

Exploring variation in courtship song throughout the *Drosophila nasuta* clade

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D. albomicans
From https://kyotofly.kit.jp/ehime/ehime_photo/alboMF.jpg

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Abstract

Courtship behavior in *Drosophila* is highly variable, and some types of behavior often display strong quantitative differentiation between closely related species. One such behavior is the “pulse song,” generated when males rapidly vibrate their wings against their abdomens as part of a stereotypical courtship ritual. The spacing of pulses, or “inter-pulse interval” differs between closely related species in several clades and is often important for species recognition. Previous research has led to methodology allowing the high-throughput measurement of pulse song parameters for *Drosophila melanogaster*; however, these methods are finely tuned for *melanogaster*-type pulse song and perform poorly in other, distantly related species. We here develop and implement a more general method for pulse song identification and parameter estimation and use it to characterize pulse song across more than a dozen species and sub-species within the *Drosophila nasuta* clade. The *D. nasuta* clade represents a recent radiation of species whose low levels of morphological and genetic divergence make them prime models for studying the genetic basis of reproductive isolation. We integrate our quantitative pulse song parameter estimation results with an estimation of phylogenetic relationships between species within the clade to assess how pulse song has evolved throughout the clade. Our results suggest that the new pulse identification method will be applicable to species with wide variation in pulse song. Moreover, we identify differences in pulse song between closely related species in the *D. nasuta* clade that show promise for mapping the genetic basis of these important behavioral traits.

Introduction

Drosophila males court using several stereotypical behaviors, including vibrating their wings against their abdomens to produce “pulse song” (1). Pulse spacing is frequently species-specific and may contribute to reproductive isolation in several clades (2,3). We were particularly interested in characterizing this courtship behavior throughout the *Drosophila nasuta* clade, which represents a recent radiation of closely related species mostly found throughout southeast Asia (4,5). However, previous methods developed for estimating pulse song in *D. melanogaster* (6) performed poorly in identifying pulse song in *D. nasuta* species.

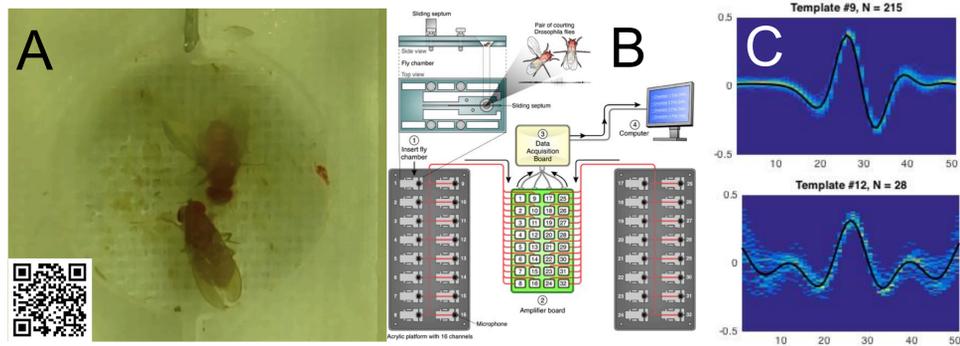


Figure 1. Measuring courtship song in non-model species. A) Snapshot of a *D. s. albostrigata* fly courting (use QR code to view video). B) Pipeline for multi-channel recording, from Arthur et al. (6). C) Heatmap histograms of example templates representing species-specific pulse song shapes.

Methods

Recording

We used the experimental setup in (6) (Fig. 1B) to record up to 32 pairs of courting flies at a time. We recorded multiple pairs from one strain apiece of 13 species or subspecies within the *D. nasuta* clade, with flies obtained from the UCSD or Ehime stock centers.

Estimating pulse song parameters

We developed a suite of tools called *FlySongClusterSegment*, implemented in the MATLAB language, to identify and characterize pulse song. In brief, we analyzed the songs as follows:

- use a subset of manually identified pulse trains from all species to **create “templates,”** or the average shape of peaks in sound corresponding to pulses (e.g., Fig. 1C)
- **optimize program parameters** for width of pulse shape, level of background noise, and degree of distinction between signal and noise, using training and validation datasets
- search all song recordings for matches to the identified template shapes of sufficient likelihood and difference from noise (**called pulses**)
- obtain the maximum density estimate of **inter-pulse interval** among trains of at least 10 pulses, from the called pulses identified above

Phylogeny estimation

We sequenced an individual from each species or subspecies to >10x depth using Illumina single-end sequencing. We applied the method in (7) to infer phylogenetic relationships, using similarity in 25-mer distributions across species as pairwise distances. We rooted the tree by inserting an outgroup at the appropriate node based on previous phylogenetic work (4,5), using the phytools package in R version 3.2.3 (8,9).

Results

Improvement in pulse calling

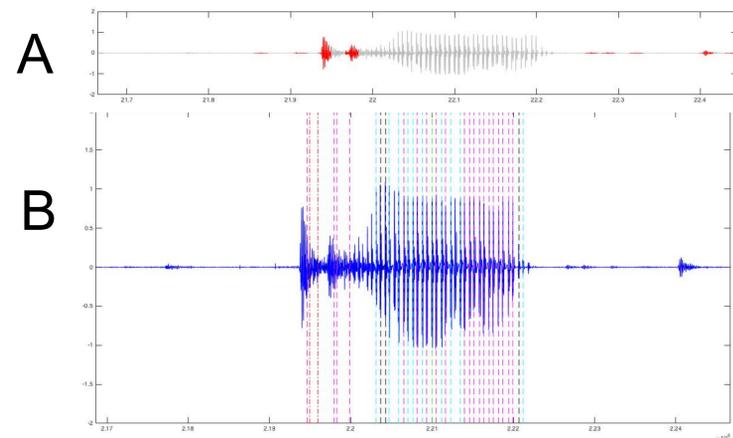


Figure 2.

A) Pulses in albomicans song identified by previous method (6), in red.
B) Pulses in the same song identified by new method (all colors).

Summary:

- The new method substantially improves the rate of pulses called in nasuta clade species.

Pulse song variation throughout the nasuta clade

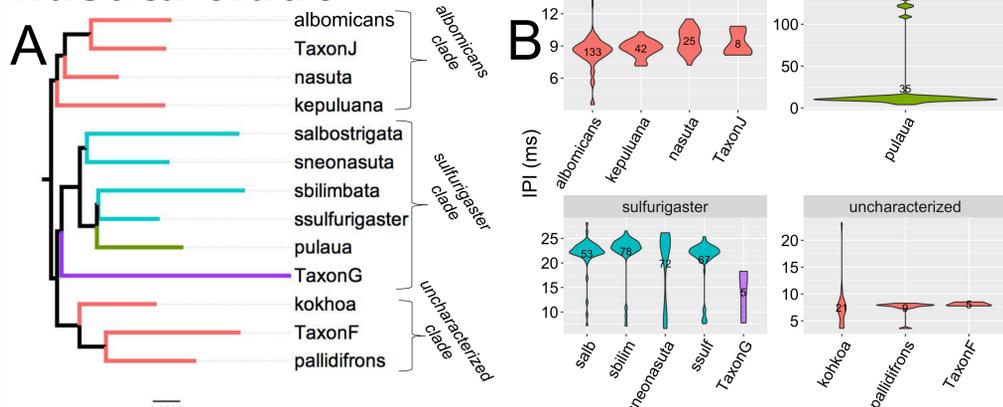


Figure 3. A) Phylogenetic relationships among species in the nasuta clade. **B)** Variation in inter-pulse interval (IPI) within and across all species, sorted by sub-clade, with pulaua shown separately. Numbers represent number of recordings analyzed. Colors in (A) and (B) represent groups of species with similar IPI.

Summary:

- Inter-pulse interval (IPI) **evolves relatively slowly** within the clade, with sister species generally showing high similarity in IPI
- IPI has **changed substantially at least twice**, varying by a factor of two to ten across sub-clades.

Conclusions & Future Directions

- Developing species-specific templates enables **highly accurate identification and characterization of pulse song** in previously unstudied species.
- Most nasuta clade species have characteristic **IPI < 30 ms**, much lower than in other clades.
- In a few species, noise still interferes with accurate pulse song parameter estimation, necessitating **future work, particularly in pulaua** (IPI > 100 ms manually verified).
- **Mapping of IPI differences** may be possible in *D. albomicans* x *D. s. neonasuta/sulfrigaster* crosses, which are partially inter-fertile.

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References

1. Cowling DE, Burnet B. 1981. Courtship songs and genetic control of their acoustic characteristics in sibling species of the *Drosophila melanogaster* subgroup. *Anim Behav* 29: 924–935.
2. Kyriacou CP, Hall JC. 1980. Circadian rhythm mutations in *Drosophila melanogaster* affect short-term fluctuations in the male's courtship song. *Proc Natl Acad Sci USA* 77: 6729–6733.
3. Miller, DD, Goldstein RB, and Patty RA. 1975. Semispecies of *Drosophila athabasca* distinguishable by male courtship sounds. *Evolution* 29:531–544.
4. Yu H, Wang W, Fang S, Zhang YP, Lin FJ, Geng ZC. 1999. Phylogeny and evolution of the *Drosophila nasuta* subgroup based on mitochondrial ND4 and ND4L gene sequences. *Molecular phylogenetics and evolution* 13(3):556–65.
5. Bachtrog D. 2006. The speciation history of the *Drosophila nasuta* complex. *Genetical Research* 88(1):13–26.
6. Arthur BJ, Sunayama-Morita T, Coen P, Murthy M, and Stern DL. 2013. Multi-channel acoustic recording and automated analysis of *Drosophila* courtship songs. *BMC Biol.* 11:11.
7. Fan H, Ives AR, Surget-Groba Y, Cannon CH. 2015. An assembly and alignment-free method of phylogeny reconstruction from next-generation sequencing data. *BMC Genomics* 16:522.
8. R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
9. Revell, LJ 2012. phytools: An R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3 217-223.