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Sex specific molecular responses of quick-to-court protein in Indian malarial vector *Anopheles culicifacies*: conflict of mating versus blood feeding behaviour



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Abstract

Understanding the molecular basis of mosquito behavioural complexity plays a central role in designing novel molecular tools to fight against their vector-borne diseases. Although the olfactory system plays an important role in guiding and managing many behavioural responses including feeding and mating, but the sex-specific regulation of olfactory responses remain poorly investigated. From our ongoing transcriptomic data annotation of olfactory tissue of blood fed adult female *An. culicifacies* mosquitoes; we have identified a 383 bp long unique transcript encoding a *Drosophila* homolog of the quick-to-court protein. Previously this was shown to regulate courtship behaviour in adult male *Drosophila*. A comprehensive *in silico* analysis of the *quick-to-court* (*qtc*) gene of *An. culicifacies* (*Ac-qtc*) predicts a 1536 bp single copy gene encoding 511 amino acid protein, having a high degree of conservation with other insect homologs. The age-dependent increased expression of

putative *Ac-qtc* correlated with the maturation of the olfactory system, necessary to meet the sex-specific conflicting demand of mating (mate finding) versus host-seeking behavioural responses. Sixteen to eighteen hours of starvation did not alter *Ac-qtc* expression in both sexes, however, blood feeding significantly modulated its response in the adult female mosquitoes, confirming that it may not be involved in sugar feeding associated behavioural regulation. Finally, a dual behavioural and molecular assay indicated that natural dysregulation of *Ac-qtc* in the late evening might promote the mating events for successful insemination. We hypothesize that *Ac-qtc* may play a unique role to regulate the sex-specific conflicting demand of mosquito courtship behaviour versus blood feeding behaviour in the adult female mosquitoes. Further elucidation of this molecular mechanism may provide further information to evaluate *Ac-qtc* as a key molecular target for mosquito-borne disease management.

Keywords: Ecology, Evolution, Genetics, Zoology

1. Introduction

Mosquitoes are the vectors for many deadly infectious diseases including malaria, dengue, chikungunya, zika fever and yellow fever claiming a few hundred million lives annually. An adult female mosquito transmits the pathogens from an infected vertebrate host to a healthy host during a blood meal. The control of mosquito-borne diseases is still dependent on the use of chemical insecticides to suppress mosquito population or to interrupt mosquito-human interaction. However, the rapid emergence of insecticide resistance poses a challenge to control vector populations effectively. An alternative to overcome this challenge includes designing molecular tools to interfere the complex feeding and mating behavioural properties [1]. Compared to the vast research concentrated on female mosquitoes, male mosquito biology is rather less explored possibly due to its indirect influence on parasite transmission. Males induce several post-mating behavioural changes in females, which also includes the blood feeding behaviour. Thus, male mosquitoes maintain the unbroken chains of the mosquito life cycle and significantly contribute in transmitting diseases in an indirect way. In nature, both males and females feed on nectar sugar for their regular metabolic energy source. Only adult female mosquitoes take blood meal to fulfil the requirement of extra nutrients for their egg maturation. However the molecular basis of evolution and adaptation of dual feeding behaviour i.e. nectar sugar versus blood meal in adult female mosquitoes is not fully understood [2, 3]. Likewise, demystifying the process through which mosquitoes manage complex mating behavioural events i.e. swarm formation, suitable mate finding and successful aerial coupling is yet a major challenge to entomologists [4, 5, 6, 7]. A few molecular markers linked to mating behaviour have been characterized in *Drosophila melanogaster* [8, 9, 10]. But, the

unavailability of any molecular marker regulating the complex mating behaviour in mosquitoes restricted our understanding.

Previous studies have indicated that neuro-olfactory system of mosquitoes regulates many complex behavioural responses such as mating, host seeking and blood feeding [11, 12, 13, 14]. Current evidence indicates that both male and female mosquito's olfactory system encodes a fairly similar number of proteins [15, 16], but how sex-specific olfactory proteins manage the conflicting demand of feeding and/or mating behaviour is not known. Modest changes occurring in the olfactory repertoire in response to blood feeding in adult female mosquitoes have been reported by various groups [17, 18]. Therefore, we hypothesize that regulation of some of the sex-specific unique genes may facilitate and manage similar functions e.g. mate partner location by adult male/female mosquitoes or host finding for blood feeding by adult female mosquitoes.

An. culicifacies is one of the major rural vectors in India accounting for more than 65% of malaria cases. Control of this mosquito species has become worse due to the rapid emergence of multiple insecticide resistance [19]. In an effort to understand the complex feeding behaviour of adult *An. culicifacies* female mosquito [3], we have identified a unique transcript from the olfactory system of the blood-fed mosquito, encoding the 'quick-to-court' (qtc) protein. *An. culicifacies* qtc protein is a homolog of *Drosophila* coiled-coil qtc (Q9VMU5) protein and shown to play an important role in driving the male courtship behaviour. In *D. melanogaster*, it is predominantly expresses in olfactory organs, central nervous system and male reproductive tract [20, 21]. Mutations in the *Dm-qtc* not only caused the males to show elevated levels of male-male courtship, but also favoured abnormally quick courtship when placed in the presence of a virgin female [18]. Recently, a qtc homolog has also been identified from whole body transcriptome of the insect *Bactrocera dorsalis* [22, 23] but yet its function is yet to be characterized.

A significant modulation of this unique transcript, *Ac-qtc* in the olfactory tissue (~5 fold up-regulation) in response to blood feeding prompted us to investigate its possible role in managing sex-specific conflicting demands of 'mate choice' and/or 'food choice' in the mosquito *An. culicifacies*. A comprehensive *in silico* function prediction analysis and extensive transcriptional profiling established a possible correlation that *Ac-qtc* might play a crucial role in the regulation and coordination of mosquito sex-specific behaviours.

2. Materials and methods

Fig. 1a represents a technical overview and workflow of the experiments to demonstrate the possible sex-specific role of *Ac-qtc* in adult *An. culicifacies*.

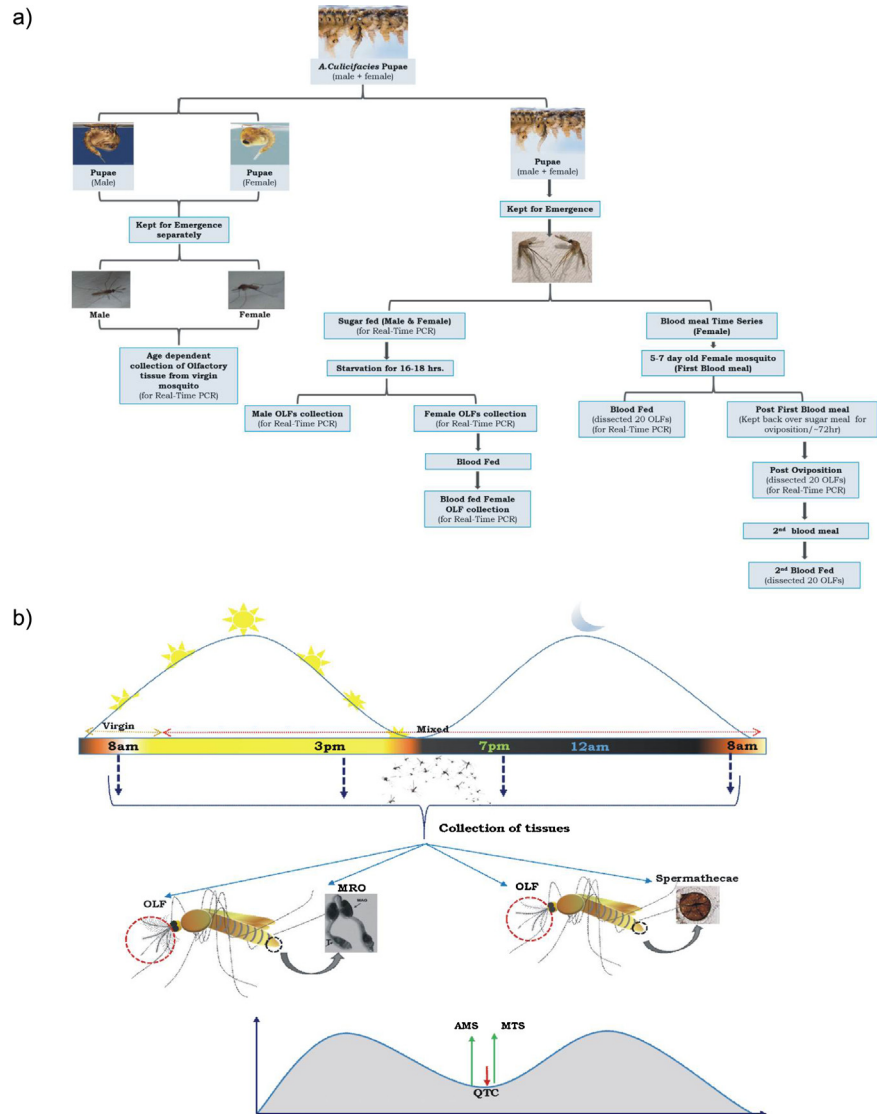


Fig. 1. Technical designing and experimental work flow: (a) Experimental overview to demonstrate the possible role of *Ac-qtz* in mosquito behavioural regulation; (b) Pictorial presentation of the assay designed to correlate the function of *Ac-qtz* in the mating success of *A.culicifacies* mosquito. OLF: Olfactory; MRO: Male reproductive organ.

2.1. Mosquito rearing and maintenance

The cyclic colony of *An. culicifacies*, sibling species A was reared and maintained in standard laboratory conditions at 28 ± 2 °C and 80% relative humidity in central insectary facility as mentioned previously [3, 24]. All protocols for rearing and maintenance of the mosquito culture were approved by ethical committee of the institute.

2.2. Tissue collection and RNA extraction

From the ice anesthetized adult male and female *An. culicifacies* mosquitoes various tissues were collected. This included olfactory tissues (including antennae, maxillary palp, proboscis and labium), brain, reproductive tissues (male reproductive organ includes testes and male accessory gland; female reproductive organ consists of spermathecae and atrium) and legs. The tissues were dissected and collected in trizol. The developmental stages of *An. culicifacies* viz. egg, larvae (stages I – IV) and pupae were also collected in trizol after removal of extra water through filter paper. Total RNA was isolated by the standard trizol method as described previously [3, 25].

2.3. Bioinformatic analysis

The putative *Ac-qtc* was identified as a partial cDNA from olfactory tissue cDNA library sequence database of the blood fed adult female mosquito (unpublished). Multiple BLAST analysis against mosquito draft genome and other transcript database were done using open source analysis tools available at www.vectorbase.org. Domain prediction, multiple sequence alignment, and phylogenetic analysis were done using multiple softwares as described earlier [25].

2.4. Behavioural and molecular assay

To track the possible role of *Ac-qtc* in mating behaviour, an assay was designed (Fig. 1b) favouring sex-specific changes of the behavioural activities occurring in response to day/night cycle in the 5–6 days old mosquitoes. As per assay design, we collected olfactory and reproductive tissues from either virgin and/or mixed cage mosquitoes of both the sexes. For the assurance of mating success, we mixed an equal number of male and female virgin mosquitoes in a single cage at early morning (0800 h) and collected tissues at 1500 h, 1900 h, and overnight/0800 h next morning from the same cage. To test and validate the completion of insemination process, we profiled and compared the expression of two independent sperm-specific transcripts in the spermathecae of adult female mosquitoes. Previously characterized sperm-specific genes (AMS/FJ869235.1 and MTS/FJ869236.1) of the mosquito *An. gambiae* [26] were queried to search and select sperm-specific homologs from the draft genome database of the mosquito *An. culicifacies*. The identified *Ac-ams* (ACUA010089) and *Ac-mts* (ACUA014389) transcripts sequences were used to design RT-PCR primers (See supplemental data for gene and primer sequence).

2.5. cDNA preparation and gene expression analysis

1 µg of total RNA was used to synthesize the first strand cDNA using Verso cDNA synthesis kit (Thermo Scientific) as described in the manufacturer protocol.

Routine differential gene expression analysis was performed by the RT-PCR. Relative gene expression analysis was done using SYBR green qPCR (Thermo Scientific) master mix and Illumina Eco Real-Time PCR machine. The four step PCR cycle included an initial denaturation at 95 °C for 5 min, 40 cycles of 10 s at 95 °C, 15 s at 52 °C, and 22 s at 72 °C. Fluorescence reading was recorded at 72 °C after each cycle. In final steps, PCR at 95 °C for 15 sec followed by 55 °C for 15 sec and again 95 °C for 15 sec were completed before driving a melting curve. Reproducibility of the result was ensured by repeating the experiments with three independent biological replicates. Throughout the experiment, *actin* gene was used as an internal control and the relative quantification data were analyzed by $2^{-\Delta\Delta Ct}$ method [27]. Statistical analysis of differential gene expression was done using Student's t-test.

3. Results and discussion

3.1. Identification, annotation and molecular characterization of *Ac-qtc*

To identify the differentially expressed genes in naïve sugar fed *vs.* blood fed olfactory system (OLF) of *An. culicifacies* mosquito, currently we are annotating large-scale RNA-Seq transcriptomic databases (unpublished). During this annotation, we identified a unique transcript from blood fed olfactory transcriptome encoding *Drosophila* homolog of quick-to-court (*qtc*) protein, which plays a crucial role in many aspects of male mating behaviour. BLASTX analysis of the partial 383 bp long transcript showed 59% identity with the *qtc* homolog of *Drosophila melanogaster*, but no putative conserved domain was identified. To retrieve full-length *An. culicifacies qtc* transcript, we performed BLASTN analysis against *An. culicifacies* genome and transcript databases using the partial transcript as a query sequence. Comparative alignment analysis of the partial and full-length transcript indicated that the identified 383 bp putative *Ac-qtc* transcript lacks both 5' and 3' sequences. The detail *in silico* analysis of 1536 bp long full-length *qtc* transcript (ACUA027268) encoding a 511 amino acid long protein showed coiled-coil domain signature at the 3' end of the sequence. *Ac-qtc* is a single copy gene, comprised of a 50 bp 5' UTR region followed by five exons and four introns followed by a 50 bp 3'UTR region as shown in Fig. 2a. A comprehensive primary structural analysis of this full-length transcript (ACUA027268) revealed that it has four coiled-coils features and one conserved GRIP domain at the 3' end of the sequence (Fig. 2b; Table 1). Multiple sequence alignment and phylogenetic analysis revealed a high degree of sequence conservation within the mosquito and other insect species (Fig. 2c). RT-PCR analysis indicated that *Ac-qtc* was constitutively expressed in all the aquatic stages of development, except in the egg of the mosquitoes (Fig. 2d). This

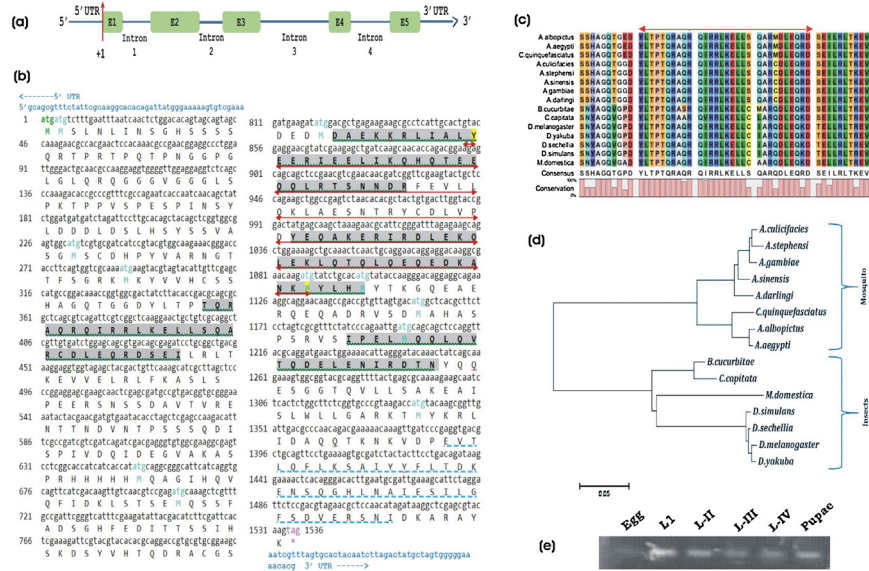


Fig. 2. Genomic and molecular characterization of *An. culicifacies* *quick-to-court* gene: (a) Schematic representation of the genomic architecture of the mosquito *Ac-qtct*. Five green coloured boxes (E1-E5) indicates the exons and +1 mark the transcription initiation site. (b) Gene organization and molecular features of full length *Ac-qtct*: The gene contains 1536 bp nucleotide, encoding 511 AA long peptides with four coiled-coils domains. Both 5' and 3'-UTR regions are highlighted (Light blue) letters. The complete coding region of 511 amino acids starts from ATG/Methionine/green colour, ending with TAG/Red/*. The different features are highlighted with different colour code, viz. Coiled-coil domains (grey colour and green underlined), Pre-folding domain (Red arrow) and GRIP-domain (sky blue dotted line). (c) Multiple sequence alignment of selected coiled coil domain (marked with green arrow) and (d) phylogenetic relationship of *Ac-qtct* with other mosquito species and *Drosophila* (e) Developmental expression of *Ac-qtct* in *An. culicifacies* by RT-PCR.

suggested that the quick-to-court protein is evolutionary conserved with the common function in the regulation of insects behavioural responses.

Table 1. Molecular features of predicted domain in the mosquito *Ac-qtct* gene.

SI. No.	Feature Type	Start Site	End Site
1.	Coiled-coils (Ncoils)	118	146
2.	Coiled-coils (Ncoils)	275	310
3.	Coiled-coils (Ncoils)	332	367
4.	Coiled-coils (Ncoils)	396	417
5.	Prefolding Domain	285	363
6.	GRIP domain	463	504

3.2. *Ac-qtc* abundantly expresses in the olfactory and brain tissues of adult mosquitoes

Sex and tissue-specific transcriptional profiling of *Ac-qtc* in the naive mosquitoes revealed the *qtc* gene expression in the olfactory tissue, brain and reproductive organs of both the sexes (Fig. 3). The previous study in *D. melanogaster* also demonstrates that *Dm-qtc* is expressed in the olfactory organs, central nervous system of both the sexes and male reproductive tract [18]. Interestingly, a relatively higher abundance of *qtc* gene in the olfactory tissue of male *An. culicifacies* indicated its possible involvement in the regulation of mosquito mating behaviour.

3.3. The sex-specific and age-dependent expression may regulate olfactory system maturation

Unlike *Drosophila*, unavailability of the proper molecular marker restricted our understanding of the complex mating biology in mosquitoes. In particular, studies on *An. culicifacies* mating behaviour are too limited [28]. However, our identification of the *quick-to-court* transcript from the blood fed olfactory tissue transcriptome data depicts that *Ac-qtc* may regulate the key molecular factors driving sex-specific behavioural modulation in *An. culicifacies*.

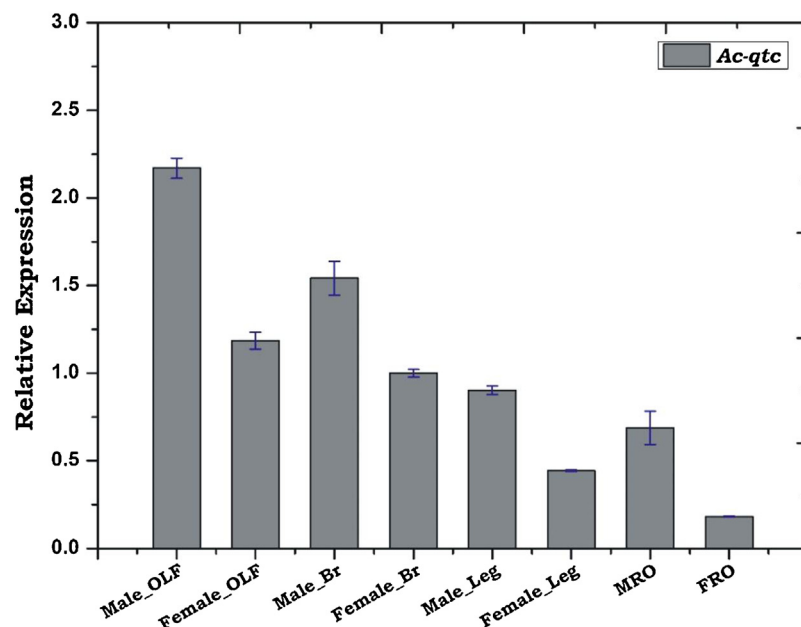


Fig. 3. Tissue and sex specific relative expression analysis of *Ac-qtc* in the adult mosquito. Male OLF: Male olfactory tissue (Antennae, maxillary palp and proboscis); Female OLF: female olfactory tissue; Male-Br: Male brain; Female-Br: female brain; Male-leg; Female-leg; MRO: Male reproductive organ; FRO: Female reproductive organ.

To test this hypothesis, we first examined the sex-specific relative expression of a pool of four transcripts including *qtc*, that were identified from the ongoing olfactory tissue transcriptomic study of blood fed adult female mosquito (Fig. 4a). Our data indicated that male olfactory system matures faster than female olfactory system even if they are at the same age. Next, we monitored the age-dependent transcriptional regulation of *Ac-qtc* in virgin male and female mosquitoes. Irrespective of the mosquito sexes, it showed age-dependent enrichment of *Ac-qtc* at the highest level (~6- fold up-regulated/ $p \leq 0.004$) on the 5th day, when compared to 1-day old virgin mosquitoes (Fig. 4b, c), followed by at least 2-fold down-regulation ($p \leq 0.0172$) on the 7th day. Interestingly after 7th-day *Ac-qtc* expression switched to up-regulation in male mosquitoes but it remained constant after 5th day in case of female mosquitoes. A similar pattern of *Ac-qtc* gene expression was also observed in the possibly mated male mosquitoes (Fig. 4d), which were collected from a mixed cage containing an equal number of male and female mosquitoes.

Although, it is yet unclear that how environmental guided non-genetic and/or genetic factors regulate the complex sexual behavioural events. But, our observations indicated that once male mosquitoes achieved the specific age of adulteration, the natural dysregulation of *Ac-qtc* by unknown mechanism may

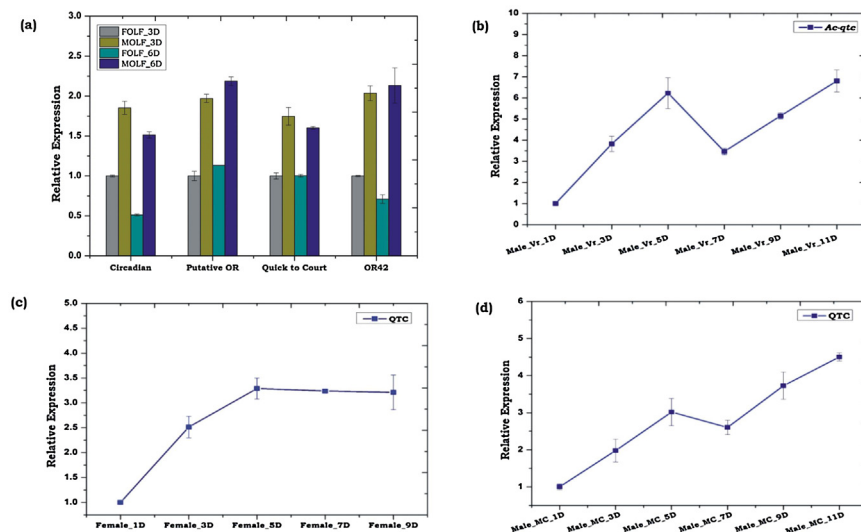


Fig. 4. Sex specific and age dependent transcriptional response of *Ac-qtc* transcripts in the olfactory tissue of *An. culicifacies*. (a) Expression analysis of four transcripts viz. *circadian*, *putative olfactory receptor* (putative OR), *quick-to-court* and *olfactory receptor 42* (OR42); FOLF: Female Olfactory; MOLF: Male Olfactory; (b-c) Age dependent transcriptional profiling of *Ac-qtc* in the virgin male and female mosquitoes (c): Male-Vr-1D: Male virgin mosquito of 1 Day old; Female-Vr-1D: Female virgin mosquito of 1 Day old. (d) Age dependent relative expression analysis of *Ac-qtc* transcript in the mated mosquito. Male-MC-1D: Male mosquito of 1 day old, collected from mixed cage (MC) containing equal number of male and female mosquitoes.

promote the courtship behaviour (see next paragraph). These results also corroborate with the previous findings in *Drosophila* where a mutation in *Dm-qtc* gene causes accelerated male-male courtship behaviour [18]. A recent study by Houot *et al.* also suggested that the *qtc* and *shaker* genes, which are abundantly expressed in the neuro-olfactory system of *Drosophila*, decrease the ability to discriminate between the sex targets [28, 29, 30, 31]. This is probably due to the declined perception to wild type female pheromone [28, 29, 30, 31].

3.4. Natural dysregulation of *Ac-qtc* may promote mating success

An alternative interpretation, of the above argument, could be that a significant downregulation ($p \leq 0.0172$) of *Ac-qtc* in the 5–7 day old virgin adult male mosquitoes may be crucial for the auto-activation of courtship behaviour in case of *An. culicifacies* mosquitoes. In the lack of any molecular marker for mating behaviour studies, we attempted to trace the possible functional correlation of male *Ac-qtc* in the mating success i.e. insemination event completion in the laboratory-reared mosquitoes. However, our initial experiments of *Ac-qtc* gene silencing using purified dsRNA injection in the thorax of male mosquito's remains unsuccessful, primarily due to a high mortality rate of these mosquitoes (data not shown).

Available literature suggests that in most *Anopheline* mosquitoes, the mating behavioural activities commenced by the onset of sunset, usually at 1700 h which may continue till 2000 h [29, 32, 33]. We hypothesize that the transcriptional modulation of *Ac-qtc* in response to dawn/dusk cycle must have a functional correlation with the mating success, especially insemination events where adult females receive and store the sperms in their spermathecae delivered by the male during copulation [34, 35]. For experimental verification of this idea, we first identified two sperm-specific transcripts from the draft genome of *An. culicifacies*, using (*ams* and *mts*) as query sequences, previously characterized from *An. gambiae* [26]. To validate sperm specificity, the primers designed against *Ac-ams* (ACUA010089) and *Ac-mts* (ACUA014389) were tested by RT-PCR in the male accessory glands (MAG) and spermathecae (SPT) collected from laboratory reared 3–4 day old virgin male and female mosquitoes, respectively (Fig. 5a). A non-specific poor amplification was visible in case of *Ac-ams* in virgin female mosquito spermathecae. This was verified by incorrect melting curve signal that appeared in Real-Time PCR data (Supplemental Fig. S1). To trace the possible functional correlation of *Ac-qtc* in mating success, we collected olfactory and reproductive tissues two hours prior or later onset of the sunset as described in methodology section (See experimental design Fig. 1b). We then analysed and compared sex-specific regulation of *Ac-qtc* in the olfactory tissue, and sperm-specific *Ac-ams/Ac-mts* genes in the male and female reproductive organs.

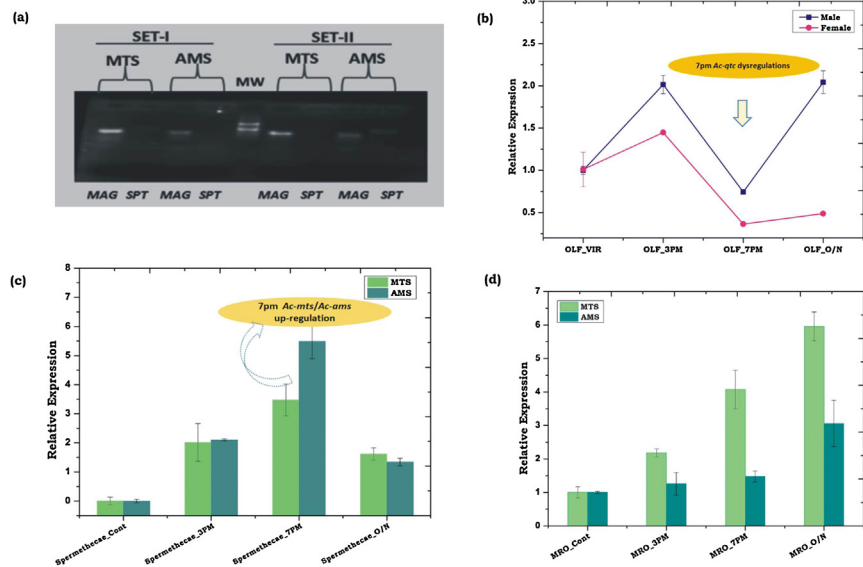


Fig. 5. (a) RT-PCR based expression validation of *Ac-ams* and *Ac-mts* in MAG (Male Accessory Gland) and SPT (Spermathecae). (b) Sex-specific transcriptional profiling of *Ac-qtc* at different circadian time in the olfactory tissues of the mosquito *An. culicifacies*; (c) Transcriptional response of *Ac-mts* and *Ac-ams* in the spermathecae of *An. culicifacies* at different circadian time. (d) Transcriptional response of *Ac-mts* and *Ac-ams* in male reproductive organ (MRO) at different circadian time. OLF: Olfactory; VIR: Virgin; Cont: Control; O/N: Overnight.

When compared to the virgin counterpart, significant down-regulation of *Ac-qtc* was observed in the olfactory system in both the sexes at 1900 h (Fig. 5b). This indicated that the lower expression of *Ac-qtc* may favour the increased mating frequency and active courtship engagement. A significant modulation of *Ac-ams*/*Ac-mts* expression in the mated female spermathecae (Fig. 5c) as well as male reproductive organs (Fig. 5d) supports the idea that a natural dysregulation of *Ac-qtc* in the late evening i.e. 1900 h may favour the copulation process by facilitating the release of unknown sex driving factors for successful insemination in the copulating couples. However, the exact molecular mechanism of *qtc* protein mediated regulation of insect's mating events is still unknown.

3.5. *Ac-qtc* regulation is independent of the nutritional status of the mosquitoes

Though feeding and mating are two mutually exclusive behavioural properties of any biological system. But those behaviours are dependent on each other to some extent at least for insects to facilitate the reproductive success and consequently their survival. The molecular basis of the sex-specific regulation of these conflicting behavioural demands (feeding vs. mating) remains largely unknown. Current studies in *Drosophila* suggested that food odour and sex-specific pheromone signals in the neuro-olfactory system work collaboratively to drive both the meal and/or mate

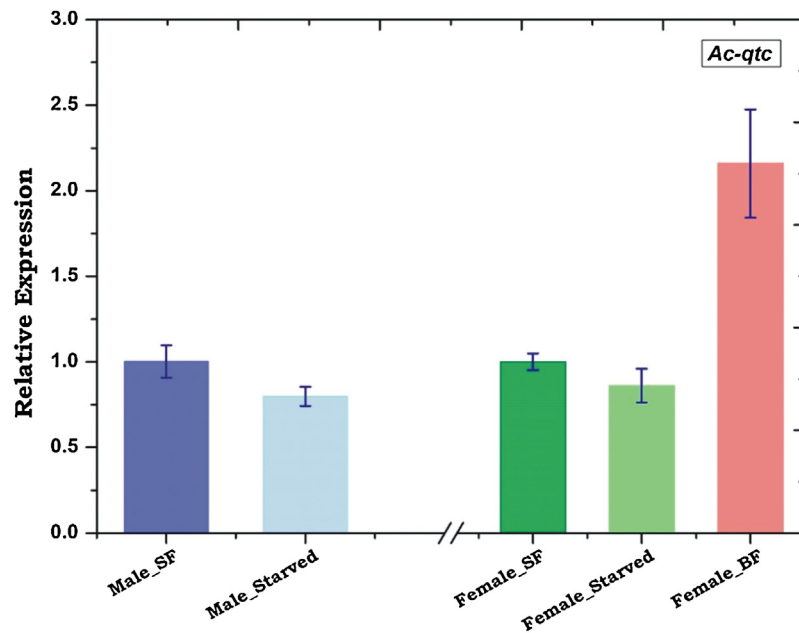


Fig. 6. Effect of starvation on *Ac-qtc* expression: both male and female mosquitoes were kept starved for 16–18 h and the olfactory tissues were collected for *qtc* expression study. Additionally blood meal was provided to female mosquitoes after starvation. SF: Sugar Fed; BF: Blood Fed.

attraction [31]. Thus to test whether *Ac-qtc* has any sex-specific relation to the nutritional status of naïve mosquitoes, we examined and compared the relative expression of *Ac-qtc* transcript in starved and sugar fed mosquitoes in both the sexes. To perform this experiment, we collected olfactory tissue from 5–6-day old sugar fed and 16–18 h starved mosquitoes. Relative gene expression analysis indicated that starvation did not affect the abundance of *qtc* transcript, but showed a two-fold up-regulation ($p \leq 0.03$) in response to immediate i.e. 30 min – 1 h post blood feeding (Fig. 6). Together these data suggested that *Ac-qtc* may not be essential for regulating mosquito sugar feeding behaviour but it may have an important role in the regulation of host-seeking/blood feeding behaviour in the adult female mosquitoes.

3.6. Blood meal alters expression of *Ac-qtc* in adult female mosquito

To further evaluate *Ac-qtc* role in response to blood feeding behaviour of female mosquitoes, we performed a blood meal time series experiment as described earlier [3]. We collected olfactory tissue from *An. culicifacies* mosquito depending on their age and blood feeding status. *Ac-qtc* expression analysis revealed increased abundance till 5th day, but significant (2.5 fold/ $p \leq 0.03$) downregulation on 7th day in the naive unfed adult female mosquitoes. This pattern is similar to the adult male mosquitoes (Fig. 7), which may probably to achieve the optimal courtship behavioural success in both the sexes.

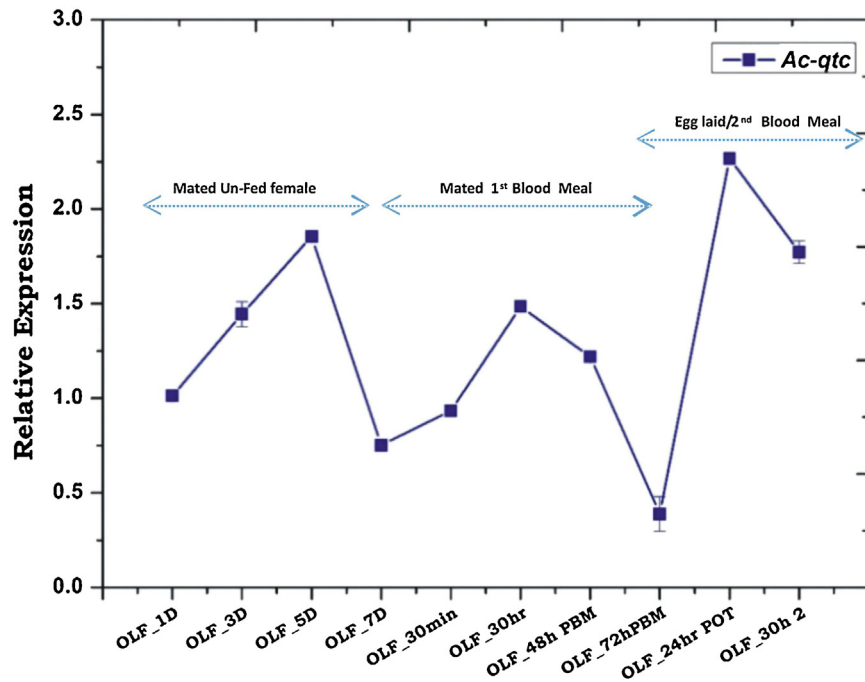


Fig. 7. Transcriptional behaviour of *Ac-qtc* in two consecutive blood meal follow up. OLF-1D – OLF-7D: Olfactory tissue from 1Day – 7 Day old female; OLF-30 min: Olfactory tissue collected from 30 min post blood fed mosquito; OLF-30 h: Olfactory tissue collected from 30 h post blood fed mosquito; OLF-48 h: Olfactory tissue collected from 48 h of post blood fed mosquito; OLF-72 h: Olfactory tissue collected from 72 h post blood fed mosquito; OLF-24 h POT: Olfactory tissue collected from 24 h of post oviposition of mosquito; OLF-30 h 2: Olfactory tissue collected from 30 h of 2nd blood meal. PBM: Post Blood Meal.

Although it is not clear whether the first blood meal transiently and/or completely pauses re-mating events, but a consistent up-regulation of *Ac-qtc* just after blood feeding (within 30 min) till 30 h post blood meal (Fig. 7) indirectly suggested that adult female mosquito may not seek any courtship event at least for the first 30 h post blood meal. Furthermore, the continuous sharp downregulation (~4 fold) of *Ac-qtc* till 72 h post first blood meal still remains questionable. It is unclear, whether *Ac-qtc* promotes host-seeking behaviour for second blood meal and/or it initiates mate partner finding. Significant up-regulation (~1.3 fold; Fig. 7b) of *Ac-qtc* after oviposition and prior to second blood meal also support our hypothesis. Second blood meal again rapidly downregulated the *Ac-qtc* expression, when examined 30 h post blood fed in the olfactory tissue. Together these data indicate that *Ac-qtc* may have a unique role in driving dual mode of behavioural responses possibly to meet the conflicting demand of sexual mate partner and/or finding a suitable vertebrate host for blood feeding.

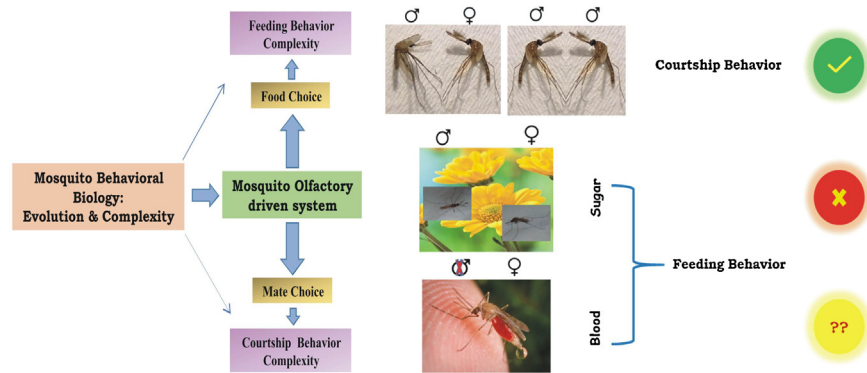


Fig. 8. Proposed hypothesis for the possible function of *quick-to-court* gene in mosquito. Photo credit to James Gathany for the blood fed mosquito picture.

4. Conclusion

Understanding the sex-specific molecular genetics of mosquito's behavioural biology is more complex. This is partly due to unique nature of blood feeding evolution and adaptation in the adult female mosquitoes, and limitation of generating mutants. Through comprehensive molecular approach, we examined the sex-specific transcriptional regulation of a unique transcript *Ac-qtc*, and predicted its possible role with 'food choice' and/or 'mate choice' behavioural performance (Fig. 8). Our data provides the first molecular evidence that *Ac-qtc* proteins may have a dual mode of action in the regulation of a cluster of mosquito olfactory genes that are linked to mating success and/or blood feeding in adult female mosquitoes. We believe these findings may guide to uncover the functional nature of *Ac-qtc* controlling complex mating and/or blood feeding behaviour in mosquitoes.

Declarations

Author contribution statement

Tanwee Das De: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Punita Sharma, Charu Rawal, Seena Kumari, Sanjay Tavetiya, Jyoti Yadav: Performed the experiments.

Yasha Hasija: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Rajnikant Dixit: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

Data associated with this study has been deposited at Genbank under the accession number KX575650.

Supplementary content related to this article has been published online at <http://dx.doi.org/10.1016/j.heliyon.2017.e00361>

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