify dozens to hundreds of behavioral modes, many of which are only subtly different

from each other. The unsupervised analysis of courtship song had the potential to produce a correspondingly complex landscape of song notes: Clemens *et al.* [3] started with tens of thousands of snippets of courtship song, each centered on a peak of acoustic intensity. That peak and the previous and subsequent 125 time points (spanning 12.55 ms) constituted a 251-dimensional representation of a single song snippet. Computing clusters, let alone visualization, in 251 dimensions is challenging, so the authors projected that data into a low (two) dimensional space for visualization and feature identification using both linear (PCA) and nonlinear (t-SNE) techniques.

After this transformation, the discovery was self-evident. There were three prominent, distinct clusters in the song data: sine, slow pulse, and the new fast pulse notes. The relative simplicity of this outcome meant that Clemens and colleagues had a clear path forward: study the biological mechanisms underlying the new fast pulse note. (In contrast,

unsupervised methods for parsing behavior as a whole typically reveal so many clusters that the path forward is less clear.) Thus, the use of unsupervised methods in finding the fast pulse note represents a standout example of how new statistical approaches can reveal biological phenomena hiding in plain sight.

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Social Behavior: A Neural Circuit for Social Behavior in Zebrafish

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A new study on the zebrafish has discovered a population of forebrain neurons necessary for social orienting, providing a foundation for dissecting social brain networks in this powerful vertebrate model.

Navigating the many social signals at, say, a dinner party requires huge information processing power. Not understanding if someone is shy or on edge, or how interested the other guests are in different topics of conversation, might quickly lead to testy exchanges, raised tempers and a spoiled evening. Many other species similarly depend on social stimuli to decide which action to take. A very fast change of direction of a conspecific, for example, may indicate the presence of some danger, while other social signals can indicate conflicts over resources and an imminent fight, or mating

opportunities. The specialized nature of the computations needed to extract relevant information from social stimuli raises the question of whether there exist neuronal substrates dedicated to processing social cues. A core social behavior network, consisting of anatomically and functionally connected regions implicated in many different social behaviors, has been described in mammals [1], and appears to be conserved across reptiles, birds, and teleost fish [2]. Shared neuroanatomical and molecular features across taxa provide the opportunity to study, in the

most experimentally tractable systems, fundamental circuit mechanisms at work in vertebrate social behavior. In a step towards this goal reported in this issue of *Current Biology*, Stednitz *et al.* [3] have identified a genetically defined population of neurons in the forebrain of zebrafish that are indispensable for social orienting.

What is the relevance of these new results? Among teleost species, zebrafish currently offer a comprehensive array of advantages for the analysis of neural circuits. Genetic tools allow the visualization and spatially and temporally targeted manipulation of different neuronal

