Examining life-history traits, behavioural and neural responses to acoustic stimuli in *Acanthogryllus asiaticus*

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Certificate of Examination

This is to certify that the dissertation titled "Examining life-history traits, behavioural and neural responses to acoustic stimuli in *Acanthogryllus asiaticus*" submitted by Ms. P Prathibha (Reg. No. MS15050) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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Dated: May 4, 2020

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Manjari Jain at the Indian Institute of Science Education and Research Mohali and Dr. Joby Jospeh at University of Hyderabad.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

P Prathibha

Dated: May 4, 2020

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Manjari Jain

(Thesis Supervisor)

Dr. Joby Jospeh

(Thesis Co-supervisor)

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List of Figures

List of Tables

Acronyms

LDMC – Long distance mating call

PTG – Pro-thoracic ganglion

HVc - Caudal hyperstriatum ventral

DTAM - Dorsal tegmental area of medulla

rRpd - Dorsal nucleus raphe

IEG *zenk* – Immediate early gene

SPL – Sound pressure level

ON1 – Omega neuron 1

AN1 – Ascending neuron1

AN2 – Ascending neuron 2

DAQ – Data acquisition card

List of contents

Abstract

Signals are units of information used by animals to communicate with each other and acoustic signals are widely used as a modality of communication. Crickets are nocturnal members of orthopteran family. They produce sound by stridulating their wings, and different calls are produced in different behavioural contexts. Mate attraction is one of behavioural context in which the sound signals are produced. In crickets, females hear the signal and respond to the call by walking towards the caller and are known to show preference for certain spectral and temporal parameters of the call. There is a dedicated neural circuitry which plays an important role in the perception and recognition of the signal. The life-history traits of cricket are known to affect its signalling, choice and reproductive success.

In this study, I have used a field cricket *Acanthogryllus asiaticus* to answer specific questions. I have looked upon the life-history traits of males and females in a lab-monitored population and compared them between sexes and the correlations within these traits in each sex. I have examined for female preference for chirp durations indicative of calls from males of different age classes. I also examined the neuronal response to heterospecific acoustic stimuli. The study suggests that in a lab-monitored population, the life-span, body-length, pronotum-length and pronotum width does not differ between sexes, whereas the parameters of hind leg size were significantly larger for females than males. In males, the wing size is found to be negatively correlated with life-span of the individual, and positively correlated with body size. In females, the ovipositor size was found to be positively correlated to pronotum length. Thus, the study suggests that there is sexual dimorphism in the hind leg size and how different sizes of body parts are correlated with each other. The female choice study indicates that females show no differential preference for the calls with chirp durations indicative of males from different age classes. This study indicates that there is no preference based on age, but it has to be further tested using other temporal parameters characteristic of the age. The study on neurons indicates that there are neurons which register heterospecific signals, but more data is required to say anything conclusive.

Chapter 1

General Introduction

1.1 Animal Communication and acoustic signalling

In the animal kingdom, it very common to find an organism producing a signal (signaller) which conveys information to another organism. Another organism receives this signal (receiver) and respond to it and this flow of information between animals is called 'animal communication' (Bradbury and Vehrencamp 1998). It is used in different behavioural contexts by both solitary and social animals and plays an important role in maintaining intra and inter-specific interactions and bonding. Social organisms communicate to maintain group cohesion, during intra and inter-group interactions, and also to pass on information regarding resource availability and potential threat. For instance, Zebra-fishes uses visual cues to join a shoal and also to maintain inter-individual distance in the shoal (Larsch and Baier 2018) and golden brown mouse lemurs use acoustic signals to maintain intra-group cohesion and coordination (Braune et al. 2005). Honeybees use dance language to communicate the location and quality of a potential food source (Frisch 1974). *Solenopsis saevissima* produces a trail substance from its Dufour's gland and uses pheromone trails to recruit members for mass foraging (Wilson 1962). Meerkats have atleast one individual positioned at an elevated position who produces alarm calls in response to potential threat (Morgan 1984) and Vervet monkeys produce predator specific alarm signals to alert their group mates (Seyfarth 1980). In golden brown mouse lemurs, olfactory cues are used to mark sleeping territories to reduce inter-group conflict (Braune et al. .2005). Solitary animals communicate to attract mates, during agnostic interactions, and so on. For instance, male tungara frogs produce auditory whines to attract females, and chucks are added to this call in the presence of other males to make the signal more attractive (Ryan 1985). Male peacock spiders, *Maratus volans*, use vibratory signals along with body ornaments and motion displays as part of courtship display (Girard 2011). In 61 species from 10 families of moths, females produce sex pheromones to attract males at long distance (Mayer and

McLaughlin 1975). In the context of aggression, both sexes of a primitive fiddler crab, *Uca vocans* use their claws to wave to other competitors (Salmon 1984), male sticklebacks may use their red belly as a visual display for aggression (Tinbergen 1951). Roaring contests in red deer is also observed in the context of male-male assessment (Clutton-Brock and Albon 1979)

Signals are the units of information for communication in animals (Smith and Harper 2003) and can manifest in multiple modalities. The signal can be an indicator of the environment around the signaller. For instance, *Anolis gundlachi* has a hump-shaped display rate with increasing temperature as the display rate increased and then decreased with increasing temperature in agreement with the performance hypothesis (Ord et al. 2017). The chirp rate of the New World thermometer crickets follows a linear relationship with temperature (Walker and Collins 2010). The signal can also provide information about the quality of the signaller and this is called honest signalling (Petak 2019). For instance, in wolf spiders *Hygrolycos rubrofasciata*, which produce audible drumming for mate attraction, the signal length might be a reliable indicator of male quality (Rivero et al. 2000). The white tail spots in the male rock sparrow, *Petronia petronia* are an indicator of male quality and is abraded when the male is in worse condition (Griggio et al. 2011). Signals sometimes provide misinformation, as seen in fiddler crabs who bluff by growing oversized claws with less mass after losing their useful claws in a fight (Backwell et al. 2000). Similarly, in a fig wasp which uses the mandible gap width (dependent on mandible length) as a cue for assessing opponents, there is a morph which has atypically longer mandible for given body size which might cause other wasps to retreat, but they are less likely to win fights and this is an example of dishonest signalling (Moore et al. 2009).

Animals use different signalling modalities to communicate like visual, auditory, tactile, and olfactory. Acoustic signalling is one of the most successful modes of communication and is observed in numerous animal taxa as it can carry information over long distances with very little signal loss (Bradbury and Vehrencamp 1998). Acoustic signals are sound waves generated by vibration of surfaces and transmitted through compression and rarefaction of particles in an elastic medium (Davidovits 2018). Invertebrates, terrestrial and aquatic vertebrates use acoustic communication, and the sound signals used by different animals span a wide range of frequencies. For instance, Asian elephants produce calls in the frequency range of 14 - 24 Hz (Payne et al. 1986), songs of red vented bulbul are in the frequency range of 0.98 - 4.5 kHz (Kumar 2004), in cichlid *Tilapa mossambica*

frequencies of the sound varied from 1 – 16 kHz (Lanzing 1974), bats and dolphin produces calls with ultrasonic frequencies (Pierce 1938, Au 2012). Acoustically active animals have specialized organs (syrinx in birds, vibrating laryngeal tissue in mammals, etc.) which produce these sound signals and have specialized neural circuitry and genetic pathways which play an important role in the sound production and reception (Herbst 2016, Goller et al. 1997, Ryan et al. 2014, Fagerlund 2004).

1.2 Life history and signal production

An organism's life history is its pattern of storing reserves, growth, differentiation and reproduction (Harper and Begon 1990). Life history traits include factors such as the number, size and sex ratio of offspring, the timing of reproduction, age and size at maturity and growth pattern, longevity, and so on (Choe 2019). The genetic parameters of life-history traits are dependent on the environment which is highly variable, so they are less heritable than physiology, behaviour, and morphology (Mousseau and Roff 1987, Price and Schluter 1991). However, life-history hypothesis suggest that these traits can be under natural selection and these traits should vary (for example, increase in fecundity, reduced mortality) if fitness is enhanced due to their occurrence (Roff 1992). For instance, a comparative study indicates that decrease in annual adult mortality and modal clutch size, like life-history traits, might have driven the evolution of cooperatively breeding in certain bird families (Arnold and Owens 1998).

Life-history traits are constrained by an animal's genetic makeup, mechanical constraints, immediate environment, resource availability, and developmental conditions, all of which can be grouped into intrinsic and extrinsic factors (Roff 1992). One of the life-history trait which is influenced by the extrinsic factors is body morphometry. The size of the body and morphological structures can be under a genetic mechanism but is heavily dependent on various extrinsic factors like the environmental stress, predation pressure and so on. For instance, individuals of *Parus major* found at high altitude have greater body weight and larger overall body length (Shao et al. 2016), tree-hole mosquito larva in drying up ponds eclose earlier as smaller individuals (Juliano and Stoffrengen 1994), and Costa Rican livebearing fish were smaller under higher predation pressures (Johnson and Belk 2001).

Body size parameters can be a symbol of quality or dominance. For example, larger females of *Anagyrus kamali* lived longer and laid more eggs when compared to smaller females

(Sagarra et al. 2001). Similarly, larger *Anolis sagrei* males defended their perch sites successfully and entered a new male's territory in comparison to smaller males (Tokraz 1985). Age is another life-history trait which can act as a symbol of quality. For instance, older males in polygynously breeding birds enjoy higher mating success than younger males (Weatherhead 1984). Similarly, age can be a symbol of dominance in some organisms. For example, in assamese macaques the younger males groom the older male and are usually subordinate to the older males (Bernstein and Cooper 1999).

Life-history traits can influence acoustic signal produced by an organism. Old individuals can either get weary, thus reducing the quality of the signal. Older Baboons produced calls with a lower fundamental frequency and "hoo" calls with shorter duration (Fischer et al. 2004). However, in some cases older individuals have better quality acoustic signals. Older male canaries have a larger song repertoire than younger, which might be because of learning (Nottebohm and Nottebohm 1978). In the banded wren *Thryophilus pleurostictus*, note consistency, bandwidth and note rate increase with age (Vehrencamp et al. 2013). Size of body and sound-producing structures can influence signal production and thus the signal. For instance, in neotropical bird species, frequency is negatively correlated with the body size of the bird (Martin et al. 2011). The average difference between successive formant frequencies is correlated with vocal tract length and body size in *Rhesus macaques* and *Colobus guereza* (Fitch 1997, Harris et al. 2006). Females can have a preference for signal parameters which correlates with life-history traits. Female red deer stags show preference to roars with lower minimum formant frequencies indicative of larger individuals (Reby and McComb 2003, Charlton et al 2007).

1.3 Female preference in acoustic signalling

Darwin defined sexual selection as either a competition between one sex for the members of other sex or as a differential choice for some members of one sex by the other sex. The sex which provides less parental investment compete within themselves to mate with the sex that provides more investment (Trivers 1972). In most animals, females invest more resources in the offspring than males (for exceptions see Fiedler 1954). If mate choice influences immediate reproductive success, then natural selection should favour females exhibiting preference that maximize this parameter of fitness (Sullivan et al. 1995). For females, mate quality is the most important factor which affects their reproductive success (Bleu et al. 2012). Females have different tactics to reject a male either pre-mating or post-

mating. In a seaweed fly, *Coelopa frigida*, females try to reject the mounted male by kicking and downward abdominal curling, thus assessing the male quality and only allowing a stronger and larger male to mate (Crean et al. 2000). Sperm and ovarian fluid interaction reduce the velocity of sperms from a related individual in guppies (Gasparini and Pilastro 2011).

In signalling organisms, the choice can be exercised before mating, as the female can perceive and evaluate these signals. Evolution of extravagant traits and signals in the context of mate attraction is explained using the differential responses of females (Zahavi 1975, Fisher 1930). Females are known to show a preference for specific signal parameters in mate attraction signals. For example, females of *Serinus canaria* showed higher response for a phrase with two brief and complex syllables (Vallet et al. 1998), females of great reed warblers showed a higher preference for the long call with higher repertoire (Catchpole et al. 1986) and females of gulf coast toad have a higher preference for male calls with longer calls and faster rates (Wagner and Sullivan 1995).

Female preference can stem from bias for a parameter. For instance, the female preference for red throat and jaw in three-spined stickleback might be an artefact of an intrinsic attraction to red objects (Smith et al. 2004). This bias might have arisen in the context of foraging as both male and female sticklebacks have showed preference for orange fruits (Rodd et al. 2002). Female also shows a preference for signal characters which relay information about the quality and dominance of a male. For instance, in *Acheta domesticus*, females show a preference for number of syllables, which is an indicator of haemocyte load, which in turn is an indicator of better immunity (Gray 1997, Ryder 2000). Females of Hermann's tortoises prefer high-pitched faster calls typical of smaller individuals who enjoy higher reproductive success (Galeotti et al. 2005). Similarly, females of red deer prefer males roaring at faster rates, which is associated with fighting ability and higher reproductive success (McComb 1991).

Female preference can also be dependent on their life-history traits and the environment. In house crickets, female preference changed with age as only younger females showed a preference for faster chirps (Gray 1999). Females from small broods showed stronger preferences for male songs than those from medium and large broods in Zebra finches (Riebel et al. 2009).

The understanding of how these signals are perceived and processed by the females, along with behavioural studies, can shed more light on female preference for different signalling parameters.

1.4 Neuroethology of acoustic signalling

Animals producing acoustic signals have neural circuitry dedicated to initiating signalling. Animals also have neural circuitry to control the locomotor muscles needed for sound production. In canaries and songbirds, a unilateral lesion in the caudal hyperstriatum ventrale (HVc) results in severe song deficits, and histological studies have traced nerves from HVc to two discrete nuclei in the forebrain which further projects to the motor nucleus innervating the motor syrinx (Nottebohm et al. 1976, Vicario 1991). In *Xenopus laevis*, the laryngeal muscles are innervated by the motor neurons of the caudal medulla, which is connected to dorsal nucleus raphe (rRpd) and candidate vocal via the dorsal tegmental area of medulla (DTAM) and this circuitry possibly initiates calling (Brahic and Kelly 2003, Wetzel et al. 1985).

Sound perception includes detecting and identifying the biological meaning and relevance of the sound (Heffner and Heffner 1992). Receivers have specialized hearing structures with receptors which can detect the sound signal. For example, tympanal organs in seven orders of insects (Hoy and Robert 1996), and Johnston's organ in the second antennal segment in *Drosophila melanogaster* (Todi et al. 2004). Similarly, anurans have basilar and amphibian papilla (Schoffelen et al. 2008), and locusts have a pair of eardrums with 70 bipolar sensory neurons on each side of the first abdominal segment (Gray 1960). The auditory receptors in these structures register the stimuli depending on how these neurons are tuned. For instance, in anurans, multiple auditory nerve fibres which innervate the papillae are tuned to different frequencies, the low and mid-frequency sensitive fibres are derived from the amphibian papilla and high-frequency sensitive fibres are derived from the basilar papilla (Feng et al. 1975, Lewis et al. 1982). Similarly, the auditory never fibres of treefrog discharge at phase following a linear relationship with the stimulus frequency (for lower frequencies) even in the absence of basilar membrane (Hillery and Narins 1984).

Different auditory interneurons receive signals from the receptors and encode different parameters of the signal. For example, in anurans, call recognition in spectral-domain is done in the central auditory system, whereas central thalamic nucleus is an important center

for call recognition in time-domain (Feng et al. 1990). In birds, the neurons in field L region and caudal mesopallium in the forebrain, and dorsal lateral nucleus of mesencephalon in the midbrain are tuned to the spectro-temporal modulations of natural sounds (Theunissen and Shaevitz 2006) In crickets, grasshoppers and bushcrickets, the sensory receptors and auditory neurons are tuned to a range of frequency and copies the temporal structure of the stimulus in their spiking pattern (Henning et al. 2004).

The perceived stimuli are associated with specific referents (individual-specific signal, conspecific signal), and is a prerequisite for decisions involved in behaviours like female choice (Genter 2004). The tonotopically organized field L complex in the principal auditory forebrain in songbirds shows a stronger response for the conspecific call (Grace et al. 2003). The caudal medial mesopallium is selective for the frequency modulation of the conspecific song and also shows an increase in the expression of immediate-early gene *zenk* (IEG zenk) in European starlings (Genter 2004). In Anurans, auditory neurons in torus semicularis (auditory midbrain) shows long term integration of response to different acoustic elements which results in strong selectivity for temporal patterns of acoustic signals that represent particular conspecific call types (Alder and Rose 1998).

Neuronal activity (such as tuning, inhibition. and overexpression) can also be useful in understanding the proximate causes driving female choice. Some neurons are tuned to a certain parameter of the signal and the tuning of neurons can influence the female choice. For example, in *Acri crepitans* the neurons of the basilar papilla are tuned to a dominant frequency lower than the mean dominant frequency of the signal and females also show a preference for lower dominant frequency calls (Ryan and Keddy-Hector et al. 1992). The other neuronal response which can influence preference is inhibition of firing in specific neurons. For example, in a synchronizing cricket, *Mecopoda elongate,* contralateral inhibition of Omega neuron1 on one side by the other side can form the sensory bias for female preference for leading signals (Romer and Hedwig 2002). The overexpression of genes present in auditory neurons can also explain the proximate cause of preference. For example, the number of ZENK (a protein expressed in response to conspecific signals) expressing cells in the ventral caudo-neostriatum was higher for longer songs and thus explaining the strong preference for longer songs in females of European starlings (Genter and Hulse 2000, Genter et al. 2001).

1.5 Summary of literature review

Animals produce acoustic signals in different behavioural contexts, and mate attraction is one of them. These signals are influenced by different life-history traits like morphometry and age. These traits are affected by extrinsic factors like nutrition, temperature, predation pressure and many more. Females perceive these signals and can have a preference for certain signal parameters which indicate different life-history traits of the males. The life-history traits of the female can also influence her choice. Acoustically active animals have dedicated neural circuitry for sound production and perception. The neural circuitry involved in sound perception encodes the information in the spectral and time domain of the signal for recognition of behaviourally relevant sounds. The selective discharge of neurons for specific parameters can help explain the proximate causes of female preference.

1.6 Crickets as a model system

1.6.1 Introduction

Crickets are nocturnal members of the Orthopteran family, which use acoustic signalling as a mode of communication. In crickets, only males produce acoustic signals, and the females are mute. A male cricket produces sound by stridulating (an action of rubbing body parts together) their forewings together. The wing has minute structures such as a plectrum, different veins, files and a harp. The sound wave of a particular frequency is produced when the plectrum of one wing progress through the files of the other wing, which is amplified by the veins which also resonate at the same frequency (Pierce 1948). Both the wings have these structures, but sound is produced when the plectrum on the left forewing is swept across a row of files in the right forewing (Sales and Pye 1974). This asymmetry in function is because the right forewing lies over the left forewing, and thus only files on the ventral surface of the right forewing can be struck against the plectrum of the left forewing (Forrest, 1987). The sound produced by this motion is further amplified by a triangular structure called harp cells (Bennet-Clark 1970, Michelsen and Nocke 1974).

Crickets produce acoustic signals in different behavioural contexts and haven categorised into:

1.Long-distance mating call (LDMC) (Alexander 1962): This is the most commonly produced acoustic signal. Males produce loud signals to attract conspecific females who might be at a distance.

2.Courtship call (Alexander 1962): This is a softer short-range call. This call is produced when a female is in the near vicinity of the male. It plays an important role in inducing the female to mount the fame in *Teleogryllus oceanicus* (Balakrishnan and Pollack 1966).

3.Post-copulatory call (Alexander 192): This call is produced after copulation in the context of mate guarding to prevent the female from removing the spermatophore (Alexander 1967).

4.Aggressive call (Alexander 1961): This call is produced during agonistic interaction between two males.

1.6.2 Acoustic characteristics of cricket call

Each closing stroke of the wings produces a sound wave (Ewing and Hoyle 1965), which is the basic unit of the cricket call called a syllable. Syllables are repeated over time to form a chirp. The chirp is repeated over time to form the Cricket call. The call can be structurally characterised using different parameters:

Temporal Parameters

- Chirp duration: time duration from the onset of a chirp to its offset
- Chirp period: time duration from the onset of one chirp to the onset of the next chirp
- Chirp repetition rate: inverse of Chirp period
- No of syllables in a chirp
- Syllable duration: time duration from the onset of a syllable to its offset
- Syllable period: time duration from the onset of a syllable to the onset of the next syllable

Spectral Parameters

- Peak frequency: the frequency of the acoustic signal with the maximum energy

Sound Pressure Level (SPL): it is a measure of the relative amount of energy present in the call.

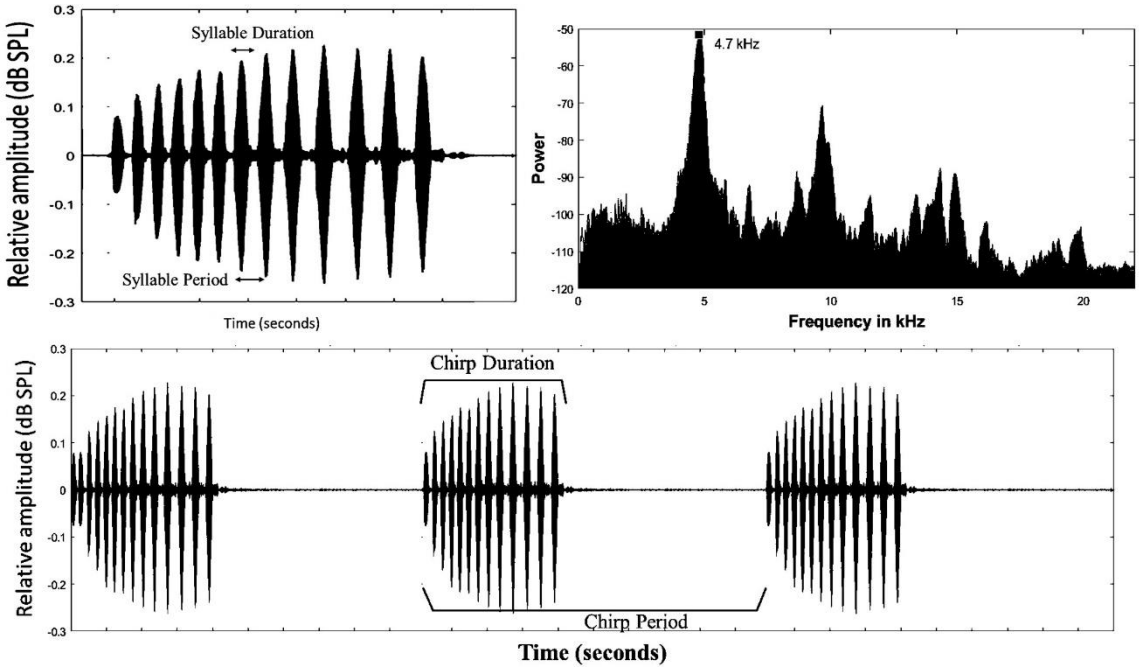

Figure 1.1: Acoustic characteristics of long- distance mating call (LDMC).

Crickets have specialized hearing structure on their forelegs to receive these acoustic signals called a tympanum (Hoy and Robert 1996). The membrane of the posterior tympanum act as pressure receptors (Huber et al. 1989). The directionality in crickets is achieved by the pressure difference between ipsilateral and contralateral spiracles and ipsilateral and contralateral tympana (Michelsen *et al.* 1994).

1.6.3 Studying life history, behavioural and neuronal response using the cricket as model system

Crickets are a good system to study life-history traits since they can be reared and monitored in the lab. The outer layer of crickets is made of chitin and the size does not change much after the final moulting, and the body measurements can be done on dead individuals (Zajitschek et al. 2009). Crickets are very susceptible to changes in the environment, and these changes are known to affect different life-history traits in crickets. For instance, temperature-dependent changes in chirp rate were steeper in individuals reared in 24 degrees Celsius compared to individuals reared in 31 degrees Celsius in a striped ground cricket (Olvido and Mousseau 1995). Similarly, male *Gryllus lineaticeps* on

high-nutrition feeding regime produced calls with higher chirp rate (Wagner and Hoback 1999). Signals produced by the male can also be a proxy of life-history traits in crickets. The frequency of the call is correlated with the size in *Requena sp.5* (Wedell and Sandberg 1995). In *Gryllus bimaculatus*, calling effort is positively correlated with body size but negatively correlated with temperature (Verburgt et al. 2011).

Since female actively responds to the signal by approaching the caller, it is a good system to study female choice. Females have shown preference for different signal parameters by responding to them differentially. For instance, females have shown to have a preference for louder calls in mole crickets (Forrest 1983). Similarly, females of *Acheta domesticus* have shown preference to calls with higher chirp rates (Stout and McGhee 1988). In *Acheta domesticus*, females have a preference for number of pulses per chirp which is an indicator of body size, but in *Oecanthus henryi*, the female does not show a preference for the lower frequency with correlates with larger a body size (Gray 1997, Deb et al. 2012).

Crickets are a good system to study neural responses to acoustic stimuli. The circuitry of acoustic neurons in crickets is well studied, and the pro-thoracic ganglion is easily accessible from the ventral side. The neurons in the pro-thoracic ganglion receive the signal from the auditory receptors in the tympanum (Huber and Thorson 1985). There are six auditory interneurons identified in the ganglion and omega neuron 1, ascending neuron 1, registers the signal with syllable level detail (Wohlers and Huber 1982). The ascending neuron 1 (AN1) is tuned to the carrier frequency, whereas the ascending neuron 2 (AN2) is tuned to high frequencies, and both the ascending neurons send the signal to the brain (Schildberger 1984). The brain recognizes the pattern by using a delay line detection, i.e., a set of neurons in the brain introduce a time delay (the time interval between two syllables in a conspecific call) to the signal from AN1 and this is correlated with the signal direct from AN1, and if both the signals correlate then another set of neurons spike (Schöneich 2015). Since the processing pattern recognition of stimuli is understood, crickets can be used to study the neuronal basis of female preferences.

1.7 Objectives

The research questions that I have tried to address in this study are,

1. To investigate life-history traits such as adult lifespan and body morphometry and their correlation with each other in a lab monitored population.

2. To examine female preference for chirp duration of LDMC of certain age classes of male.

3. To standardize extracellular tetrode recording from pro-thoracic ganglion and to investigative neural responses to heterospecific acoustic stimuli.

1.8 Study system

The system used to address the objectives mentioned above is a field cricket, *Acanthogryllus asiaticus*. The morphological features of this species are (Gorochov 1990);

"Body size small for genus. Head large, red along entire length and angularly bent clypeal suture, apex of angle approximately at level or lower margins of antenna] pits. Color of head dark brown, with 6 distinct short longitudinal pale lines on posterior part of vertex. Pronotum dark brown, with pale spots in posterolateral angles of disk. Elytra with rather transverse stridulatory ridge, more or less rounded speculum, and distinctly bent diagonal vein, area between diagonal vein and oblique veins relatively wide. Color of elytra pale brown, with dark brown stripe along upper margin of lateral area. Legs, abdomen, and cerci brownish, more or less unicolorous. Genitalia without process in middle part of posterior margin of epiphallus, with very short epiphallic apodemes, and with middle processes extending from distal half or ectoparamere and only slightly extending beyond anterior ends of ectoparameres."

Acanthogryllus asiaticus produces long-distance mating call, courtship and post-copulatory calls, the mating call have chirps with 13-14 syllables, and their peak calling time is 9-12 pm in the night (Singh and Jain 2020). The females in this species have a preference of a call louder by at 6dB (Jain master thesis dissertation, 2019).

A taxonomic characterization of *A. asiaticus* is as follows:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insects

Order: Orthoptera

Suborder: Ensifera

Superfamily: Grylloidea

Family: Gryllidae

Genus: *Acanthogryllus*

Species: *Acanthogryllus asiaticus*

Figure 1.2: *Acanthogryllus asiaticus*, left-male, right- female.

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Chapter 2

Examining the correlation of body morphometry with adult lifespan

2.1 Background

The life-history theory suggests that the life-history traits which are under natural selection will increase or decrease depending on their fitness benefits and is dependent on both extrinsic and intrinsic factors (Roff 1992). In crickets, body size and lifespan are two life-history traits which have been shown to affect reproductive success. For example, larger males of *Gryllus bimaculatus* and *Gryllus firmus* enjoy higher lifetime mating success (Simmons 1988, Saleh et al. 2014). Similarly, in *Gryllus pennsylvanicus*, males who lived longer showed higher calling effort, which females preferred (Judge et al. 2008). Various extrinsic factors, like rearing and monitoring conditions, can also affect body morphometry and lifespan. For instance, high protein level diet in *Teleogryllus commodus* increases and decreases longevity in females and males respectively (Hunt et al. 2004). Individuals reared at higher temperature had longer wings (macropterous), and rate of macroptery was found positively correlated with nymphal population density (Olivido et al. 2003).

Since selection pressure on both the sexes can be different (dimorphic niche hypothesis-Slatkin 1984), the morphological traits and longevity of both males and females can be different from each other. For example, males of *Acheta domesticus* had a larger head in comparison to females (Walker et al. 2008). Similarly, females of *Teleogryllus commodus* lived longer than males (Zajitschek et al 2009). Body parameters can also be correlated to each other, allowing morphometric measurements of certain structures to act as a reference for the selection pressure. For example, harp area, and wing area are positively correlated with pronotum width in four cricket species and their wing morphologies are under the stabilizing selection since females show strong preference for frequency (Moradian and Walker 2008).

Singh et al. 2020 have already looked at the correlation of morphometry (pronotum length, pronotum width, wing length and size of sound producing structures) and call parameters of males of this species. The effect of rearing temperature on morphology and development also have been investigated (Singh et al unpublished). In this objective, I have looked at the body morphometry of males and females in a population of crickets grown in lab along with crickets collected as nymphs from the field. I have looked at femur length, femur width, and tibia length along with the other parameters already looked at. All the adults were given standard temperature, humidity and diet and these parameters were compared across sexes. I have also looked at how age and different morphometric traits correlate with each other.

Objective 1: To investigate life-history traits such as adult lifespan and body morphometry and their correlation with each other in a lab monitored population.

2.2 Methodology

2.2.1 Collection and housing crickets

Sub-adults were collected from the field in IISER Mohali, Punjab, India (30°39'N, 76°43'E), and Mohali has a humid sub-tropical climate and habitat is predominantly grass. Sub-adults were also collected from lab grown cultures. The sub-adults were then kept in a large plastic container (35 X 25 X 12 cm) until their date of final moulting. The lid covering the plastic container had a (10 X 10 cm) hole covered using mosquito screening mesh to allow air circulation. On that date, adults were separated into boxes (diameter 12 cm and height 5 cm) with a mesh (9 X 8 cm) lid (ensuring social isolation) with dog food and wet cotton balls for food and nourishment (temperature at 24 degrees Celsius and humidity at 40-70 per cent). Each adult was given an individual identification code.

2.2.2 Adult lifespan and body morphometry measurements

The date of final moulting and of death was noted for each individual. Adult lifespan is defined as the total number of days an individual survived after its final moult till its death. When the individual died, they were preserved in 70 percent ethanol to prevent the degradation of tissue. Body morphometry measurements were done on these preserved specimens since body measurements do not change after final moult (Zajistschek et al.

2009). The morphometric measurements were done using a digital camera (Leica MC120HD, Leica Microsystems GmbH, Wetzlar, Germany) connected with Leica Stereo Zoom Microscope (M 205C, Leica Microsystems GmbH, Wetzlar, Germany). The parameters considered were: Body length, Pronotum length, Pronotum width, Wing length, Femur length, Femur width, Tibia length and Ovipositor length for females.

2.2.3 Statistical analysis

All Statistical analyses were conducted in R Studio (R version 3.6.2). Shapiro-Wilk test was done to check the normality of adult lifespan and other morphometric parameters. A t-test was done to compare the body length, pronotum length, wing length, femur length, femur width and tibia length across sexes. A Wilcoxon rank-sum test was done to compare the adult lifespan and pronotum width across sexes. The correlation between different parameters was checked using Pearson's coefficient and Spearman's rank-order correlations.

2.3 Results

Figure 2.1: Images of male and female cricket (scale bar - 2mm): A) Left: an adult male, Right: right hind leg of the same individual. B) Left: an adult female, Center: right hind leg of the same individual and Right: The ovipositor of the same female.

Life history trait	Males	Females	Statistical test	p-value
Adult lifespan (days)	77.42±34.79	66.5±23.06	Wilcoxon rank-sum test	0.081
Body length (mm)	16.52±1.46	16.47±1.25	t-test	0.925
Pronotum length (mm)	2.66±0.23	2.74±0.20	t-test	0.347
Pronotum width (mm)	4.66±0.36	4.82±0.29	Wilcoxon rank-sum test	0.333
Wing length (mm)	10.02±0.81	9.35±0.57	t-test	0.016
Femur length (mm)	8.71±0.69	9.24±0.46	t-test	0.025
Femur width (mm)	3.32±0.27	3.64±0.25	t-test	0.003
Tibia length (mm)	5.86±0.50	6.26±0.54	t-test	0.046
Ovipositor length (mm)	-	5.78±0.34	-	-

Table 2.1: Mean ± SD (Standard deviation) of adult life span (for 109 males and 92 females) and six morphological traits in males (N=14) and females (N=16) and comparative analysis for these traits between sexes.

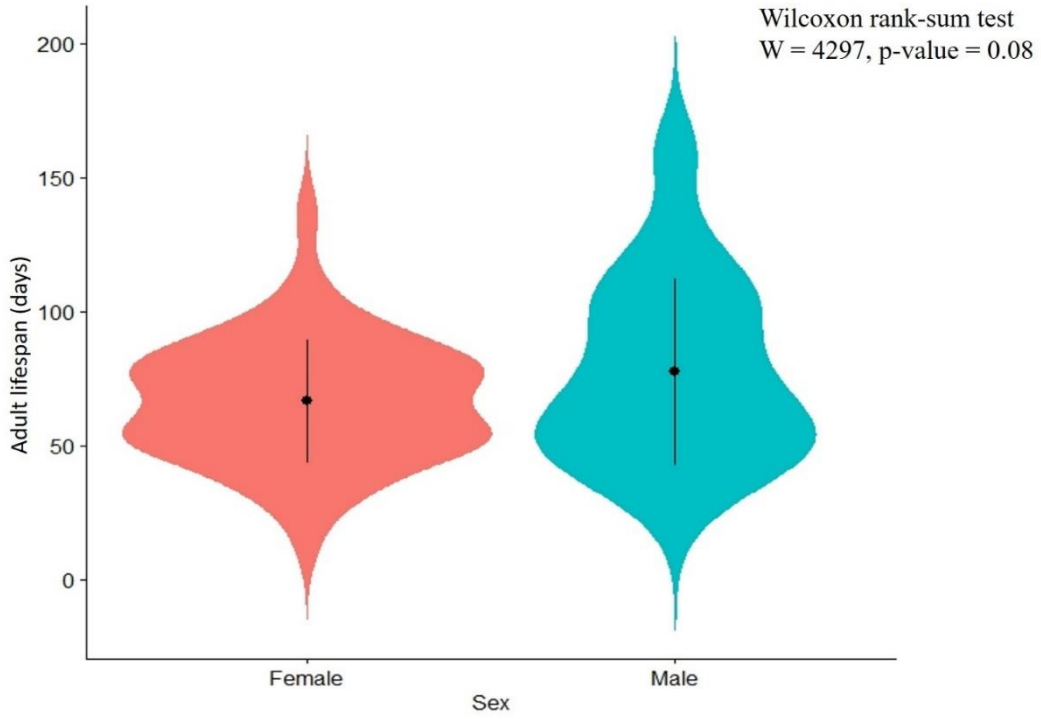

Figure 2.2: Violin plot of adult lifespan of lab monitored males (N=109) and females (N=92): this graph shows the distribution of lifespan in both the sexes. Both, males and females survived for similar number of days in lab conditions.

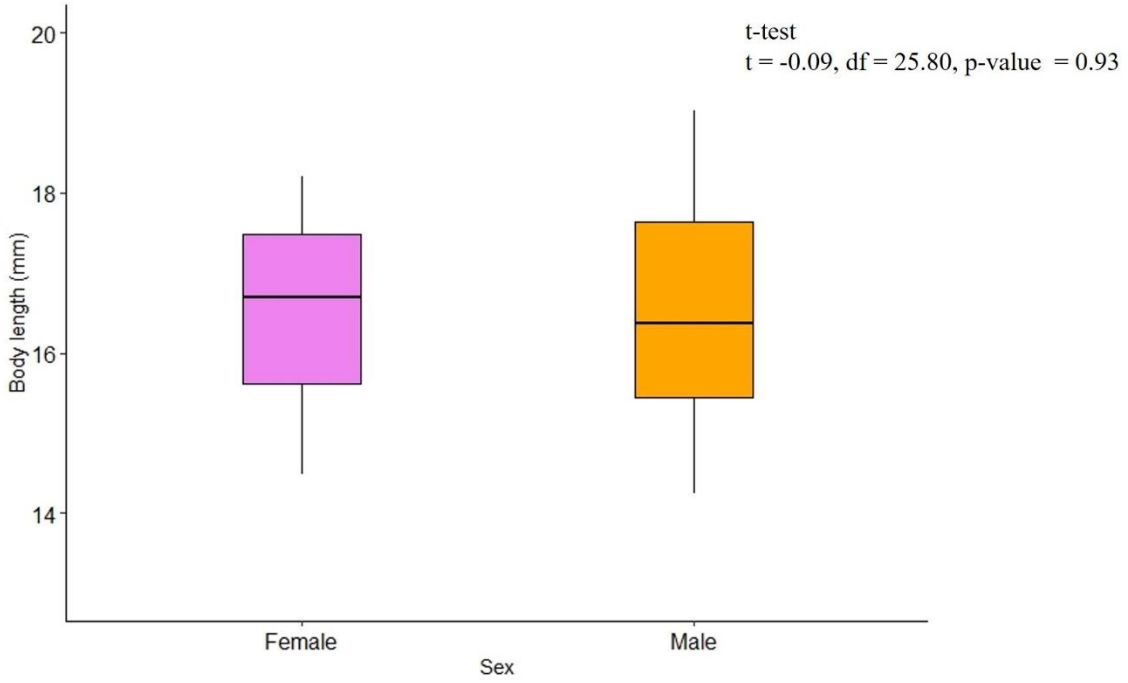

Figure 2.3: Boxplot of body length of lab monitored males (N=14) and females (N=16): body length of females was not significantly different from males.

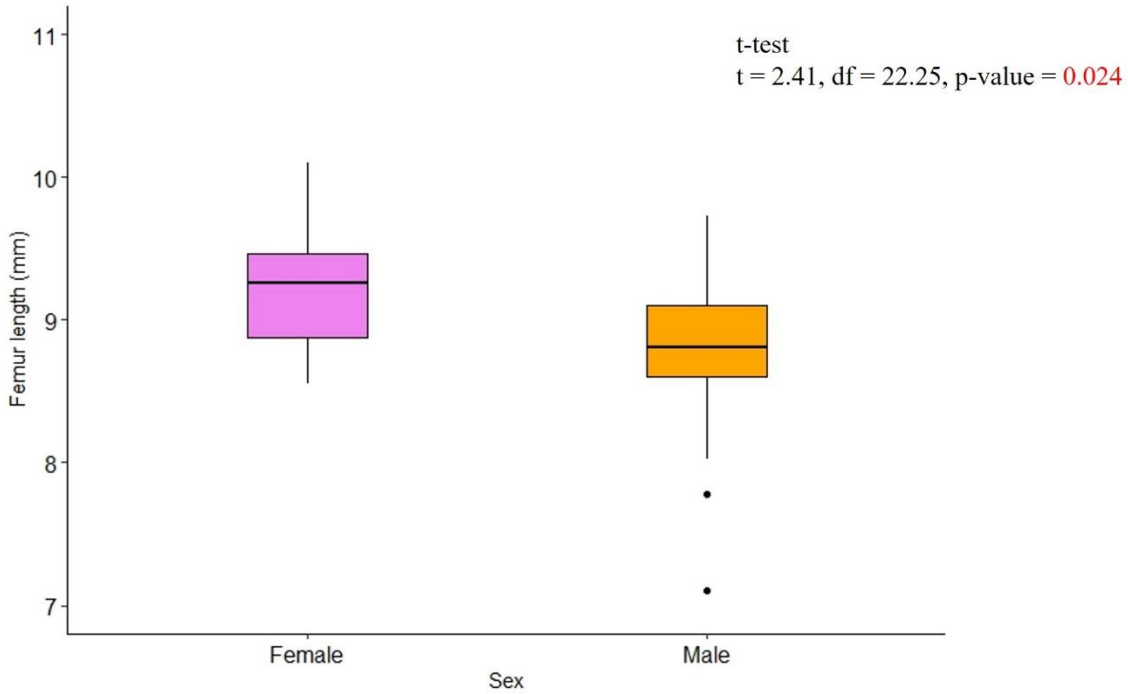

Figure 2.4: Boxplot of femur length of lab monitored males (N=14) and females (N=16): femur length of females was significantly larger than males.

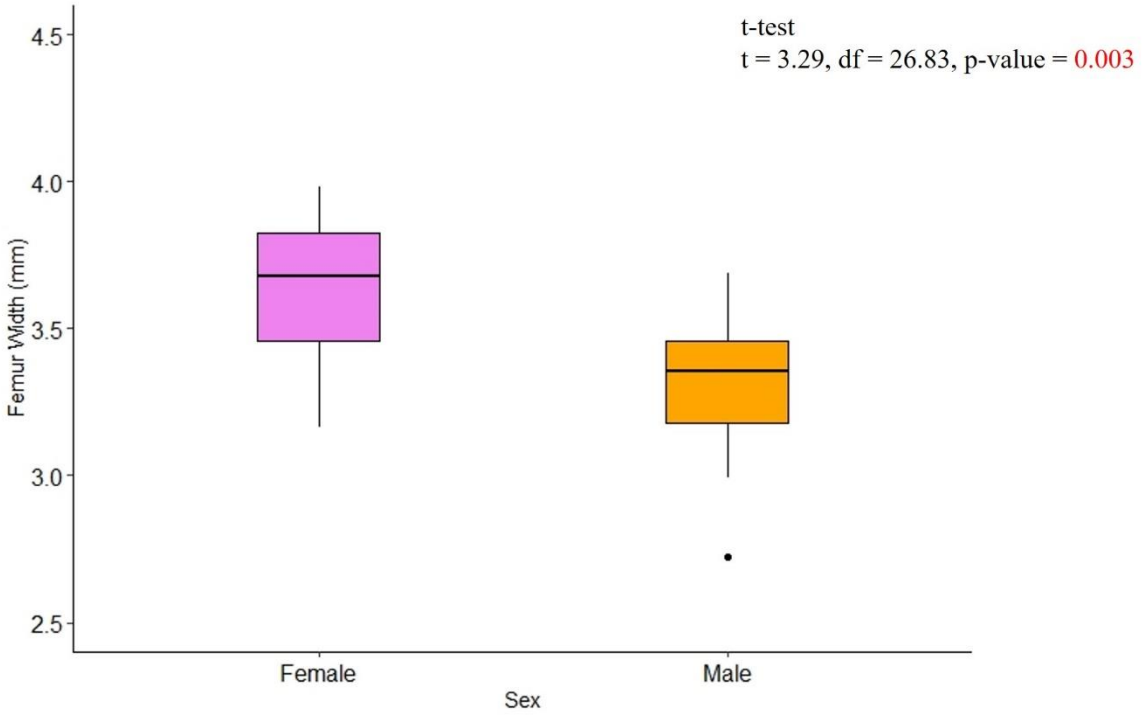

Figure 2.5: Boxplot of femur width of lab monitored (N=14) and females (N=16): femur width of females was significantly larger than males.

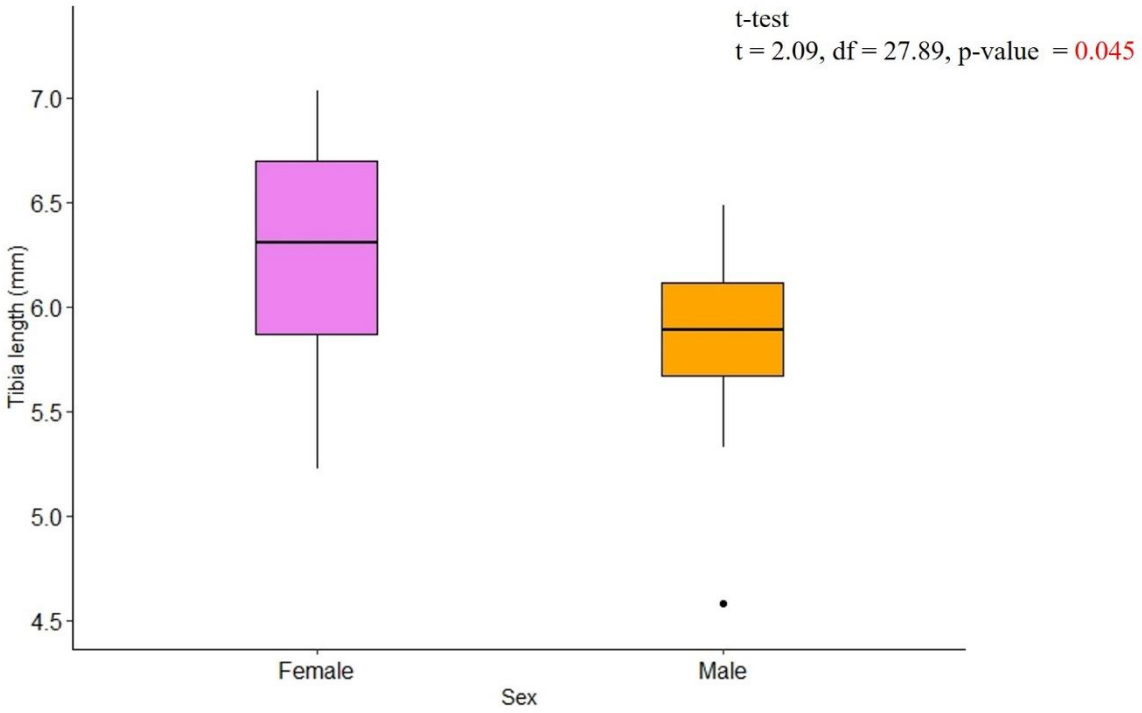

Figure 2.6: Boxplot of tibia length of lab monitored males (N=14) and females (N=16): tibia length of females was significantly larger than males.

		Adult lifespan	Body length (mm)	Pronotum length (mm)	Pronotum width (mm)	Wing length (mm)	Femur length (mm)	Femur width (mm)	Tibia length (mm)
Adult lifespan	Correlation								
	rho/cor								
	p-value								
Body length (mm)	Correlation	SR							
	rho/cor	-0.38							
	p-value	0.181							
Pronotum length (mm)	Correlation	SR	P						
	rho/cor	-0.16	0.64						
	p-value	0.594	0.014						
Pronotum width (mm)	Correlation	SR	SR	SR					
	rho/cor	-0.25	0.41	0.76					
	p-value	0.39	0.146	0.003					
Wing length (mm)	Correlation	SR	P	P	SR				
	rho/cor	-0.56	0.83	0.59	0.45				
	p-value	0.038	2.38E-04	0.028	0.104				
Femur length (mm)	Correlation	SR	P	P	SR	P			
	rho/cor	-0.06	0.57	0.73	0.55	0.48			
	p-value	0.844	0.033	0.003	0.044	0.081			
Femur Width (mm)	Correlation	SR	P	P	SR	P	P		
	rho/cor	-0.002	0.58	0.77	0.56	0.46	0.96		
	p-value	1	0.029	0.001	0.040	0.102	2.72E-08		
Tibia length (mm)	Correlation	SR	P	P	SR	P	P	P	
	rho/cor	-0.06	0.41	0.60	0.42	0.24	0.92	0.90	
	p-value	0.844	0.144	0.024	0.137	0.406	3.17E-06	1.06E-05	

Table 2.2: Correlation between adult lifespan and seven morphological traits in males (N=14). Adult lifespan is not correlated to any of the body size parameters except wing length. Life span is negatively correlated with wing length. Wing length and femur length is correlated with other body size parameters (body length and pronotum length). All size parameters of hind leg (femur length, femur width and tibia length) are correlated with each other.SR- Spearman's rank correlation, P- Pearson's product-moment correlation.

		Adult lifespan (days)	Body length (mm)	Pronotum length (mm)	Pronotum width (mm)	Wing length (mm)	Femur length (mm)	Femur width (mm)	Tibia length (mm)	Ovipositor length (mm)
Adult lifespan	Correlation									
	r									
	p-value									
Body length (mm)	Correlation	P								
	r	-0.15								
	p-value	0.568								
Pronotum length (mm)	Correlation	P	P							
	r	-0.09	0.52							
	p-value	0.737	0.040							
Pronotum width (mm)	Correlation	P	P	P						
	r	-0.02	0.41	0.79						
	p-value	0.955	0.114	0.0003						
Wing length (mm)	Correlation	P	P	P	P					
	r	-0.57	0.36	0.33	0.11					
	p-value	0.020	0.175	0.218	0.674					
Femur length (mm)	Correlation	P	P	P	P	P				
	r	0.18	0.44	0.76	0.90	0.07				
	p-value	0.507	0.091	0.001	1.48E-06	0.789				
Femur width (mm)	Correlation	P	P	P	P	P	P			
	r	0.34	0.33	0.71	0.88	-0.12	0.90			
	p-value	0.202	0.208	0.002	7.60E-06	0.671	2.37E-06			
Tibia length (mm)	Correlation	P	P	P	P	P	P	P		
	r	0.19	0.41	0.78	0.94	-0.04	0.95	0.95		
	p-value	0.472	0.118	0.0003	7.58E-08	0.886	1.49E-08	9.53E-09		
Ovipositor length (mm)	Correlation	P	P	P	P	P	P	P	P	
	r	0.30	0.68	0.55	0.63	-0.18	0.75	0.77	0.79	
	p-value	0.267	0.004	0.028	0.010	0.514	0.001	0.001	0.0003	

Table 2.3: Correlation between adult lifespan and eight morphological traits in females (N=16). Adult lifespan is not correlated to any of the body size parameters except wing length. Wing length is not correlated to any body size parameters. Ovipositor length is correlated to all body size parameters (body length, pronotum length, pronotum width). Femur length is correlated with protontum length which in turn is correlated with body length. All parameters of hind leg (femur length, femur width, and tibia length) size correlated with each other. All body parameters are correlated with each other except pronotum width and body length. P- Pearson's product-moment correlation.

2.4 Conclusion

There was no significant difference in the average lifespan, body length, pronotum length and pronotum width between the sexes. Contradicting results were found in another study in exclusive lab-grown population as body length of females were more than the body length of males (Singh and Jain unpublished). Femur length, femur width and tibia length of females were significantly larger than males. The average length of ovipositor was 5.78 ± 0.34 mm.

The wing length of males correlated with body length and pronotum length. The femur length of males correlated with their body length, pronotum length, pronotum width, femur width, and tibia length. The wing length of males was negatively correlated with adult lifespan, implying that males with larger wings have a reduced lifespan. In another study conducted on the same species, similar results were found as pronotum width, and wing length were positively correlated with prontoum length (Singh et al. 2020).

The ovipositor length of females correlated with all body size parameters except wing length. The femur length of females correlated with their pronotum length, pronotum width, femur width, and tibia length. The wing length of females was not correlated with any of the other body size parameters but was negatively correlated with life span.

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Chapter 3

Female preference for chirp duration

3.1 Background

Various studies in crickets have shown that different temporal and spectral parameters of the male mating call can serve as proxies for male quality (Ryder 2000, Gray 1997, Brown et al. 1996, Simmons 1998). Females are also known to show preference for theses signal parameters. For instance, in *Acheta domesticus* female showed preference for calls with higher chirp rates produced by larger male (Charlton 2007). Similarly, in *Gryllus bimaculatus* females showed a preference for the louder call which correlates with male body size (Simmons 1998). Age is one life-history trait of crickets which has been reported to influence both signalling and female response. For example, older males in *Oecanthus nigriconis* produce calls with shorter pulse duration (Brown et al. 1996), while older females in *Gryllus integer* are more motivated to mate and did not discriminate among different pulse rates within the natural range of calling song (Prosser 1994). Similarly, older males of *Gryllus campestris* called at a lower carrier frequency and had shorter chirps (Jacot et al. 2007). Females can also show a preference for the calls of males from a particular age class. For instance, female bushcrickets had a mating preference for calls of intermediate-aged and younger males (Ritchie et al. 1995) and females of *Gryllus bimaculatus* also showed a preference for the mating call of younger male with higher chirp rate (Verburght et al. 2011).

In our study system, it has been found that the chirp duration increase with age from an average of 371 milliseconds for one-week-old to 400 milliseconds for a four-week-old male (Chaudhuri and Jain unpublished). In this objective, I wanted to examine whether female crickets showed any preference for the chirp duration for either one-week-old or four-week-old male's mating call.

Objective 2: To examine female preference for chirp duration of LDMC of certain age classes of male.

3.2 Methodology

3.2.1 Phonotaxis arena

All phonotaxis experiments were conducted in a Y-shaped arena. Each arm of the arena was 50cm long, two arms of the arena with the playback speakers were 17cm wide, whereas the arm where the female cricket was released was narrow, and was 4 cm. The narrow arm was to ensure that the females started walking in a straight line to the decision-making point, which is the point where this arm splits into two. Identical Speakers (JBL GO, JBL) were kept at the end of the two arms to avoid visual biases if any. The whole arena was kept inside another Y- shaped chamber lined with acoustic foam to reduce the signal dampening. All the trials were recorded from outside the releasing arm of the arena using an infrared camera (Cannon model - XA20, Japan).

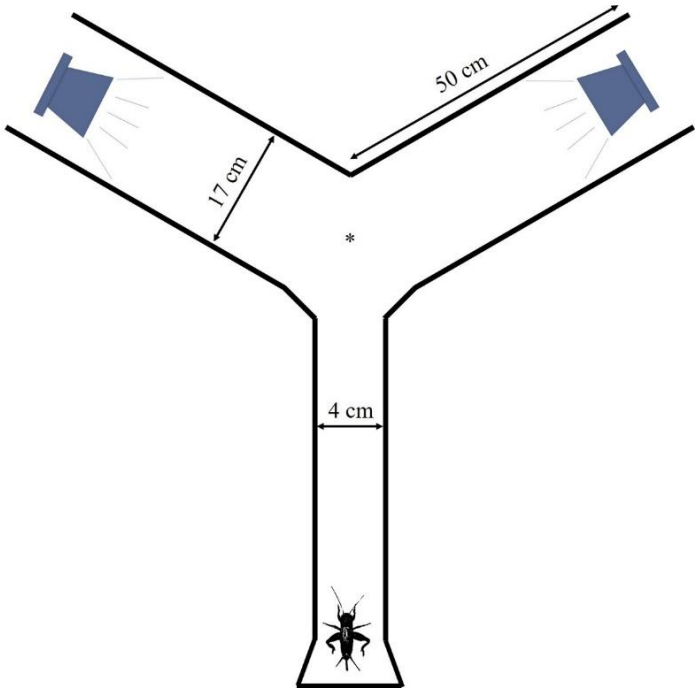

Figure 3.1: A schematic representation of the arena used for all phonotaxis trials: all females were released at the end of the narrow arm. Two identical speakers were kept at the end of the other two arms. The asterisk shows the decision making point. A camera was kept outside the Y-maze to record the trials. The whole arena was kept inside a Y-shaped chamber lined with acoustic foam.

3.2.2 Phonotaxis playback experiments

A total of 19 socially and acoustically isolated lab grown females were used for all two-choice experiments. All the females used for the trials were virgin females and their age was less than 5 weeks.

Female preference for chirp duration was tested using 'two-choice paradigm'. In this paradigm, two calls were presented as choices for the female by playing two stimuli simultaneously. For this set of trials, we used calls of two different chirp duration (371 and 400 milliseconds) representative of the songs of males from two different age classes (1 week and 4 weeks old males) (Chaudhuri and Jain unpublished). The chirp duration of 1 week old individual was 0.371±0.05s and the values ranged from 0.22s to 0.516s, similarly, the chirp duration of 4 weeks old individual was 0.400±0.06s and the values ranged from 0.255s to 0.574s (Chaudhuri and Jain unpublished). For the playback all other temporal and spectral parameters except chirp duration of these calls were maintained as population average. Both calls were played back at same loudness and kept at 62 dB, well above their behavioural hearing threshold recorded as 46 dB in quiet and ambient noise conditions (Jain master thesis dissertation, 2019). The loudness of the calls was measured at a distance of 50 cm from the speaker using a Bruel and Kjaer 1/2" microphone, Type 4189 (20Hz to 20kHz), attached to a Sound Pressure Level Meter, Type 2730 (Bruel and Kjaer, Naerum, Denmark). Since both the calls are presented at the same time minimal or no overlap is achieved between the calls by alternating the two signals (out of phase).

The side of presentation was switched after each trial to avoid any side bias. After every trial, the Y-maze was wiped with 70% ethanol to remove any olfactory cues. The final choice of these trials and the latency of response was recorded and used for further analysis.

3.2.3 Statistical analysis

All statistical analyses were done using R Studio (R version 3.6.2). Shapiro-Wilk test was done to check the normality of the data. To check for preference, responses and no-responses were compared using chi-square distribution with a p-value. The latency of response for the stimuli was compared using the Wilcoxon rank-sum test (Mann-Whitney U test).

3.3 Results

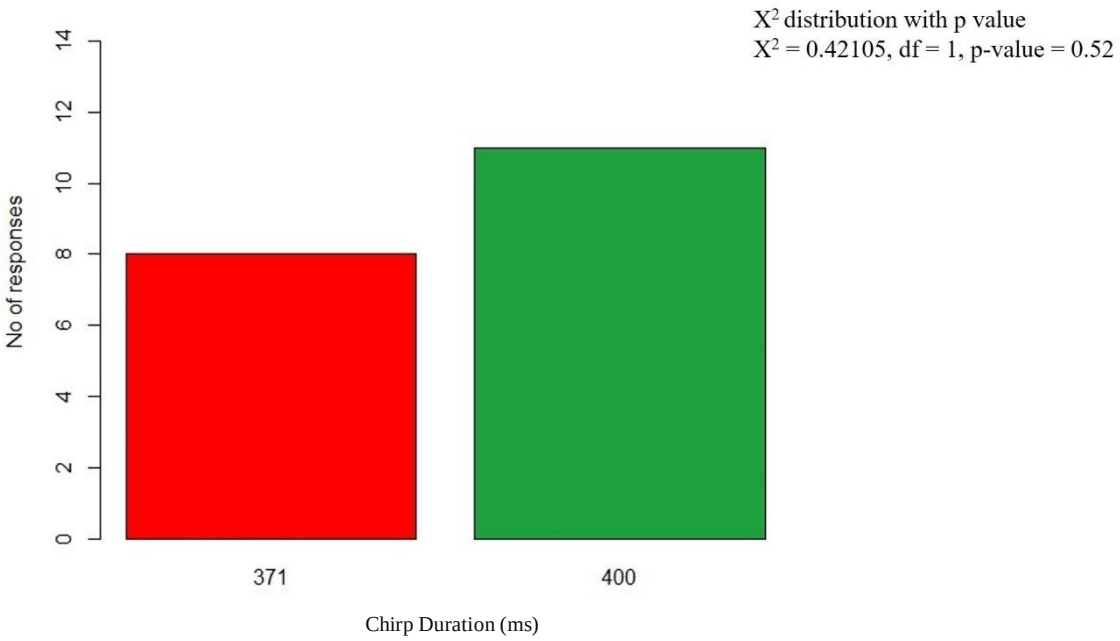

Figure 3.2: Response towards different chirp durations: females did not show a preference for either of the two chirp durations. 8 out of 19 females preferred 371ms chirp duration and rest of the 11 females preferred 400ms chirp duration.

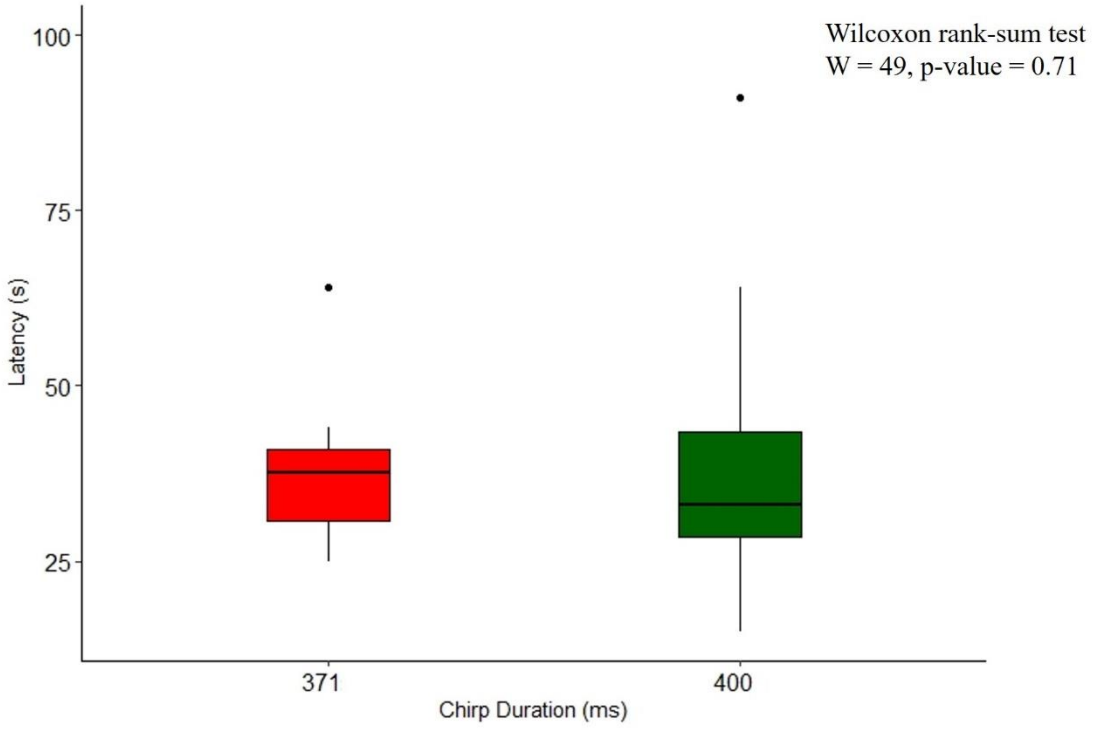

Figure 3.3: Boxplot of latency of response towards different chirp durations: females did not show any difference in latency while responding to either of the chirp durations.

3.4 Conclusion

The number of responses for chirp duration 371 milliseconds (N=8) was not significantly different from 400 milliseconds (N=11) suggesting that females do not seem to have a preference for either of the chirp duration. The time taken to respond to either of the chirp durations was also not significantly different from each other.

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Chapter 4

Neural response for heterospecific acoustic stimuli

4.1 Background

Crickets have specialized structures called tympanum on their foreleg, which have around 70 auditory receptor cells which fire in response to a wide range of frequencies from 1 – 2 kHz to over 100kHz (Pollack 1998, Young and Ball 1974). The auditory receptors have sensitivity for different frequency ranges, for example, less than 5.5kHz, 10 - 12kHz and greater than 18kHz are the frequency sensitivity peaks for auditory receptors of *Teleogryllus oceanicus* (Imaizumi and Pollack 1999). The acoustic signals received by the tympanum in the foreleg is sent to the pro-thoracic ganglion (PTG) (Huber and Thorson 1985). The auditory inter-neurons in PTG registers the stimuli before sending it to the brain for further processing (Zaretsky 1971, Hill 1974). Several studies have shown that auditory inter-neurons in the pro-thoracic ganglion are tuned to a particular range of frequency, which is the carrier frequency of the conspecific call. The firing of omega neuron 1 (ON1), ascending neuron 1 (AN1) is highly tuned towards the carrier frequency (Popov 1973, Wohlers and Huber 1982). Whereas, ascending neuron 2 (AN2) is tuned towards higher carrier frequency, possibly for anti-predatory responses (Wohlers and Huber 1982). But most of these studies have focused on individual neurons and not on the population level information, and very few studies have used extracellular recording techniques to study auditory neurons (for exception look at Stout and Huber 1972, Kostarakos and Hedwig 2017). In this objective, I have attempted to acquire extracellular neural recordings from the pro-thoracic ganglion. Furthermore, I examined how heterospecific calls with similar spectral parameters, but different temporal parameters from the conspecific calls are registered in pro-thoracic ganglion.

Objective: To standardize extracellular tetrode recording from pro-thoracic ganglion and to investigative neural responses to heterospecific acoustic stimuli.

4.2 Methodology

4.2.1 Dissection

Female crickets used for the experiment were either lab bred or collected from the field in IISER Mohali, Punjab, India and the University of Hyderabad, Telangana, India. All females were kept in acoustic isolation at least one day before the experiments. Before dissection, females were immobilized by giving a cold shock (4 degrees Celsius) for around 5 minutes. Once she was immobilized, her mid and hind legs were removed to improve accessibility to pronotum. The female was then mounted on an elevated platform made of clay with her ventral side facing upwards. The head was stretched using metal wire and fixed on a clay platform, whereas both the forelegs were stretched to expose tympanum. Heads were stretched to expose more region around the pro-thoracic ganglion and this also restricted the movement of the individual. A wax cup is made from beyond the compound eyes till the end of pronotum around the legs. Wax cup was used to prevent the leaking of the saline (Ringer's reagent - used for perfusing the tissue). Ringer's reagent is prepared using many salts (KCl, NaCl, $CaCl_2$, $MgCl_2$, $NaHCO_3$) and compensates the loss of fluids due to dissection and maintains a constant ionic concentration around the tissue after dissection which is equivalent to insect body fluid.

The pro-thoracic ganglion and its nerve connectives are exposed by carefully removing the cuticle, muscles and fat bodies around it using spring scissors and extra-fine forceps. A metal wire was passed beneath the ganglion to lift it, which separated the ganglion from the gut and its movements. A protein sheath protects the insect ventral nerve cord, and electrodes cannot penetrate this sheath. Protease was applied on the surface of ganglion for some time and washed away later to soften this sheath which was then removed with the help of fine forceps.

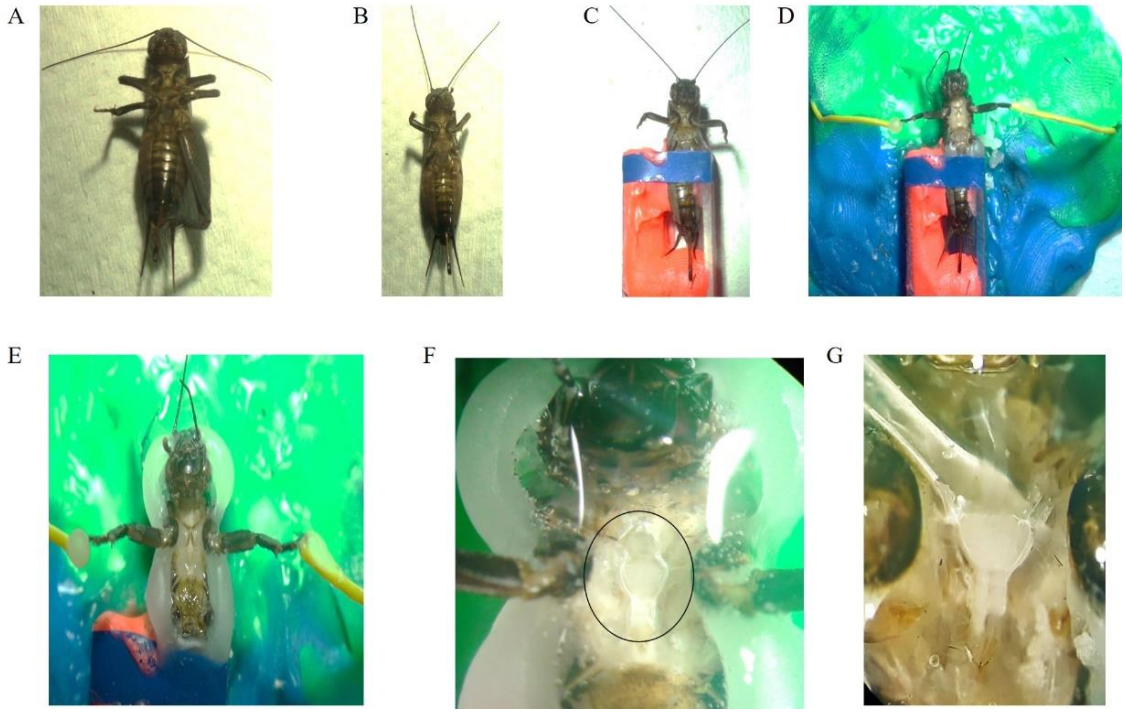

Figure 4.1: Dissection procedure for exposing pro-thoracic ganglion: A) Female lying ventral side up after 5-minute cold shock. B) Both the hind and mid legs were removed. C) Tied down to a custom made chamber with fore legs free. D) Chamber was attached to a clay platform with forelegs stretched and fixed to expose tympanum. E) A wax cup was made around the pro-thoracic ganglion and head to hold saline and restrict movement F) The cuticle was removed to expose pro-thoracic ganglion and is encircled in this case G) The ganglion was lifted using a metal platform.

4.2.2 Tetrode winding

For the extracellular multi-unit recording, a tetrode was used. The tetrode was made using insulated nickel-coated chromium wire. The wire was wrapped for ~ 6-8 times along the long-side of a rectangular cardboard winding board. After wounding, the wrapped wires were slit at both the short edges giving two long stretches of wire. One stretch of wire was hung from the short side of the cardboard. The other end of the wire was clipped to a rectangular weight using an alligator clip. This weight was then inserted between the parallel plates of a rotating winder. The wire was then rotated for ~ 100-120 times at a particular voltage using a DC motor for winding them together. The polyimide coating of these wound wires was fused by blowing a heat gun over them from different angles for 5 seconds each. This single stretch can be used to make two tetrodes, thus cut in the middle.

The wire was then inserted into a capillary tube which is attached to the centre of an eight-pin IC socket. The wires at this end were inserted individually into the socket after removing the insulation by burning the wires slightly and fixed in place using IC base pins. This IC socket was then coated with epoxy to hold the strands inserted into sockets and capillary in position. Epoxy was also applied to the other end of the capillary tube to hold the wire in place.

4.2.3 Multi-unit extracellular recordings

The dissection setup was observed under a stereo-microscope to direct the tetrode to the region of interest in prothoracic ganglion. The IC socket of the tetrode was plugged into pre-amplifier on the headstage which is attached to a manipulator. Chlorided - silver wire was inserted near the tissue of interest and immersed in the saline and the other end was attached to a pre-amplifier. This wire acted as a reference for all the recordings from the tetrode. This setup was then connected to a filter + gain amplifier. The tetrode was directed using a manipulator to enter the neural tissue and relax into the tissue. The recordings from the tetrode were observed in real time using an oscilloscope. The analog signals from the amplifier were then digitised at 15kHz with Data Acquisition Card (DAQ card USB 6211, National Instruments) using custom 'Lab view program'.

For the playback experiments, the acoustic stimuli were played back through a JBL Speaker (JBL) connected to a computer. Via a parallel connection this audio signal was sent to the DAQ to be simultaneously acquired with the physiology signals. This was then used to correlate the neural recordings and the acoustic stimuli later during analysis. Each stimulus lasted for 10-12 seconds and was repeated over multiple trials (5-10 trials) on the same animal. Two spectrally and temporally different heterospecific calls of *Teleogryllus rohinae* and *Teleogryllus occipitalis* were used as the acoustic stimuli.

4.2.4 Analysis

The multi-unit recordings were sorted into possible distinct cells using Igor Pro 4.3 (Wavemetrics), a spike sorting software based on Pouzat et al.2002. Multiple statistical tests suggested in Pouzat et al. 2002 were used to check the reliability of this sorting. The time points of the firing of these cells and their correlation with the stimuli were checked using a custom-written code in MATLAB 2018a.

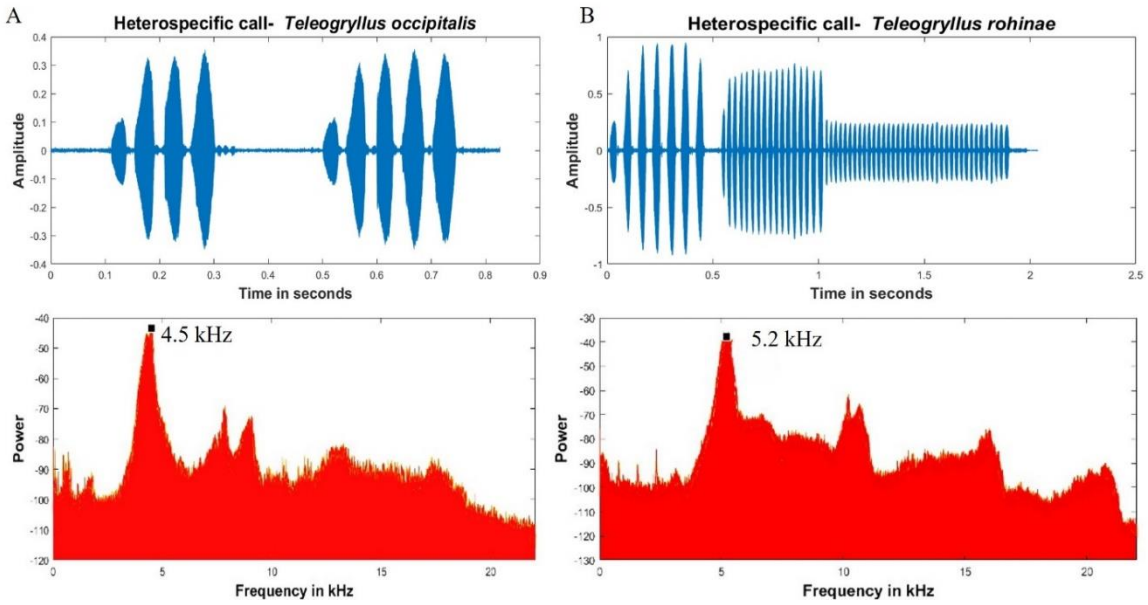

Figure 4.2: Oscillograms and power spectra for hetero-specific playback stimuli: A) Top: the oscillogram of the LDMC of *Teleogryllus occipitalis*, Bottom: the power spectrum of the same call and the peak frequency is 4.5kHz. B) Top: the oscillogram of the LDMC of *Teleogryllus rohinae*, Bottom: the power spectrum of the same call and the peak frequency is 5.2kHz.

4.3 Results

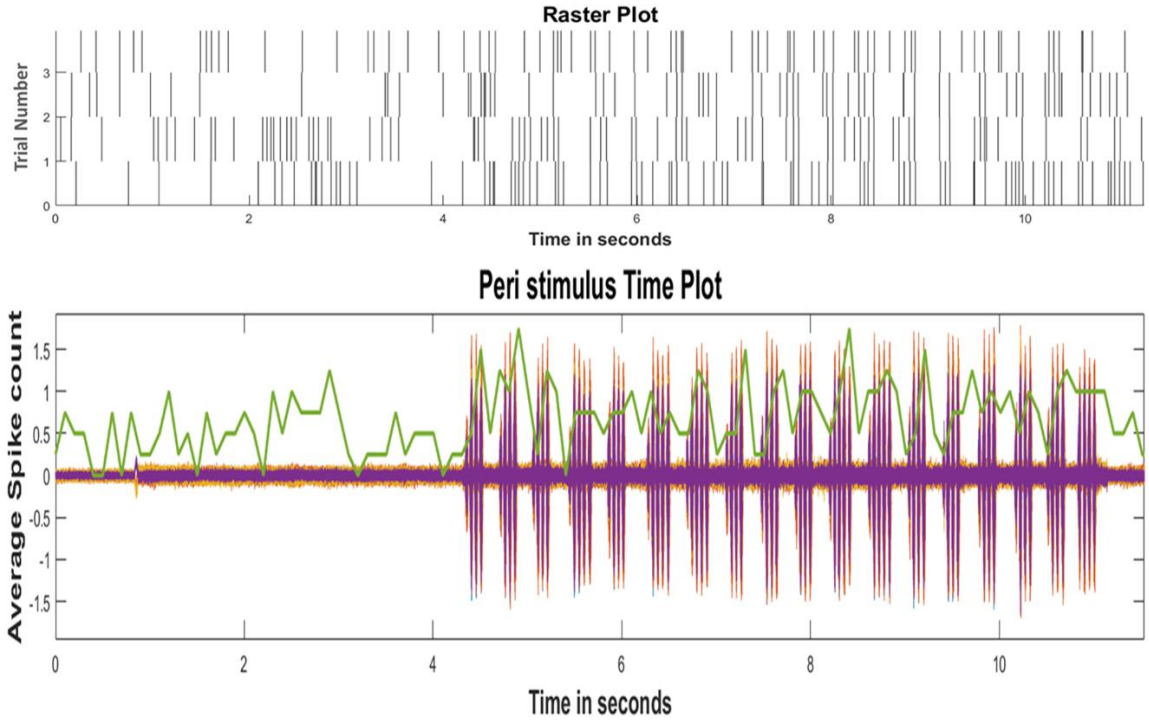

Figure 4.3: Neural response for *Teleogryllus occipitalis* call stimuli: Top: A raster plot for four trials, each row represents a single trial and the vertical lines in a row is representative of neural firing, the x-axis is the time point where the firing happened. Bottom: A Peri-stimulus Time Plot, the neural firing is averaged over four trials and plotted over the stimulus provided, the average spike count seems to follow the pattern of stimulus presentation.

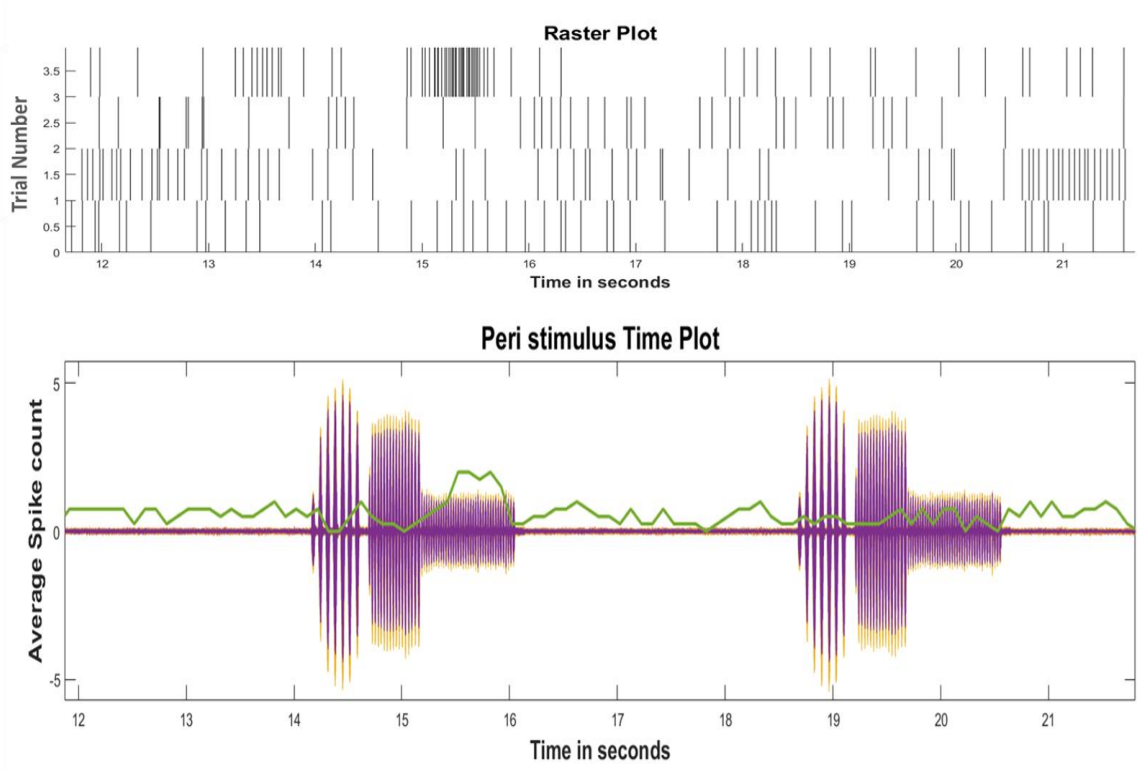

Figure 4.4: Neural response for *Teleogryllus rohinae* call stimuli: Top: A raster plot for four trials, each row represents a single trial and the vertical lines in a row is representative of neural firing, the x-axis is the time point where the firing happened. Bottom: A Peri-stimulus Time Plot, the neural firing is averaged over four trials and plotted over the stimulus provided, the average spike count does not seem to follow the pattern of stimulus presentation.

4.4 Conclusion

The raster plot suggests that the procedure mentioned above could be used as a procedure to collect neuronal recordings from a group of neurons in the pro-thoracic ganglion. The peri-stimulus histogram suggests the presence of a group of neurons in the pro-thoracic ganglion which responds to *Teleogryllus occipitalis* and does not respond to *Teleogryllus rohinae*. Since this is from only four trials on one female, the results are not conclusive.

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Chapter 5

Discussions and Future directions

Objective 1: In the lab monitored population of *Acanthogryllus asiaticus*, the adult lifespan, body length, pronotum length, pronotum width of males were not significantly different from that of the females. This might be because of the same standard conditions provided for both the sexes in the lab. However, the femur length, femur width and the tibia length of females were significantly more than that of males. Similarly, females of *Gryllus pennsylvanicus* also have longer femur than males (Judge and Bonanno 2008). We can speculate that this difference might be because, in crickets, females mount the males for courtship. In another study conducted in the same species it has been shown that rearing temperature influences the body size parameters (body length, pronotum length, pronotum width, wing length and ovipositor size), adult lifespan and developmental time of both males and females (Singh et al. unpublished).

In females, the ovipositor length correlates with all the other body size parameters except wing length. Similarly, in males, the wing length correlates with the body size parameters. I speculate that larger individual invest more resources to these structures because the reproductive success these structures can provide. Ovipositor in females is important for laying eggs and wings in males is important to attract females. In case of males, a previous study in this species conducted in the lab showed similar correlation between pronotum length and pronotum width and the wing length was also positively correlated with both these body size parameters (Singh and Jain 2020). In females, the wing size is negatively correlated with life span but the wing size was not correlated with any other body size parameter, so we cannot speculate that larger females die early. Whereas, in males, the wing size is negatively correlated with lifespan and positively correlated with body size parameters which suggests that males with larger wings have a reduced life-span. In *Teleogryllus commodus* males with increased calling effort have reduced longevity (Hunt

et al. 2004). So in future, it would be interesting to check how calling effort correlates with wing size and longevity.

It would also be interesting to check whether there is a difference in body mass of males and females from the lab population and whether it correlates with lifespan or other body size parameters in either of the sexes.

The analyses were done in a mixed population of both lab-bred and lab-reared (sub-adults are collected from the field) crickets. So, the nymphal conditions of these crickets are different, and it would be interesting to investigate whether the pattern changes if these populations are considered separately. A different trend was observed in the exclusively lab grown population of the same species at 25 degrees Celsius as body length of female was significantly more than males (Singh and Jain unpublished) and the combined lifespan of males and females were similar to the lifespan one found in this study (Singh et al unpublished) so this warrants a thorough investigation in this regard.

Objective 2: The experiments using the two-choice paradigm indicates that female does not have a preference for the chirp duration of a particular age class. In different species of crickets, the preference for call parameters which indicates age were different. For instance, in *Gryllus pennsylvanicus* and *Gryllus veletis*, females are attracted to older males, but in *Gryllus bimaculatus* females are attracted to younger males (Zuk 1987, Verburgt et al. 2011). The absence of any preference might also be because chirp duration of the calls used in the two-choice paradigm differed by only 29ms (one syllable) and this difference by might not be sufficient to elicit any differential response. In cricket species which showed a preference for chirp duration, the longer chirp duration differed by 125ms from the shorter chirp duration (Wagner 1966).

In our species, it is also found that the chirp period decreases with age (Chaudhari and Jain unpublished). So in future, we can look whether females have a preference for a faster chirps, which also indicates an older male. Context-dependent choice has been seen in crickets, in *Gryllus bimaculatus*, the preference for the unattractive call of an old male increase if they are louder (Zhemchuzhnikov et al. 2017) so in future research, it would be interesting to check whether the combination of both chirp duration and chirp period (longer and faster chirps against shorter and slower chirps) have a differential response. In

Acheta domesticus, younger females show a preference for faster chirps and older females do not show any preference (Gray 1999). So, it would also be interesting to investigate how the age of females in our species influences their preference for chirp duration.

Objective 3: Extracellular recording using tetrode can be used to record spikes/discharges from a population of neurons in the pro-thoracic ganglion. The initial set of trials suggest that there is a population of neurons in pro-thoracic ganglion which might respond to heterospecific calls with similar spectral parameters as the conspecific calls. It is known that omega neuron 1 and ascending neuron 1 in the pro-thoracic ganglion is tuned to a particular frequency but registers the temporal features of the stimuli presented (Schildberger et al. 1984 and Wohlers and Huber 1982). The recognition of the conspecific call using temporal features is achieved in the cricket brain by delay line detection (Schöneich et al. 2015).

From the limited data available from the neurons recorded in these trials it seems that they respond to the stimuli but does not seem to encode syllable level information of the stimuli. Yet, this warrants a more thorough and extensive investigation in the future.

For future directions, it would be interesting to look at how individual interneurons register heterospecific signals using an intracellular recording setup. It would also be interesting to see how noise (ambient and traffic) affects the registering of conspecific calls in the pro-thoracic ganglion.

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Appendix A: for chapter 2

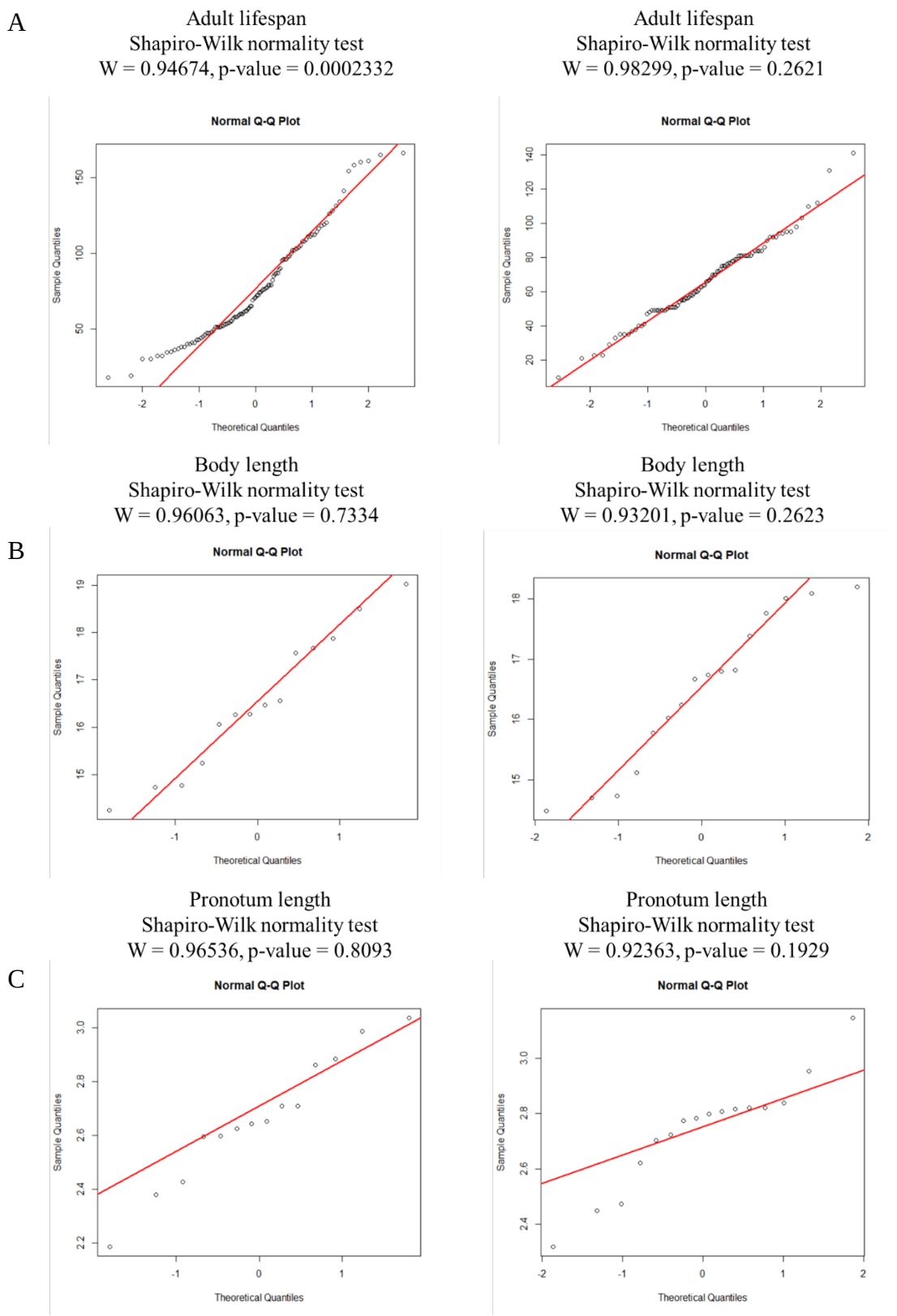
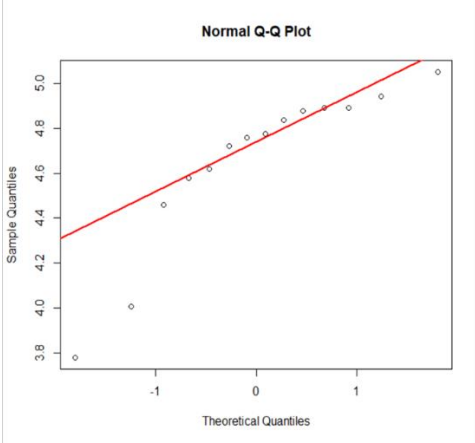


Figure 1: Shapiro Wilk test and QQ plots for life-history traits of males(left) and females(right). A) Adult lifespan, B) Body length and C) Pronotum length.

D

Pronotum width
Shapiro-Wilk normality test
W = 0.82313, p-value = 0.009784



Pronotum width
Shapiro-Wilk normality test
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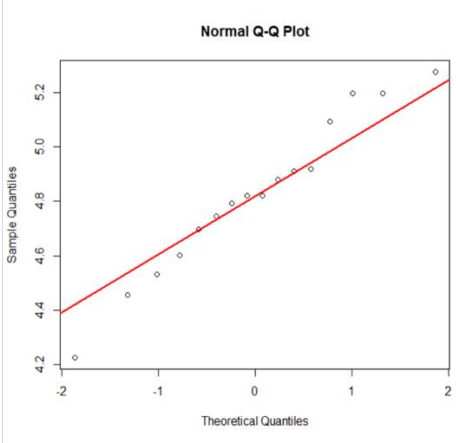
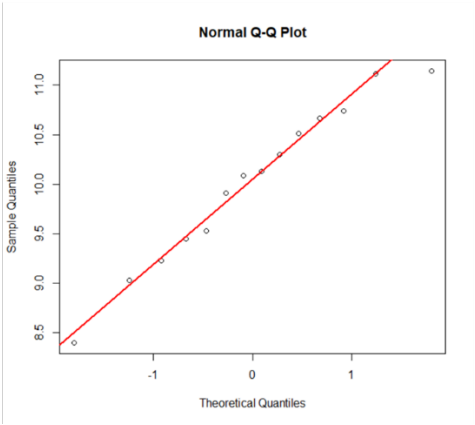


E

Wing length
Shapiro-Wilk normality test
W = 0.9673, p-value = 0.8386



Wing length
Shapiro-Wilk normality test
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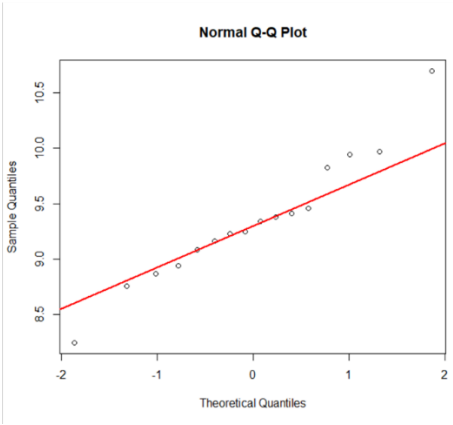
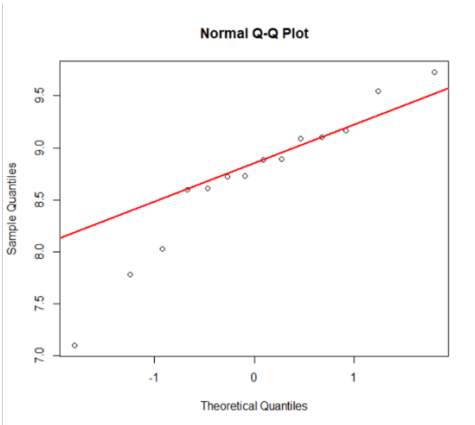


F

Femur length
Shapiro-Wilk normality test
W = 0.93098, p-value = 0.315



Femur length
Shapiro-Wilk normality test
W = 0.95505, p-value = 0.5736

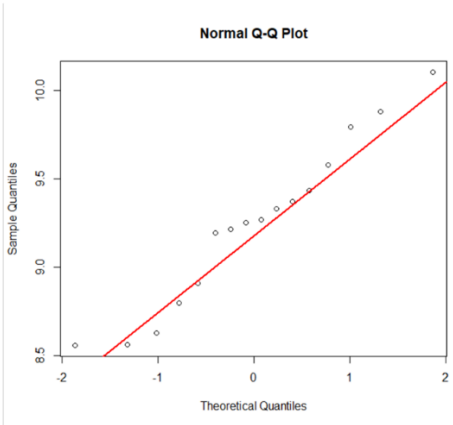


Figure 2: Shapiro Wilk test and QQ plots for life-history traits of males(left) and females(right). A) Pronotum width, B) Wing length and C) Femur length.

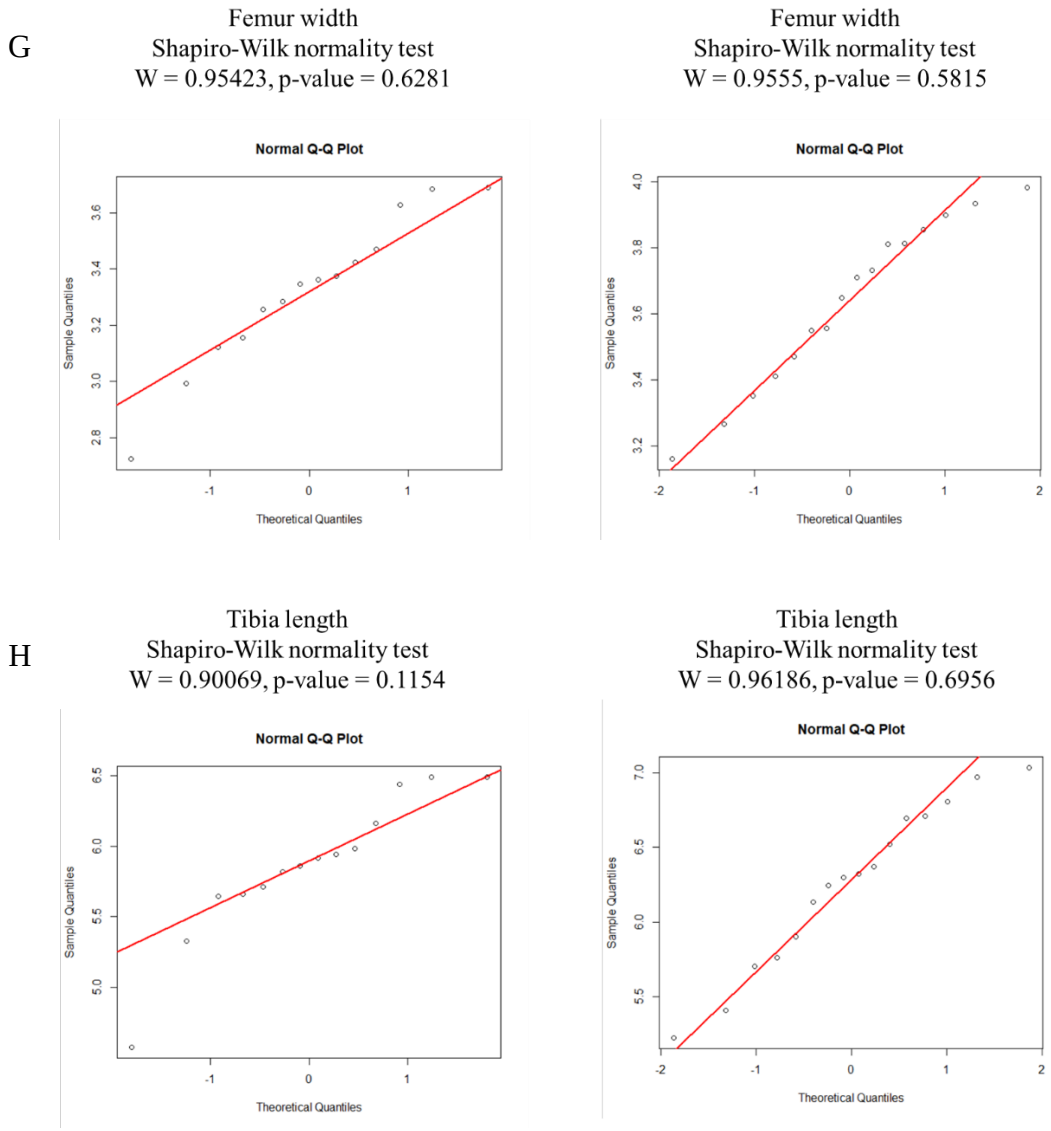


Figure 3: Shapiro Wilk test and QQ plots for life-history traits of males(left) and females(right). G) Femur width, H) Tibia length

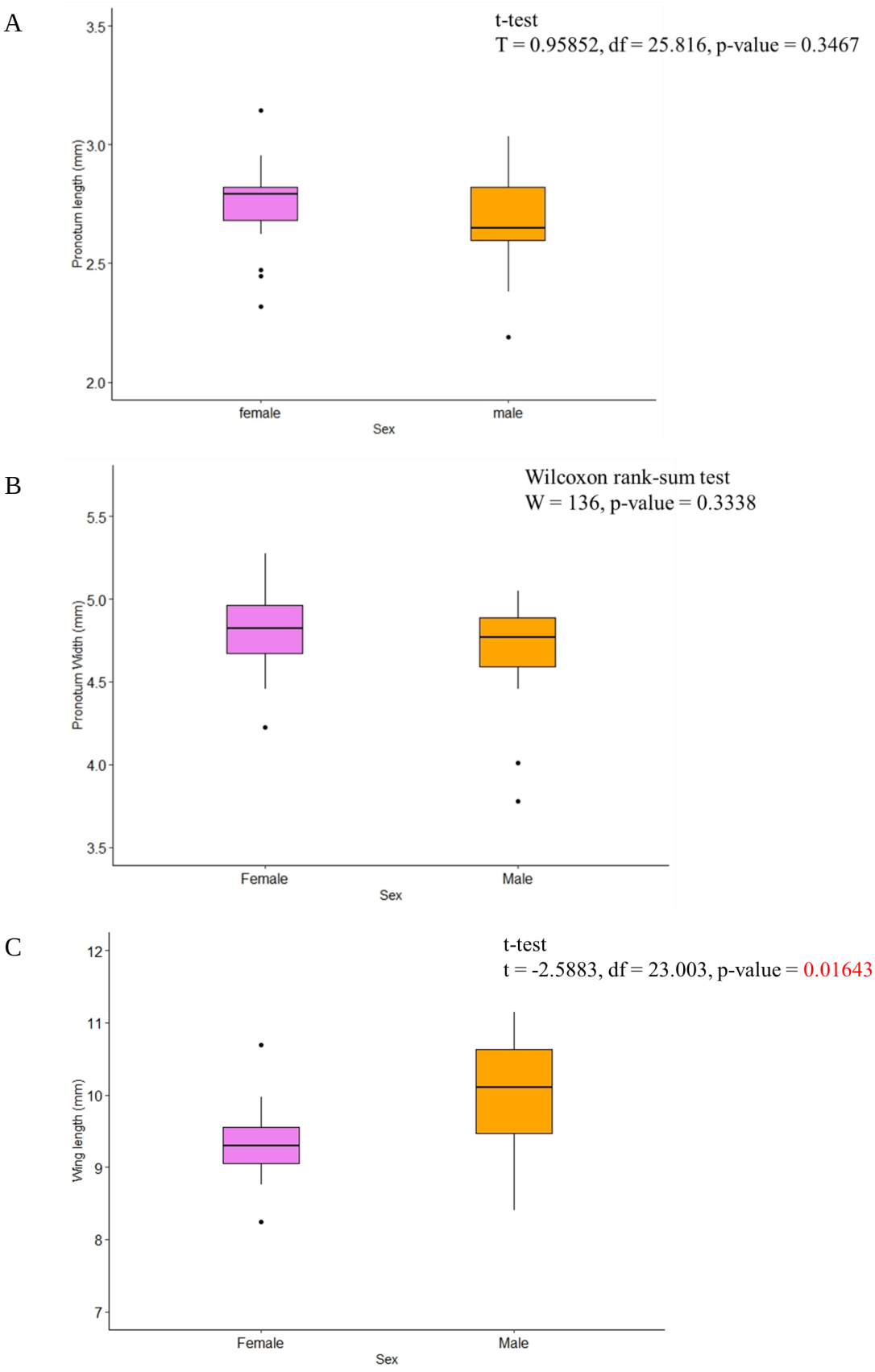


Figure 4: Boxplots of life-history traits of lab monitored males and females. A)Pronotum length, B)Pronotum width, C)Wing length.

Life-history trait	Males (N= 14) (Mean ± SD)	Females (N= 16) (Mean ± SD)	t	df	p-value
Body length (mm)	16.52±1.46	16.47±1.25	-0.09	25.80	0.93
Pronotum length (mm)	2.66±0.23	2.74±0.20	0.96	25.82	0.35
Wing length (mm)	10.02±0.81	9.35±0.57	-2.58	23.00	0.02
Femur length (mm)	8.71±0.69	9.24±0.46	2.41	22.25	0.02
Femur width (mm)	3.32±0.27	3.64±0.25	3.29	26.83	0.003
Tibia length (mm)	5.86±0.50	6.26±0.54	2.09	27.89	0.045

Table 1: A comparative analysis of life-history traits between sexes using t- test

Life-history trait	Males (Mean ± SD)	Females (Mean ± SD)	W	p-value
Adult lifespan (in days)	77.42±34.79	66.5±23.06	4297	0.08112
Pronotum width(mm)	4.66±0.36	4.82±0.29	136	0.3338

Table 2: A comparative analysis of adult lifespan and pronotum width between sexes using Wilcoxon rank-sum test. For adult life-span, males (N=109) and females (N=92) and for pronotum width, males (N=14) and females (N=16)

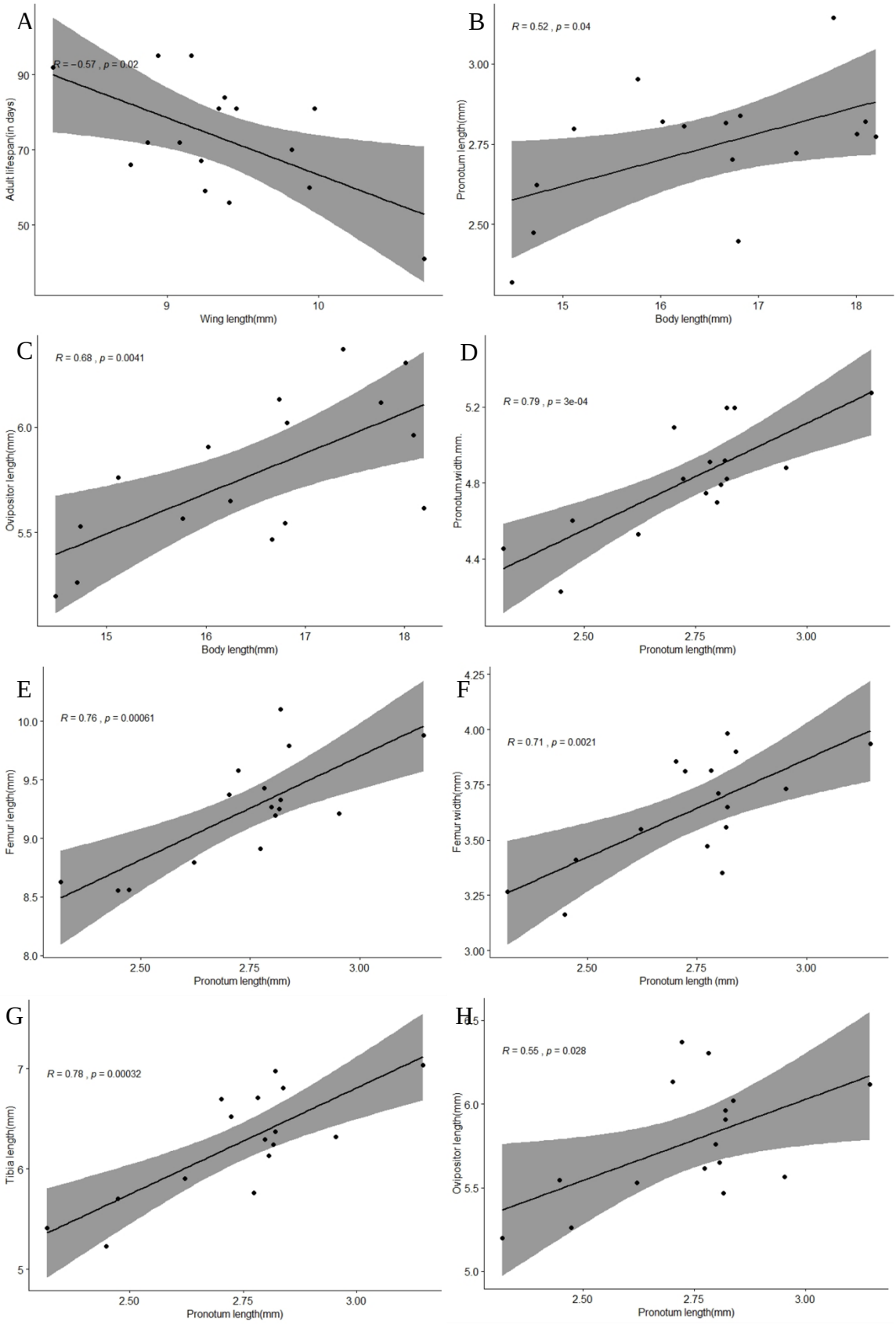


Fig 5: Correlation between life history traits in females: (A)Adult lifespan vs Wing length,(B, C)Body length vs pronotum length and ovipositor length, (D,E,F,G, H) pronotum length vs pronotum width, femur length, femur width, tibia length and ovipositor length,

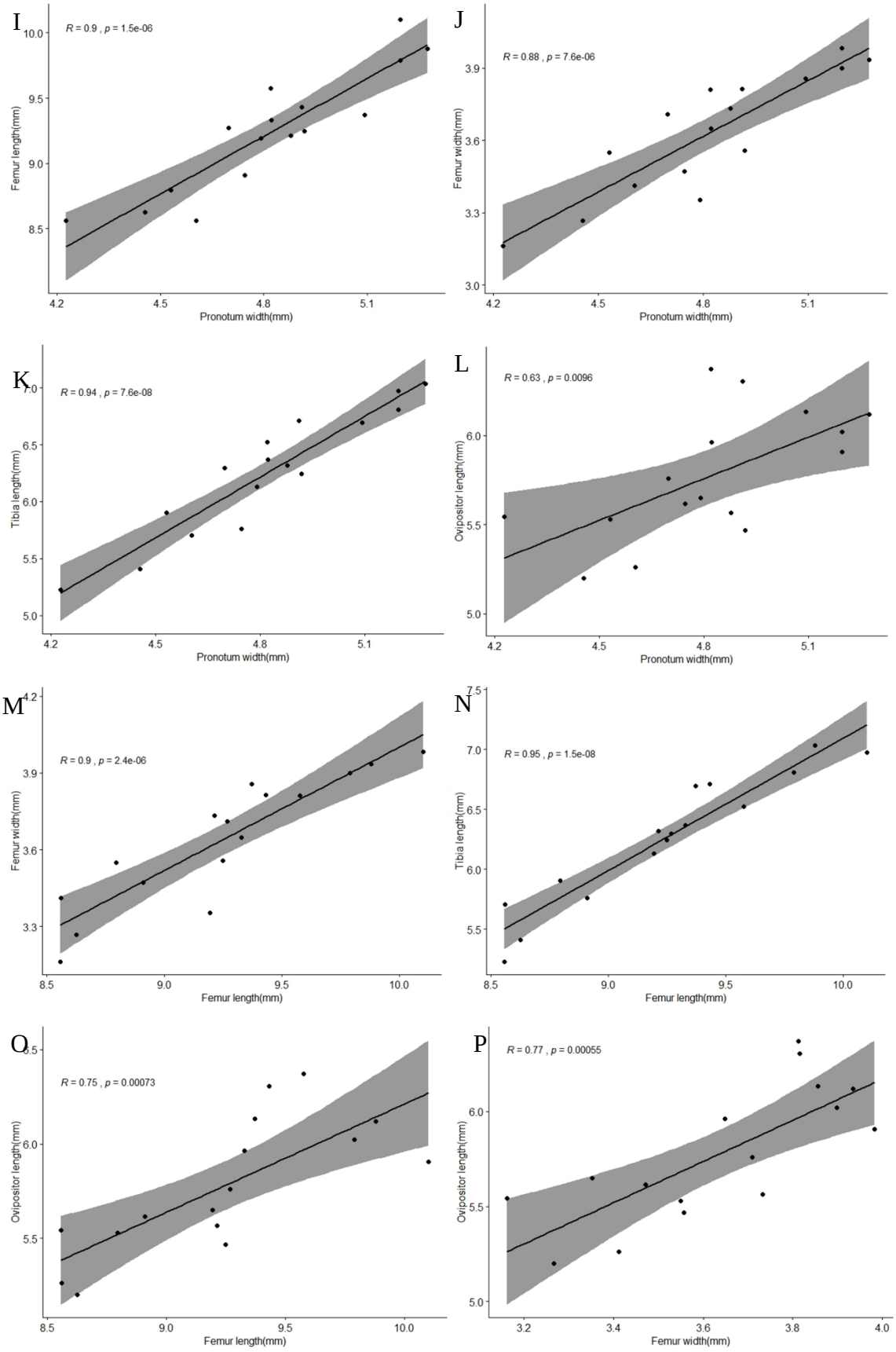


Fig 6: Correlation between life history traits in females: (I,J,K,L) pronotum width vs femur length, femur width, tibia length and ovipositor length, (M,N,O)femur length vs femur width, tibia length and ovipositor length, (P) femur width vs ovipositor length.

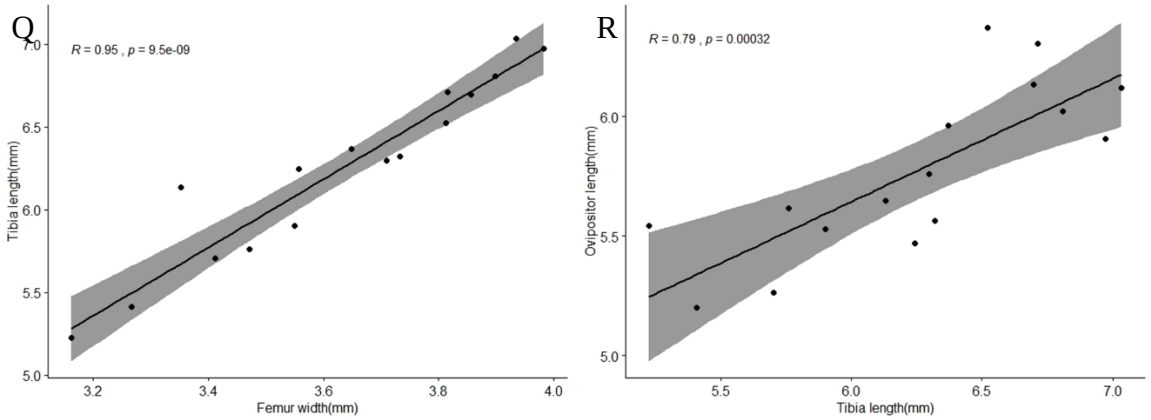


Fig 7: Correlation between life history traits in females: (Q) femur width vs tibia length and (R) tibia length vs ovipositor length.

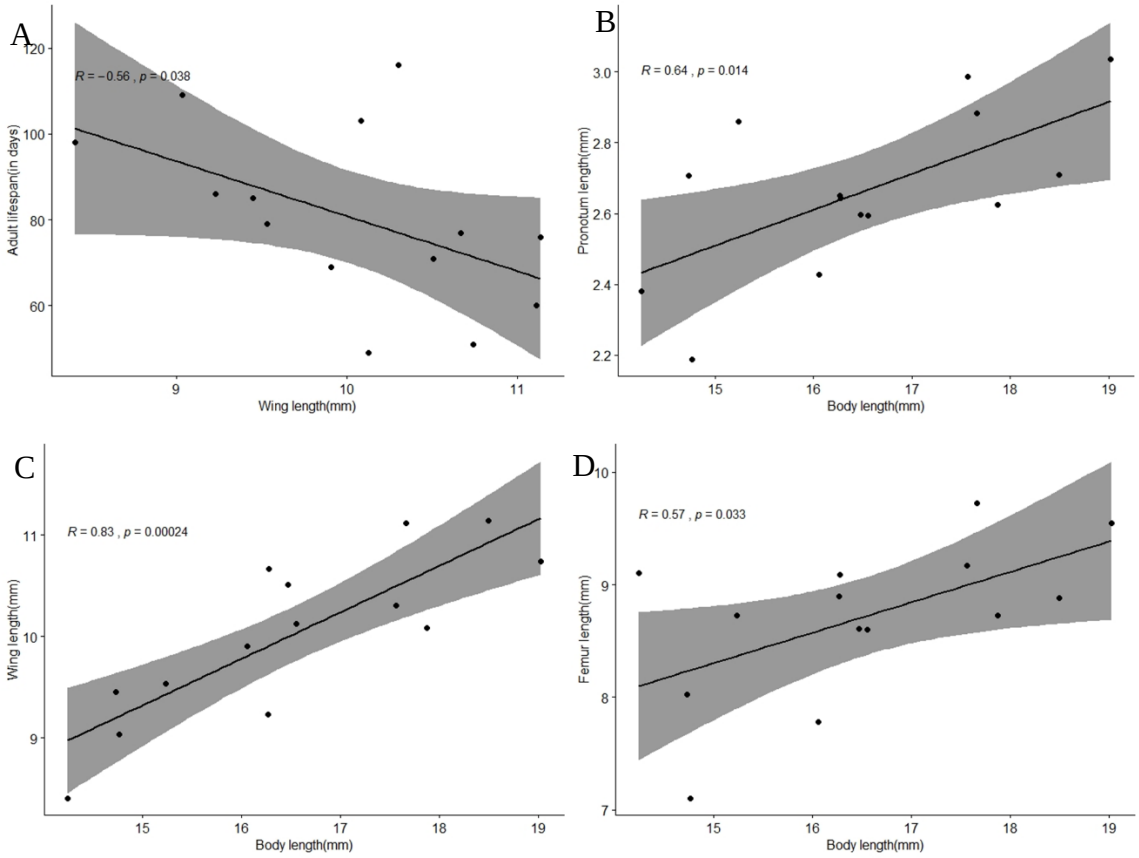


Fig 8: Correlation between life history traits in males: (A) wing length vs adult life span, (B, C, D) body length vs Pronotum length, wing length and femur length.

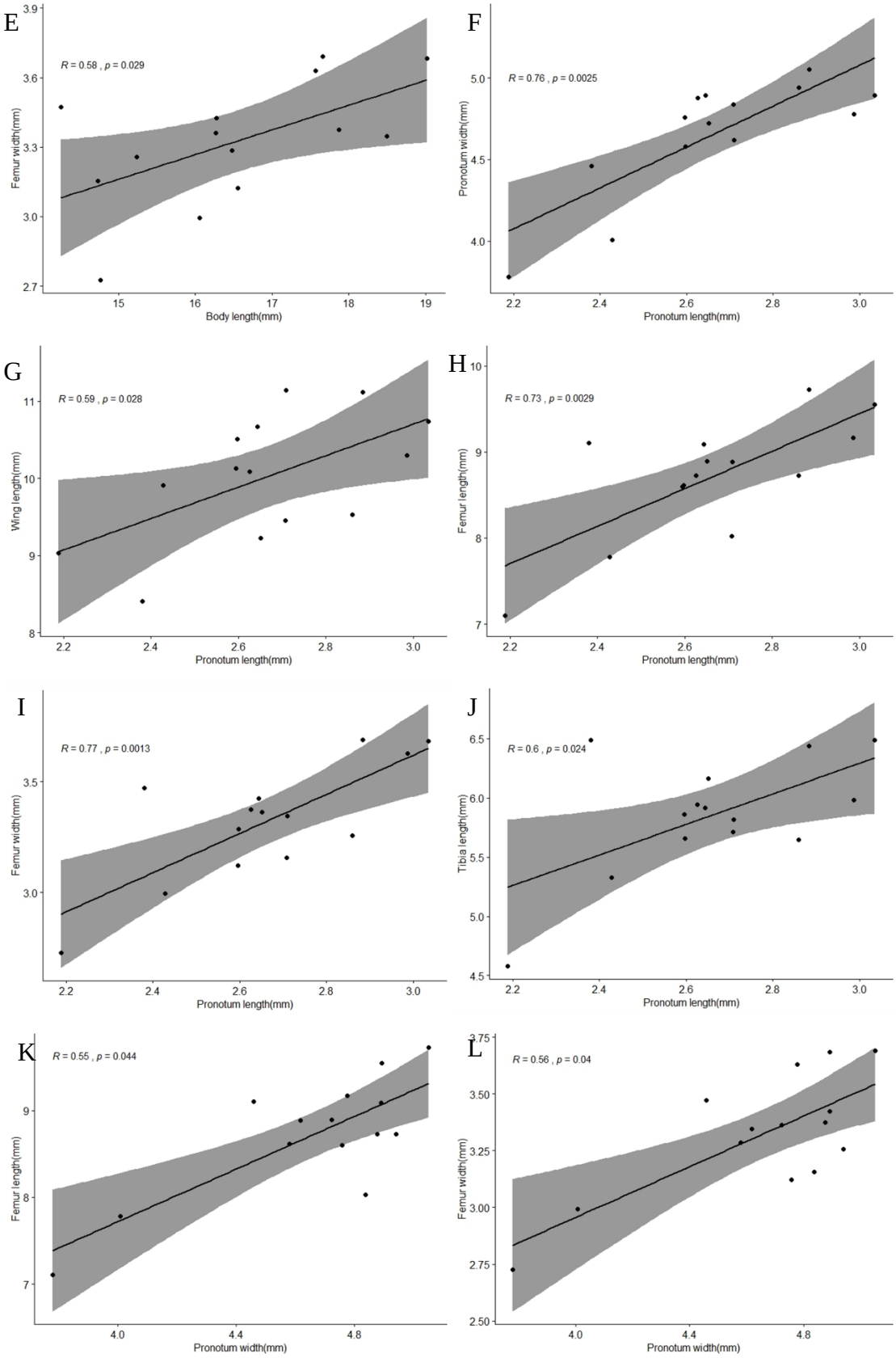


Fig 9: Correlation between life history traits in males: (E) body length vs femur width, (F, G, H, I, J) pronotum length vs prontoum width, wing length, femur length, femur wdith, and tibia length, (K, L) pronotum width vs femur length and femur width.

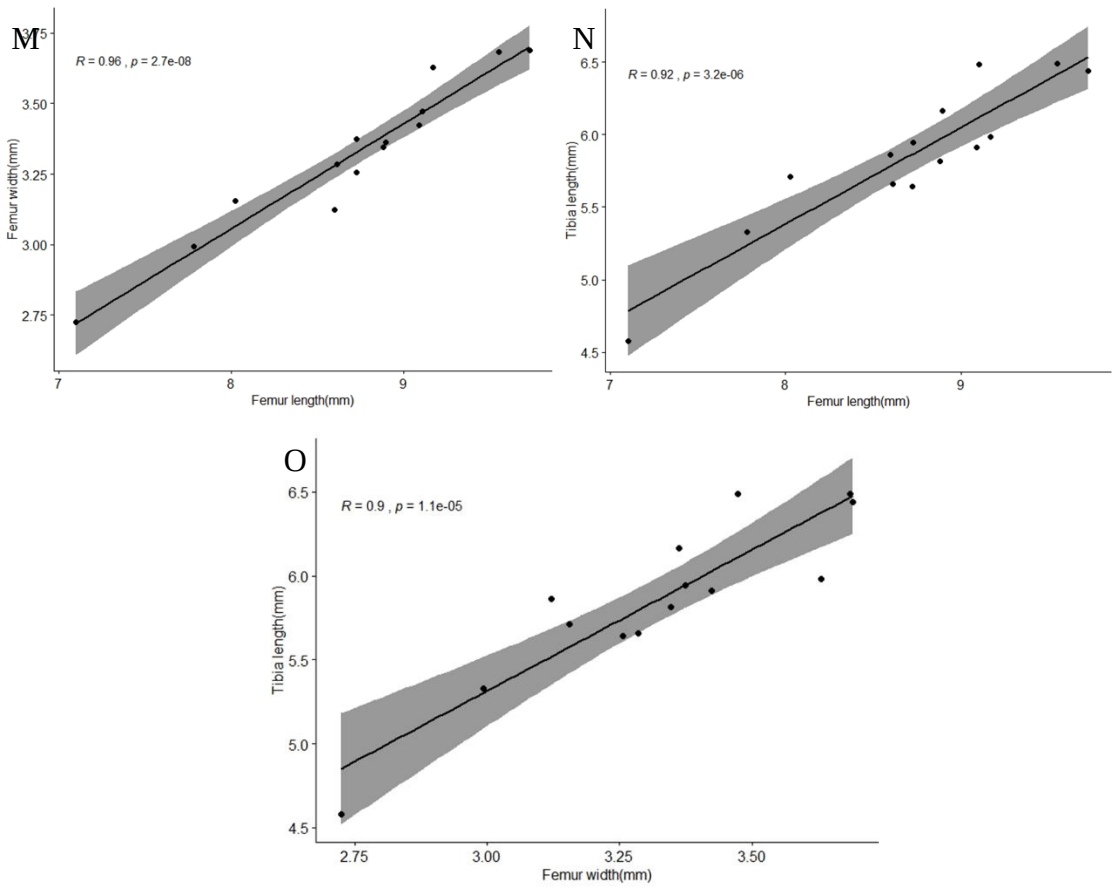


Fig 10: Correlation between life history traits in males: (M, N) femur length vs femur width, and tibia length, (O) femur width vs tibia length.

Appendix B: for chapter 3

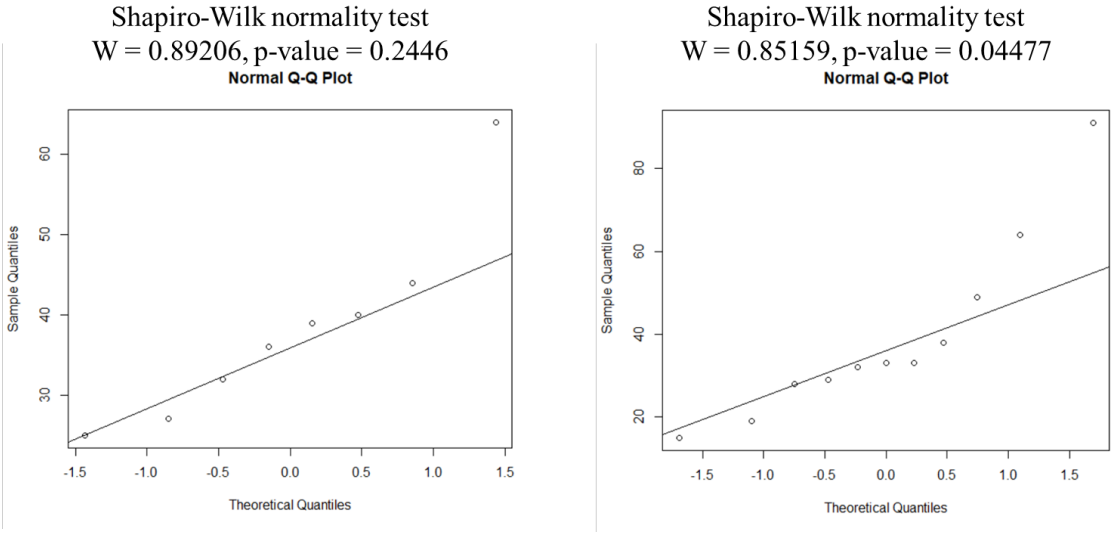


Figure 1: Shapiro Wilk test and QQ plots for latency for responses for 371ms chirp duration (left)

	Chirp duration (371ms) (N= 8) (Mean ± SD)	Chirp duration (400ms) (N= 11) (Mean ± SD)	W	p-value
Latency of response (s)	38.38±12.22	39.18±21.79	49	0.71

Table 2: A comparative analysis of latency of response to two different chirp durations using Wilcoxon rank-sum test.

Appendix C: for chapter 4

A) Code for aligning the neural firing with the acoustic stimuli and plotting the raster plot and per-stimulus plot.

```
s = read_data_tet_setup_multi('C:\Users\HP\Desktop\Prathibha_MS15050\Raw Data\Neural recordings\NR_4\experiment\experiment',7,4,7,[]);

result=[];

o=0;

op=[];

for i=1:size(s,1)

[v, lags]=xcorr(s(1,:),s(i,:));

[q,I]= max(v);

w=lags(I);

op=[op;w];

z=w*(70/size(s,2));

result = [result z];

end

FNAME='C:\Users\HP\Desktop\Prathibha_MS15050\Analysed\Neural\NR_4_Analysed\NR_4\experiment_C1.txt';

Fs=15000;

T_trial=70;
```

```
STIM_TIME=1;

eval(['K=load('" FNAME "',"-ascii");'])

X=[K(:,7:end)];

time_lag = repmat(result,size(X,1),1);

D= X+time_lag;

for n=1:size(D,2)

    Y{n}=setdiff(D(:,n),0)*15000;

    Yorig{n}=setdiff(K(:,n),0)*15000;

end

clf

subplot(211);

plot_raster(Y,Fs,T_trial,STIM_TIME);

xlabel('Time in seconds','fontsize',15,'fontweight','bold')

ylabel('Trial number','fontsize',15,'fontweight','bold')

title('Raster Plot','fontsize',18,'fontweight','bold')

ds=zeros(size(s,1),abs(min(op)));

de=zeros(size(s,1),abs(max(op)));

is=[ds s de];

fs=[];
```

```
for i = 1:size(op)

ss=circshift(s(i,:),op(i));

fs=[fs;ss];

end

subplot(212)

y=linspace(0,70,size(s,2));

plot(y,detrend(fs)/400)

xlabel('Time in seconds','fontsize',15,'fontweight','bold')

ylabel('Amplitude','fontsize',15,'fontweight','bold')

title('Peri stimulus Time Plot','fontsize',18,'fontweight','bold')

hold on

fd=X';

fdk=setdiff([fd(1,:) fd(2,:) fd(3,:) fd(4,:)],0);

[fdkh xh]=hist(fdk,linspace(0,70,700));

kf=fdkh/4;

plot(xh,kf,'linewidth',2)

hold off

ylabel('Average Spike count','fontsize',15,'fontweight','bold')
```

B) Code for the function read_data_tet_setup_multi used in the above code for alignment and per-stimulus plot.

```
function [X] = read_data_tet_setup_multi(ODOR,TET,number,start,skip)

%function [X] = read_data_tet_setup_multi(ODOR,TET,number,start,skip)

%start          -> Starting trial

%number              -> Number of trials

%skip          -> List of Tirals to be skipped



%INTRA_path_gain=1;        %Gain in the path of Intracellular recording channel

%ADC_bits=12;              %Numer of bits resolution of the ADC

%ADC_MAX_in=2.5;

%Q_levels_swing=2^(ADC_bits-1); %Onsided maximum ampitude of the ADC stored vlaue

%CELL_val=ADC_MAX_in*X{1}{2}/(Q_levels_swing*INTRA_path_gain);
            %Intracellular signal dtrended

xa=1;

X=[];

x_cnt=0;

for x=1:number

  xa=x+start-1;              %The file number under consideration
```

```
%warning off
if isempty(intersect(xa,skip))
  x_cnt=x_cnt+1;
  if TET<10
    if xa<10
      suffix=['_t0' int2str(xa) '.0' int2str(TET)];
    else
      suffix=['_t' int2str(xa) '.0' int2str(TET)];
    end
  else
    if xa<10
      suffix=['_t0' int2str(xa) '.' int2str(TET)];
    else
      suffix=['_t' int2str(xa) '.' int2str(TET)];
    end
  end
  %name=[prefix, '.', suffix];
  name=[ODOR,suffix];
%    disp(['     ',int2str(x),'/',int2str(number),'  -  loading ' name]);
```

```
    FID=fopen(name,'rb','ieee-be');
    data=fread(FID,[1,inf],'int16');
    X=[X; data];
    fclose(FID);
  end
end
```