

# Calmodulin-binding transcription factor shapes the male courtship song in *Drosophila*

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論文題目	Calmodulin-binding transcription factor shapes the male courtship song in <i>Drosophila</i> （雄の求愛歌の特性決定に関与する <i>croaker</i> 遺伝子の 同定とその機能の解析）
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## 論文内容の要旨

### Introduction

Many animals utilize acoustic signals or songs to attract mates. During courtship, *Drosophila melanogaster* males vibrate a wing to produce characteristic sounds called pulse and sine song, respectively. However, genetic and neural mechanisms regulating courtship songs have not been fully elucidated. Males of the *Drosophila melanogaster* mutant *croaker* (*cro*) generate a polycyclic pulse song dissimilar to a monocyclic song typical of wild-type males during courtship. However, *cro* has not been molecularly mapped to any gene in the genome. In this study, I molecularly and functionally characterized the *cro* locus to understand how the nervous system shapes the male courtship song.

### Results

In this study, I demonstrated that *cro* is a mutation in the gene encoding the Calmodulin-binding transcription activator (*Camta*). A previous study mapped *cro* to the chromosomal location 45DE on the 2nd chromosome. Based on this knowledge, I performed genetic complementation tests with chromosomal deficiencies located around this cytological region. Some of the tested deficiencies produced the *cro* like phenotype when placed in trans to *cro*. The deficiency mapping data placed the *cro* locus between the cytological positions 45D8 and 45E3. In this cytological interval, three genes have been annotated, i.e., *bruchpilot* (*brp*), *Wnt2* and *Camta*. To narrow down the gene responsible for the *cro* phenotype, I carried out a rescue experiment by introducing an intact *cro* locus into the mutant chromosome with a BAC clone (BAC-CH321-22B08) that contains the entire *Camta* locus, but neither *brp* nor *Wnt2* locus. The BAC clone rescued the *cro* mutant phenotype, suggesting that *cro* is allelic to *Camta*.

Since *cro* is a P-element insertion mutant, I next determined the P-element insertion point by two independent methods: plasmid rescue and inverse PCR. Both methods suggested that the *cro* mutant

chromosome has a P-element insertion in the fourth intron of the *Camta* gene. To further validate this result, I collected several available fly lines having a transposon insertion in the *Camta* locus to conduct genetic complementation tests with *cro*. Indeed, some of the chromosomes carrying a transposon insertion (e.g., MiMIC<sup>M104570</sup>) failed to rescue the *cro* phenotype, indicating that the *cro* phenotype is rescued from deficits of *Camta*. This notion is supported by the qPCR experiment that showed that the *Camta* mRNA amount is significantly reduced in the *cro* mutant.

I performed a knockdown experiment with *UAS-Camta RNAi* using several brain cell specific GAL4 drivers. Two pan-neural GAL4 (*elav-GAL4* and *nSyb-GAL4*) induced polycyclic songs typical of *cro* mutants whereas Glia specific *repo-GAL4* did not when used to drive *UAS-Camta RNAi*, indicating the role of *Camta* in neurons. To further narrow down the neural subset responsible for the *cro* phenotype, I performed *cro* knockdown in *fru-GAL4* or *dsx-GAL4* positive cells, because the core portion of the male courtship circuit is composed of *fru* and/or *dsx* expressing neurons. *fru-GAL4*-driven *Camta RNAi* expression induced polycyclic songs, indicating that *fru*[+] neurons are involved in inducing the *cro* phenotype. Cells positive for *dsx-GAL4* had no effect on pulse song upon knocking down of *Camta*.

It has been postulated that courtship decision-making is made in the brain, whereas the motor pattern generator for courtship song resides in the ventral nerve cord (VNC). To determine where in the central nervous system (CNS) *cro* is required for normal song generation, I performed head specific knockdown of *Camta* in *fru*[+] cells with *Otd<sup>FLP</sup>*, which releases GAL4 from GAL80-mediated repression by flipping the *STOP* sequence out from *tub>GAL80>* only in the head. Knockdown of *Camta* in *fru*[+] neurons in the head induced polycyclic pulse song, indicating their role in pulse song generation.

Taking into account the observation that the head-restricted *cro* knockdown in *fru-GAL4*-expressing neurons induced polycyclic pulse song, I tested whether *cro* knockdown in a few *fru*-expressing brain neurons known to have a pivotal role in courtship song generation can induce polycyclic pulse song. I first examined pIP10 neurons, which represent laterally paired descending interneurons with the

command fiber-like function to initiate male courtship upon artificial activation. These interneurons have been reported to trigger pulse song generation when forcibly activated. Notably, *cro* knockdown specifically targeted to pIP10 neurons by the *VT40556-GAL4* did not induce polycyclic pulse song during male courtship toward a female. Next, I examined the effect of *cro* knockdown in mcALa neurons, which have been implicated in the ordering of courtship elementary actions. Interestingly, the mcALa-specific intersectional combination *p52a-GAL4* and *fru<sup>FLP</sup>* led to the generation of polycyclic pulse song, suggesting a new role for mcALa, i.e., shaping pulse song. In contrast, P1-targeted *cro* knockdown by the intersection of *NP2631-GAL4* and *fru<sup>FLP</sup>* did not induce polycyclic pulse song during male courtship. To decipher whether *cro* function is required for shaping pulse song during courtship actions or in the development of the courtship song circuit, I also conducted a stage-restricted *cro* knockdown experiment. Knockdown of *Camta* in developmental stages resulted in *cro* phenotype indicating its role in shaping the song circuit in developmental stages.

## Conclusion

I demonstrated that the *cro* chromosome carries a P-element insertion in the fourth intron of the *Camta* locus, which decreases the amount of *Camta* mRNA in the *cro* mutant. *Camta* knockdown in a defined set of cells suggested that *cro* acts in *fru*[+] mcALa neurons in the brain to shape monocyclic pulse song. This finding points to the role of brain in pulse song shaping, which has been ascribed to the motor pattern generator for courtship song locating in the mesothoracic segment of the ventral nerve cord (e.g., the TN1 cluster). It would be thus interesting to examine the neural circuit mechanism whereby mcALa controls the action of the song pattern generator in the VNC to generate monocyclic songs. Since *cro* has been implicated in the generation of the species difference in courtship songs between *D.sechellia* and

*D.simulans*, it would also be interesting to examine an evolutionary divergence in the *cro* locus as a substrate of different courtship songs among species. This possibility can be tested by the experiment, where the entire *Camta* locus of one species replaces the counterpart in the other species to see whether this *cro* gene exchange is accompanied by the exchange of song characteristics.

## 論文審査結果の要旨

ショウジョウバエ属では行動の種差が、種間の生殖隔離に寄与していることが少なくない。特に、雄の発する求愛歌の特徴を雌が聞き分け、それにより交尾をするか否かの意思決定が左右されることが知られている。しかし、その重要性にも拘らず、求愛歌を生み出す遺伝子や神経回路の種差については、その多くが未解明である。本研究では、キイロショウジョウバエを用いて求愛歌の生成に寄与する遺伝子を同定するとともに、その機能の場を細胞レベルで特定することを目指した。

キイロショウジョウバエの求愛歌はパルス歌とサイン歌の2タイプからなる。このうち、パルス歌に異常の生ずる突然変異体として、*croaker (cro)*が知られている。野生型の雄ではパルスのピークは一個 (monocyclic) であるが、*cro* 変異体雄のパルスには二つのピークを持つ (polycyclic) ものが高い頻度で含まれる。プラスミドレスキュー法と inverse PCR により *cro* 原因遺伝子をクローニングし、Calmodulin-binding transcription factor (*Camta*) をコードする遺伝子を特定した。実際、正常型 *Camta* を含む BAC clone を *cro* 変異体に導入すると、パルス歌の異常が救済され、*Camta* 座を欠く欠失染色体は *cro* 変異を相補しなかった。*CamtaRNAi* を組織特異的に発現させて *cro* 変異体と同様の表現型を誘導する実験から、ニューロンでの *Camta* ノックダウンが有効であることを見出した。加えて、雄の求愛行動回路形成においてマスターレギュレータとして働く *fruitless (fru)* 遺伝子に着目し、その発現ニューロンに限定した *Camta* ノックダウンを行うと、*cro* 様表現型が現れた。そこで *fru* 発現細胞の一部のみを標的としたノックダウンする実験を行い、mcAL と呼ばれる30個のニューロンでの *Camta* のノックダウンが、*cro* 様のパルス歌の異常を生じさせることが明らかとなった。

この様に、求愛歌産生に寄与する遺伝子と神経細胞の特定に大きな成果を挙げており、この事実は Tanveer Ahsan が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって、Tanveer Ahsan 提出の論文は、博士 (生命科学) の博士論文として合格と認める。