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Aggressive Behavior and DNA Sequencing of *Acheta domesticus* (Gryllidae: Orthoptera)

Ambreen Afghan[1*], Riffat Sultana[1] and Naheed Baloch[1]**

[1]Department of Zoology, University of Sindh, Jamshoro
Corresponding author's Email:
*riffat.sultana@usindh.edu.pk
**ambreenakhtarafghan05@gmail.com

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Abstract

Male crickets show intensive aggressive behavior when they encounter another male. During this study comprehensive analysis on aggressive behavior in *Acheta domesticus* (Linnaeus) was investigated. It was noticed that male adopted six behavioral responses i.e., pre-established dominance, antennal railing, uni-lateral and bi-lateral mandible dispersal, engagement of mandibles and grappling in order to gain contact to females and to defend his territory was significantly prominent. This aggressive behavior was ignored yet attention was not paid towards this. Beside this, Illustrative Barcode, Nucleotide Sequence, Sequences producing significant alignment, and Distance distribution graph of *Acheta domesticus* was also presented for correct identification of sibling species.

Key Words: Crickets, *Acheta domesticus*, aggressive behavior, Six, dominance, Antennal, DNA sequencing

Introduction

Males in many species of crickets exhibit specialized aggressive behavior. (Laufer, 1972) fighting of crickets has actually has been a popular sport in the Orient region for thousands of the years Kato, & Hayasaka1958). There has been no extensive analytical or comparative study of this parameter, though, recorded a dominance in crickets in the laboratory, (Huber, 1950) explored the physiological and genetic bases for aggressive and sexual behavior in *Gryllus campestris* L. and *G. bimaculatus* De Geer. Sexually responsive females are attracted to the stationary males by their loud, rhythmical stridulations, which are inevitably distinctive among naturally sympatric and synchronic species. Earlier, worked on the inheritance of morphological characters in hybrids between various European and neotropical species of *Acheta* and *Gryllus.* (Khalifa, 1949) sexual behavior of the house cricket, *Acheta domesticus* Gabbut, (1954) behavior and life history of *Nemobius sylvestris* (Sellier, 1956) has briefly summarized on wing polymorphism in Gryllinae and Nemobiinae in Gryllinae and Nemobiinae; (Ghouri & Mcfarlane,1957) investigated macropterous concept in *Gryllodes sigillatus* (Walker and demonstrated reproductive isolation between house crickets of Pakistani and Canadain species which were not previously known to be different (Alexander & Bigelow, 1960) studied aggression between male house crickets, *A. domesticus*, with the goals of knowledge of its six stages determining the potential information content of aggressive displays and the factors determining the initiation, intensity and eventual outcome of aggressive contests. House crickets are considered as a model for learning as animal aggression behavior. Males in many cricket species, including: *A. domesticus*, produce aggressive songs with distinct temporal and frequency structures when they encounter a rival male in their permissive. These aggressive songs production was studied in detailed by Alexander & Bigelow, (1960) in addition they also reported brief-sharp signal that elicits fighting behavior, reciprocal aggressive stridulation, or retreat. Beside this it was noted that aggressive songs are different from one to other cricket songs in their brevity.

Materials and Methods

Sampling: Cricket was collected from different fields such as sugarcane, rice, maize and wheat throughout year. Insect were picked by hand and insect net. Large numbers of specimens were collected from different district of Sindh. Collection was brought to laboratory for further experimental purpose.

Culturing: Cricket *A. domestics* used were raised under laboratory conditions (25°25'0.73"N, 68°16'27.5"E). Specimens were reared to mature at 28±2°C to 39±2°C with relative humidity 28-61% (RH), and photoperiod of 12:12 (L: D) and fed a diet of insect pellets, alfalfa pellets and raw vegetable scraps. We used sexually mature male crickets for all experiments. To reduce the influence of prior fighting experience and to motivate fighting, each cricket was individually separated in a 100 ml glass jars lined with filter paper for 2 days before the experiments. House crickets display peak activity within 3 h of dusk; this life cycle focused their peak activity in the early afternoon, which was convenient for data collection. Male crickets show intensive aggressive behavior when they encounter another male.

DNA Sequence:

Sample collection and preparation: Insects were sampled from rice field between 2018–2020 using both active and passive collecting methods including sweep nets, hand collections, Malaise traps, and pitfall traps. The specimens were sorted to the order and, where possible, to lower taxonomic ranks. Large specimens were either pinned and preserved dry or placed in Matrix tubes filled with 95%

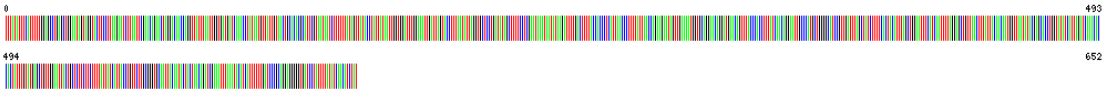
ethanol. Small specimens were individually placed in a well containing 30 µl of 95% ethanol in 96-well microplates. Specimen metadata and an image were submitted to BOLD where the information can be accessed on both the specimen page and on the corresponding BIN page. Voucher specimens are archived at the department of Zoology, university of Sindh (DZSUJ), Pakistan (with sample ID prefix RS-USJ) or at the Centre for Biodiversity Genomics (CBG), Guelph, Canada (with ID prefix BIOUG). Specimens were shipped to Centre for Biodiversity Genomics (CBG), Guelph, Canada for barcoding.

DNA barcoding: *Acheta domesticus* samples were shipped to Centre for Biodiversity Genomics (CBG), Guelph, Canada where specimens were successful barcoded following standard protocols. In brief, a leg was removed with sterile forceps from each large specimen and transferred to a well preloaded with 30 µl of 95% EtOH. As smaller specimens were already in plates, they were ready for analysis, but vouchers were recovered after DNA extraction (Porco et al. 2010). DNA extraction, PCR amplification, and sequencing were performed at the Canadian Centre for DNA Barcoding (CCDB) following established protocols (Ivanova et al. 2006, Hebert et al. 2018, De Waard et al. 2019b). PCR reactions were either 6 µl or 12 µl (Hebert et al., 2013). Three quarters (73%) of the specimens were Sanger sequenced while the rest were analyzed using SMRT sequencing on a Sequel platform (Pacific Biosciences). Sanger sequencing employed Big Dye Terminator Cycle Sequencing Kit (v3.1) on an Applied Biosystems 3730XL DNA Analyzer. Sequences were assembled, aligned and edited using Codon Code Aligner before submission to BOLD. SMRT sequencing employed protocols described by Hebert et al. (2018). The resultant sequences were uploaded to mBRAVE (Multiplex Barcoding Research and Visualization Environment; www.mbrave.net) for editing (sequence trimming, quality filtering, de-replication), identification, and generation of operational taxonomic units (OTUs). The edited sequences were subsequently exported to BOLD for BIN assignment and reference library development. The specimen records, sequence data, electropherograms, and primer details are available in the dataset "DS-INSCTPAK" (dx.doi.org/10.5883/DS-INSCTPAK). All DNA extracts are stored within a DNA archive facility at the

Results

Male crickets exhibit intensive aggressive behavior towards other males in order to gain contact to females and to defend their territory basically there are six aggressive behavior of *A. domesticus* was investigated during this study: Pre-established dominance, Antennal railing, uni -lateral and bi-lateral mandible dispersal and Engagement of mandibles and Grappling. It was examined that in the first stage which is pre-established dominance in this overriding male aggressive is an arrangement of mounting behavior. Aggression levels decline at diverse stages and initiation with two male crickets in the presence of every, lacking act between them. Shared escaping and absence of escalation into aggressive, displays there is no tenacity to display prevailing violence. At times, pre-established domination in a male cricket will display its hegemony by conflicting the other cricket unswervingly. The contrasting male cricket will fly directly lacking mounting the condition to fight while both males fronting each other in antennal railing second stage in this when two aggressive males meet head-on and neither retreat with the initial antennal contact, each then begins to "lash" his antennae rapidly upon the other. This is generally associated with rearing the fore-body, drawing the palpi up and back, spreading the mandibles, and stepping forward. Antennae also display readiness to fight and fighting skill during this time, one antenna will whip continually at the antagonist while the other antennae was relaxed skimming movement. Throughout antennal railing unilateral mandible dispersion occurs in uni –lateral and bi-lateral mandible third and fourth stage of aggressive behavior of insect indicated that males involved in the conflict will show spread mandibles. They similarly use together uni – lateral and bi-lateral mandible (mandible dispersion) beside this in fifth stage insect initiates the engagement of mandibles promptly afterward the scattering of mandibles, the two male crickets drive against each other with force in the last stage i.e., formation of grouping in which tussling consists both crickets' male in an all-out fight. Both challengers mesh mandibles and thrust each other by means of their power and control. This competition was continued until final decision (who is winner and who lost). Males also bite the rival enemy's body parts trying to cause maximum damage. The wrestling phase carries on with repetitive activities, re-engaging and meshing mandibles till there is a strong leader or winner. It was noticed that this flight amongst the male was very interesting and fascistic. Measurement of various body of *Acheta domesticus* has been shown in (Table 1).

***DNA Sequencing of Acheta domesticus Linnaeus*, 1758**



Illustrative Barcode (Table 2 Fig.1)

Nucleotide Sequence:

TTTATATTTCATTTTTGGAGCCTGAGCTGGAATAGTAGGTACTTCCTTAAGAATCTTAATTCGAACAGA
ACTAGGACAACCAGGTTATTTAATTGGTGATGATCAAACCTATAATGTTATTGTAACTGCACACGCATTCG
TAATAATCTTTTTTATAGTTATACCTATTATAATTGGTGGATTTGGAAATTGATTAGTACCTTTAATATTAG
GTGCTCCTGATATAGCTTTTCCTCGAATAAATAATATAAGATTTTGACTTTTACCACCCTCATTAACCCTTT
TATTAACCAGAAGAATAGTTGAAAATGGTGCAGGAACAGGATGAACAGTTTACCCACCTTTATCAACAGG
AATTGCCCATGCTGGGGCATCCGTTGATTTAGCTATCTTTTCACTACATTTAGCTGGAATTTCATCAATTTT
AGGAGCTGTTAACTTTATTACAACAATGGTTAATATACGAGCACCTGGTATATCATTAGATCAAACACCA
CTATTTGTATGAGCTGTTGGAATTACTGCTCTTCTTCTTTTATTATCATTACCTGTTCTTGCGGGTGCAATTA
CAATACTACTAACAGATCGAAATTTAAATACATCATTTTTTGATCCCGCAGGAGGGGGTGATCCTATTTTA
TATCAACATCTAT

Table: 1: Morphometric analyses of various body parts of *A. domesticus.*

Body Parameters	**Mean ± SD (mm)**	
	♂ **(n=5)**	♀ **(n=5)**
Length of head	2.25 ± 0.15	3.26 ± 2.8
Width of head	2.56 ± 0.35	4.2 ± 0.72
Length of pronotum	3.5 ±1.4	3.83 ± 1.50
Width of pronotum	3.15 ±0.35	3.5 ± 0.40
Length of femur	11.0 ± 2.08	14.0 ± 4.11
Width of femur	3.33± 0.57	4.5 ± 1.0
Length of tibia	6.01 ± 1.0	7.33 ± 2.06
Width of tibia	2.0 ± 0.0	2.0 ± 0.0
Length of mirror	4.5 ± 1.73	----------
Width of mirror	4.5 ± 1.73	----------
Length of ovipositor	----------	10.66 ± 2.94
Total body length	15.33 ± 4.2	16 ± 3.05

Table: 2 Sequences producing significant alignment of *Acheta domesticus*

S#	**Species**	**Marker**	**Maximum score**	**Query cover**	**Percent Identification**	**Accession number**
1	Gryllidae sp.	CO1	1206	100%	100.00%	JF838680.1
2	*Melanogryllusbilineatus*	CO1	891	100%	91.27%	MK344775.1
3	*Gryllusbimaculatus*	CO1	856	99%	90.35%	KJ850234.1
4	*Acheta domesticus*	CO1	848	99%	90.28%	MG458975.1
5	*Gryllus campestris*	CO1	846	100%	90.05%	GU706083.1
6	*Gryllusbimaculatus*	CO1	845	99%	90.05%	MW085273.1

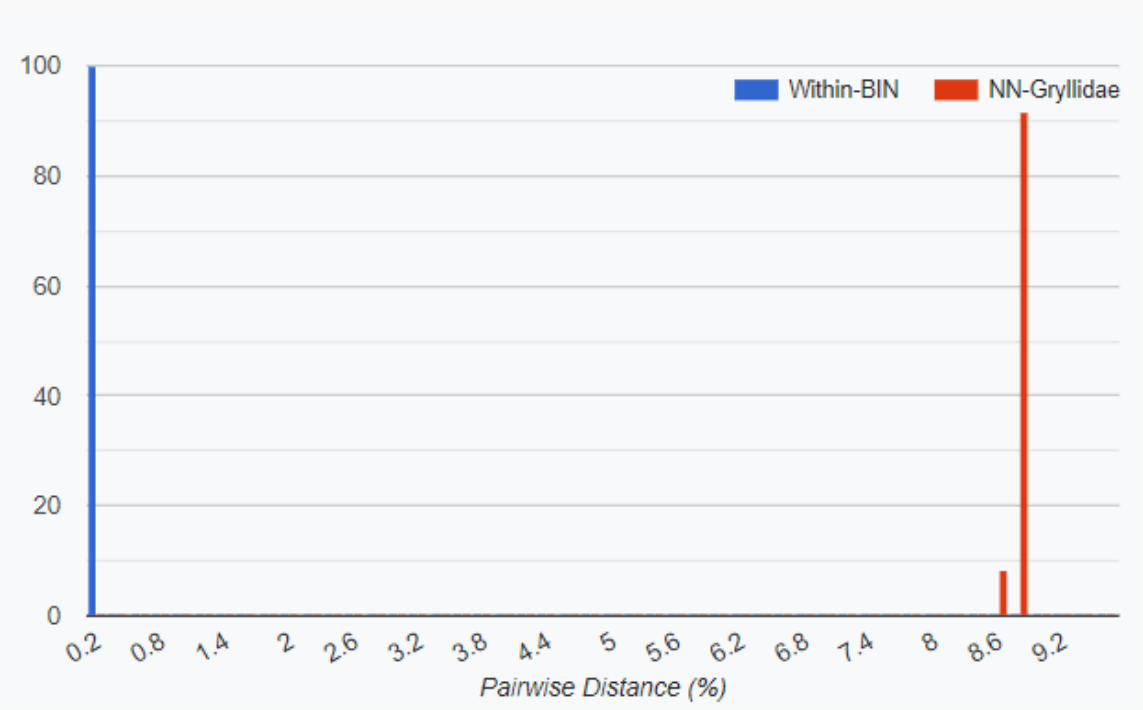


Figure: 1 Distance distribution graph of *Acheta domesticus*

Table:3: Collection of Acheta domesticus various Localities of Larkana from rice field in 2019.

Species	**Locality**						
	Bakrani	**Dokri**	**Ratodero**	**Berochandio**	**Village pathan**	**Village Allahdinopathan**	**Total**
June	639	149	96	101	98	203	1,286
July	423	98	49	193	103	51	917
August	73	13	26	32	7	_	151
September	63	14	8	26	36	_	147
October	6	_	_	_		_	6
November	32	46	36	29	169	53	365
December	3	2	1	2	3	_	11
January	1	_	_	_	_	_	1
February	3	6	1	1	_	_	11
March	6	3	3	2	1	1	16
April	7	3	3	3	_	_	16

Discussion

Insect behavioral interactions, whether with the environment or other animals, have fascinated researchers for a long time. However, over the last four decades, fighting behavior has been studied (Kato & Hayasaka 1958) extensively from an evolutionary point of view using evolutionary game theory At present aggressive behavior of cricket was examined Alexander (1961) it was noted that this aggressive ability is greater in cricket rather than another insect investigated that aggressive behavior in crickets is released by antennal contact detecting cuticular substances between two conspecific males when

a male cricket encounters another male by chance, it exhibits intensive aggressive behavior. (Hofmann & Stevenson, 2000) reported that once the fighting between insects is settled down, the loser (subordinate) will refuse to fight again for a while. According to in insect biogenic amine system is closely linked with agonistic behavior when Octopamine level of the hemolymph might mediate aggression level. Biogenic amines in the insect brain work as neurotransmitters. However, it remains unclear how the biogenic amine system in the central nervous system is mediated during a fight it need further investigation. It was noted that male sing aggressive song that is called rivalry or triumph song a very loud trill was produced during or after combat with another cricket however, cricket females do not sing; however, they are attracted to this signal and approach it in a directed orienting response towards the source of the song CNS these sensory inputs are processed and integrated. At present, no information is available regarding the tactile sensors on the body surface. With regard to antennal sensory integration, mechanosensory neurons in the antennae are considered to project into the ventral area of flagellar afferents which is located in the posterior deutocerebrum in the cricket Staudacher and (Schild berger, 2000) carpenter ant, the female-specific *Basiconic sensilla* on the antennae that are responsible for detecting cuticular hydrocarbons project to specific glomeruli in the dorsomedial region of the antennal lobe (Nakanishi, et al., 2009) anatomical study indicated that the chemical and tactile information pathways from the antenna are definitely dissociated at least at the level of second-order neurons (Yoritsune, & Aonuma 2012) house crickets are considered as a model system for both studies of animal aggression studies its acoustic signaling. Yet remarkably little work has been done to note aggressive songs in *Acheta domesticus* with distinct temporal and frequency structures when they encounter a rival male. However, this aggressive song is described by Aggressive songs differ from other cricket songs in their brevity, often being only a single chirp, although in some instances they may be repeated frequently. These songs are thus a signal of aggressive intent, but beyond their use in the context of aggression, we currently know almost nothing about the information content of aggressive songs in crickets present study recommends that it should be investigated in future.

Table 3 shows monthly update of *Acheta domesticus* of in Larkana Sindh which Start from June up to April 2019 the large and small number of were recorded in different month of year the huge quantities of insect were recorded in June and July and small quantities of insect recorded in January. *Acheta domesticus* destroyed the crop of rice. During field survey also observed aggressive behavior of *Acheta domesticus* displayed in competition for food resources. Table 2 Shows the study and comparison of Different body's parameters of males and females like Width of head, Width of pronotum, length of head, pronotum, Length of femur, width of femur, Length of tibia, Width of tibia, tegmina, Length of mirror, Width of mirror, Length of ovipositor, total body length with tegmina.

Conclusion

The large number of *Acheta domesticus* were collected from various localities of District Larkana in the month of June to July ,2019 The greater numbers were captured from Village Allahdino pathan and village pathan, among them, males were unexpectedly greater in numbers than females. The different body's parameters of both males and females like Width of head, Width of pronotum, length of head, pronotum, Length of femur, width of femur, Length of tibia, Width of tibia, tegmina, Length of mirror, width of mirror, Length of ovipositor, total body length with tegmina were studied which shows that male body parameters are longer than females. The impact of different environmental factors like temperature, rainfall on the abundance of *Acheta domesticus* were also studied.

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