Examining intrasexual conflict in male field cricket, *Acanthogryllus asiaticus* in relation to their morphology, prior experience and behaviour

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Certificate of Examination

This is to certify that the dissertation titled **"Examining intrasexual conflict in male field cricket, *Acanthogryllus asiaticus* in relation to their morphology, prior experience and behaviour"** submitted by **Ms. Saumya Gupta (Reg. No. MS11005)** for the partial fulfilment of BS-MS dual degree program 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.

Dr. N.G. Prasad | Prof. Somdatta Sinha | Dr. Manjari Jain (Thesis Supervisor)

Dated: April 22, 2016

Declaration

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

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.

Saumya Gupta

Dated: April 22, 2016

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)

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Acronyms

RHP: Resource holding potential

RV: Resource value

LDMC: Long distance mating call

VC: Victory call

Pf: Peak frequency

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CHAPTER 1

INTRODUCTION

1.1 General introduction on aggressive contests in animals

Conflicts in animals are related to competition over resources such as food, mates and territory (Lindenfors & S.Tullberg, 2011). These conflicts may be intersexual, intrasexual, between parents and offspring or between juveniles. Intrasexual competition is the most widely studied among all types of agonistic interactions in the context of sexual selection. This type of competition is much more apparent among males than in females because generally males invest heavily as compared to females. The winners of these male-male competitions get access to resources while losers are chased away to look for other opportunities. Clearly, the outcome of these conflicts directly affects an individual's survivorship and reproduction making aggression an important life history trait (Smith & Price, 1973). It is expected that tactics involving the use of vicious weaponry would get selected in order to overthrow the opponent in a combat but surprisingly, escalated contests which inflict serious injury are rarely seen in nature. Contests mostly consist of strategies which seldom have any lethal effects on contestants such as ritualized tournaments and displays which turns out to be an an evolutionary stable strategy (ESS) as predicted by Maynard Smith in his game theory model 'war of attrition' (J M Smith, 1974). Other evolutionary game theory models such as 'sequential assessment game' have further shown that the function of such ritualized behavioural display is to help contestants to assess asymmetries in fighting ability and motivation without incurring costs of physical fighting (Enquist et al., 1990). Studies also show that animals use physical and behavioural cues for the assessment of opponent's fighting ability and motivation (Clutton-Brock et al., 1979; Hofmann & Schildberger, 2001). The two major intrinsic factors that largely affect fighting outcomes include resource holding power and motivation. Resource holding power (RHP) or fighting ability (G. A. Parker, 1974) is generally related to attributes known for physical strength such as size, weight, weaponry. Size and weight related asymmetries between individuals have been documented to be the most well-established factors in aggressive contests (M. A. Hack, 1997; Morris et al., 1995). Empirical studies have also shown that even aggressive songs can be used as indicators of RHP (Brown et al., 2006). These are good indicator but generally not true predictors of fighting outcomes (Brown et al., 2006; Hofmann & Schildberger, 2001). Other less commonly studied intrinsic factor is resource value (RV) or motivation of individuals. This is related to the differences in perceived value of resources. High motivation levels can help weaker individuals (with low RHP) to gain an upper hand over individuals that have relatively high RHP but low motivation. For example, studies have shown that differential mating success leads to asymmetries in motivation which in turn affects male aggressiveness (Brown *et al.* 2006). Apart from intrinsic factors, extrinsic factors such as residence ownership and prior winning/losing experiences are also important in determining contest outcomes. Effect of residency on contest outcome has been seen in territorial animals where the prior owner of a resource mostly wins a contest (M. Hack *et al*., 1997; Haley, 1994). Many theories have been suggested to explain this effect. While some researchers believe that prior ownership effects come into role only because of high intrinsic fighting ability and motivation of owner (Leimar *et al.*, 1984), models based on game theory suggests that this effect could be because of an arbitrary decision rule adopted by population (John Maynard Smith *et al.*, 1976). Past experiences are also known to have a significant effect on future contest outcome in many taxa. Such effects are typically known as winner and loser effects. It is said that prior winning/losing experience may increase the probability of winning and losing a subsequent contest, respectively (Chase, 1994). This theory is based on the assumption that experienced individuals will be able to analyse costs and benefit much better than naïve individuals and hence, they would modify their behaviour to maximize benefits and minimize the costs involved in fighting. Recently, a lot of attention has been given to the effect of past experiences by means of theoretical modelling as well as empirical studies. In fact, one of the major focus of this study is to examine the effect of prior winning/losing experience on future contest outcome and behaviour in crickets and hence, the following section is dedicated to a literature review on winner/loser effects with a special focus on arthropods which will illuminate the current knowledge, gaps, caveats and contradictions presented by different studies.

1.2 Literature Review: Winner-loser effects

Winner-loser effects are ubiquitously seen across animal taxa: fishes (Chase 1994;Y Hsu et al., 1999; Huang et al., 2011; Oliveira et al., 2011), arthropods (Bang et al., 2015; Bergman et al., 2015; Kasumovic et al., 2010; Lee et al., 2014; Suzuki, 2013) and mammals (Lehner et al., 2011; Oyegbile et al., 2005). Huge inter-species variation has been documented in studies with respect to experience since many species show both winner and loser effects while some show only loser effect. A large body of evidence also shows that these two effects may differ in magnitude and retention time because of the underlying psychophysiological mechanisms acting just after an experience (Yuying Hsu et al., 2006).

Many game theoretical models have been established to dwell on evolution of aggressive behaviour and winner and loser effects as evolutionary stable strategy (Parker, 1974; Lindenfors et al., 2011; Van Doorn et al., 2003). Other than these, extensive models given by Dugatkin (1997, 2001, 2004) reveals importance of contest experience in the formation of dominance hierarchies.

1.2.1 Theoretical framework on past experiences

Theoretical models have been built on the effect of past experiences in the context of animal conflicts. These models explore the functions and consequences of winner-loser effects.

(a) Ultimate explanation of winner loser effects

Individuals assess cost and benefit associated with engaging in a contest. The decision of how much to invest in a contest is critical for individuals and a strategy which increases the overall fitness of an individual is preferred by natural selection. Winner-loser effects are seen in groups where individuals can potentially or actually modify each other's fitness. Tug of war model shows that in groups where winner-loser effects are apparent, the mean fitness of all individuals is always greater than in groups without such effects (L A Dugatkin & Reeve, 2014). Hence, an evolutionarily stable strategy of using information from prior interactions in order to make decisions about future contests could be adopted by animals.

(b) Formation of dominant hierarchies

Dominant hierarchies are mostly formed in group living animals where antagonistic interactions are frequent. Intrinsic factors responsible for fighting ability alone are often insufficient to explain these hierarchies. Models have shown that both intrinsic and extrinsic factors can explain the formation of linear hierarchies (Landau, 1953; L. A. Dugatkin et al., 2004) . These models are based on combined winner and loser effect and do not take into consideration the case where individuals can assess each other's RHP. Dugatkin (1997) incorporated the ability of individual to assess its own as well as other's RHP in his models and found that the type of hierarchy formed actually depends on whether winner or loser effects act independently or in combination. He showed that winner effect alone engenders hierarchy where ranks could be assigned to all individuals whereas, loser effect alone engenders hierarchies where only clear alpha individuals exist (Lee Alan Dugatkin, 1997).

Other plausible extrinsic factors such as bystander effects and audience effects along with winner and loser effects can also possibly bring a change in dominance hierarchies of animals (Chase, 1982; Dugatkin, 2001). All these models need empirical data for validation but till date most of the empirical studies focus on winner loser and bystander effects as pairwise interactions. Only handful of empirical studies have tried to explore hierarchical formation with respect to winner loser effects (L. A. Dugatkin et al., 2004; Yurkovic et al., 2006; Bell et al., 2015).

(c) Modulation of winner loser effects

Past winning/losing experiences most likely change the actual or perceived fighting ability of individuals. This change in fighting ability based on success or failures affect different individuals differently, based on their age and experience. Hawk and dove models show that aggression is highest in young and naïve individuals who are unsure of their fighting ability since losing initial fights will have a greater impact on their decision to escalate future contest. But for older individuals, winning would bring about greater impact as the weaker individuals would adopt dove (non-aggressive) strategy and a win could prompt them to switch their strategy (Fawcett & Johnstone, 2010). This model gives a testable prediction and such studies could be easily carried out in laboratories but researchers generally either ignore the effect of age or they select individuals of the same age to look for the winner-loser effect.

1.2.2 Debate on changes in actual fighting ability VS perceived fighting ability

Prior experience effects are known to cause changes in aggressive behaviour and contest outcome by changing the fighting ability of individuals involved in a combat. There are two leading theories on these apparent modulation of fighting ability. These suggest that fighting ability can be affected either because of changes in actual fighting ability by changing RHP of an individual (Parker, 1974) or because of changes in perceived fighting ability by underestimation or overestimation of RHP (Mesterton-Gibbons, 1999) .

1.2.3 Empirical evidences

Across animal taxa, a number of experiments have been performed to test the effect of past experiences but the extant literature on this topic presents many contradicting results.

(a) <u>Differences in methodologies and experimental designs</u>

Discrepancies in results obtained from winner and loser studies may be attributed to the differences in methodologies and designs adopted by various researchers.

The procedure adopted to select individuals for the fighting experience certainly have an impact on the outcome of experience effect (Chase et al., 1994). There are typically two types of procedures that are followed: '**self-selection method**' and '**random selection method'**. In self-selection method, equivalent individuals (size or weight matched) are pitched against each other and the winner/loser is then allowed to meet a new opponent. This method although widely used has been in scrutiny since it confounds the fighting experience with the inherent fighting ability of individuals (winner of matched fights is expected to have higher fighting ability than average). Simulations have shown in such cases, an appropriate null hypothesis of 0.67 rather than 0.5 should be used to account for intrinsic differences. Hence, many studies based on this methods should be carefully interpreted before reaching any conclusions (Adamo & Hoy, 1995; Lee et al., 2014; Whitehouse, 1997). In random selection method, individuals are chosen randomly to gain a predetermined winning/losing experience either by pitching them against asymmetric individuals or against habitual winners/losers (individuals with extremely high/low intrinsic ability) (Bang et al., 2015). This method tries to randomize the intrinsic fighting differences between individuals so as to see the effect of experience in isolation.

Second methodological difference involves experience and age of selected individuals. Theoretical model has already shown that experience and age might modulate winner loser effects (Fawcett & Johnstone, 2010). Many studies rear the animals in lab conditions and isolate them during birth so as to eliminate any effect of previous experience while other studies use wild caught individuals and isolate them for long intervals to allow previous perceptions of individuals from the field to disappear. In the latter case, the isolation time is critical to eliminate immediate experience effects. Age is also a factor that is often difficult to control in studies, especially those that use wild-caught animals. According to predictions, loser effect will be more prominent in young individuals while winner effect will be more prominent in old individuals (Fawcett et al., 2010). Very few studies meticulously separate these two categories and examine the effects in both the age groups. While most of the studies on wild caught animals simply ignore age effects, studies on lab-reared animals generally take into consideration only young individuals. There is a dearth of studies comparing winner-loser effects on the two age groups.

Differences in experimental designs could also lead to variation in outcomes after experience effects. Many studies expose the contestants for long durations while other studies keep a short time duration of interaction between contestants (Chase et al., 1994). Long-time durations may confound the effects of past experiences as the probability of physical injury, exhaustion and energy depletion might increase with exposure time (Yuying Hsu et al., 2006). This could lead to negative impact on winners/loser involved in a contest with naïve males. Apart from duration of contest, recovery time given to winners/loser before exposing them for next contest is also important (Dugatkin et al., 1994). Some studies use a very long recovery time to eliminate the possibility of physical exhaustion and energy depletion which is not an appropriate design since it has been established that these effects are primarily transient (Chase et al., 1994).

(b) Transient nature of winner / loser effects

It is widely known that winner/loser effects are only short term effects. The duration for these experience effects are important since it allows us to explore the effect of past experiences meaningfully. Studies on other taxa (mostly fishes) suggest that loser effect lasts for longer when compared to winner effects (Hsu et al., 2006) and very few studies on arthropods have verified these results (Kasumovic et al., 2010). Yuying Hsu recently showed that the general conception about high retention time of loser effect when compared to winner effect may not hold true as it depends on the type of contest behaviour that is observed (Huang et al., 2011).

1.2.4 Proximate mechanisms governing winner/loser effects

Modulation of behaviour such as change in frequency of attacks, latency of attack/retreat, intensity and duration of competition and ultimately, determination of contest outcome due to prior winning/losing experience is known to be induced because of physiological mechanisms. In fact, the transient nature of these experience effects can be explained in terms of the rapid changes in neuromodulators and hormones in response to experience effects. In insects, the adrenaline/nonadrenergic analogue: amine octopamine is found to be correlated with high aggression level and generally serotonin is responsible for suppression on aggressive behaviour (Rillich et al., 2011; Stevenson et al., 2013). In other arthropods like crustaceans, even though the key players are similar but the mechanism seems to be different. While octopamine levels decrease after winning and increase after losing as compared to resting values, dopamine and serotonin levels surge after winning experience (Sneddon et al., 2000). This study has revealed a link between the concentration of biogenic amines in resting state and fighting ability suggesting a possible predictor of fighting outcomes.

1.2.5 Conclusions

In the light of present literature, as discussed above, the following point may be concluded:

i) Prior winning/losing experience may influence future contest outcome in arthropods although the effect is not same for all taxa.

ii) Some taxa exhibit only loser effect while other exhibit both winner and loser effect with loser effect being much more prominent.

iii) Some of the observed variation in existence, magnitude, duration and behavioural influence caused by these effects may be partly because of artefacts due to methodological differences between studies for which standardized procedure needs to be laid down and partly because of different environmental contexts of different species where social animals are expected to have much greater implications of winner/loser effects.

iv) It should be noted that even though experience effects are found to be a very important factor related to resource holding potential, there are still other factors that also play crucial role in deciding fighting outcomes such as resource value, aggressiveness, prior ownership of a resource etc.

1.3 Crickets as a model organism to study aggression

There are innumerable examples of intrasexual competition in nature but crickets provide an excellent and convenient model to test hypothesis on aggression in lab conditions (Judge & Bonanno, 2008). Frequent male-male agonistic interactions over territories and mates have led to the evolution of a large number of behavioural tactics (M. A. Hack, 1997). Escalated male fights comprise of highly ritualized sequence of aggressive behaviours starting from individuals making a contact via antennal fencing to mandible spreading and mandible entanglement (Hofmann et al., 2001). A whole range of exotic behaviours can be seen during this process such as rock body, kick, threat posture, bites, aggressive calls etc (Adamo & Hoy, 1995). Finally, the decision about the winner is made, and the loser is chased away followed by a rivalry song by winner which is often termed as "victory call" (fig 1.1).

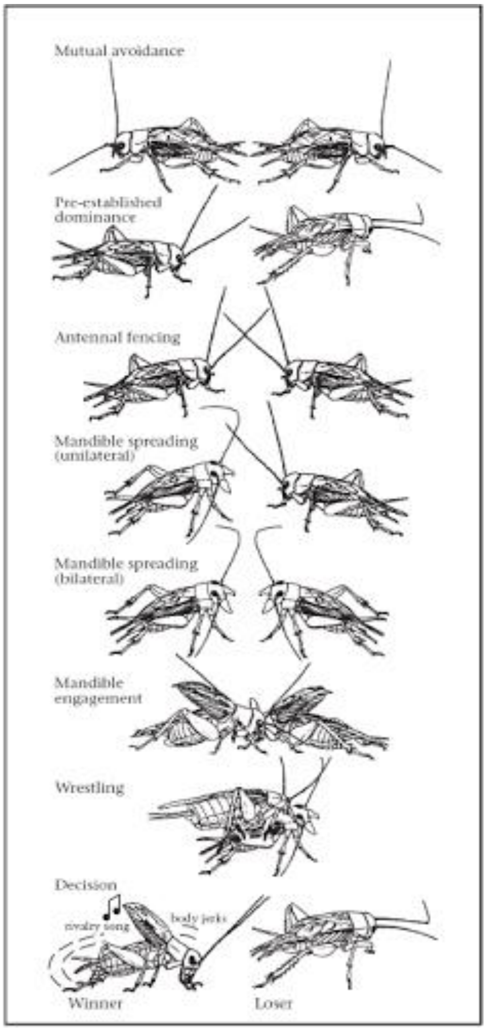

Figure 1.1: Stereotype sequence of aggression during contests in field cricket, *Gryllus bimaculatus*

Reference: Hofmann et al., 2001

Apart from the dramatic visual display, many crickets also use acoustic communication during contests. Male crickets produce sound by rubbing their elytra (modified hardened forewings) with each other (Bennet-Clark, 1970). They produce sounds primarily in order to attract females (calling songs) over long distances. These calls are typically very loud, however, they also produce calls at the time of mating (courtship calls) and during or after agonistic interactions (aggressive calls / victory calls). These aggressive calls are loud trills which have shorter chirp duration and high frequency than any other call produced by crickets (Brown, Smith, et al., 2006).

1.4 Objectives

In this study, I mainly examined the factors responsible for contest outcome in field cricket, *Acanthogryllus asiaticus.* Following are my objectives:

i) To test if characteristics indicating RHP such as body weight and other morphological features of *Acanthogryllus asiaticus* are indicators of male-male aggressive outcomes.

ii) To see how past experiences influence the behaviour and outcome of subsequent aggressive contests in this species.

ii) To perform a posthoc analysis of victory calls and long distance mating calls of *Acanthogryllus asiaticus* in order to characterize both the calls.

1.5 Study system

In order to address the above-mentioned objectives, I used a field cricket species, *Acanthogryllus asiaticus* (fig. 1.2). It has been described by Gorochov (1990) as "small body-sized (av. length= 15mm), large brown head with 6 distinct short longitudinal pale lines on posterior part of vertex, dark brown pronotum (length= 2.9 mm) with pale spots in posterolateral angles of disk, pale brown elytra with transverse stridulatory ridge, distinct bent diagonal vein, relatively wide area diagonal vein and oblique vein and unicolorous legs, abdomen and cerci". Their taxonomical position is as follows:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insects

Order: Orthoptera

Suborder: Ensifera

Superfamily: Grylloidea

Family: Gryllidae

Genus: *Acanthogryllus*

Species: *asiaticus*

Figure 1.2: *Acanthogryllus asiaticus* male

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This species is quite abundant on IISER Mohali campus and pilot experiments in laboratory conditions revealed that males when pitched together in arena show a stereotypical aggressive behaviour (as described above) making them ideal model organisms to study aggression.

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CHAPTER 2

Investigating the factors responsible for fighting success in *Acanthogryllus asiaticus*

2.1 Materials and methods

2.1.1 Collection and housing of animals:

Acanthogryllus asiaticus adult males were collected mainly from two areas: IISER Mohali campus (lat 30°39.820', long 76°43.619') and a nearby area (lat 30°39.800', long 76°43.870') starting from August 2016 till mid-October 2016 (n = 113) (fig. 2.1).

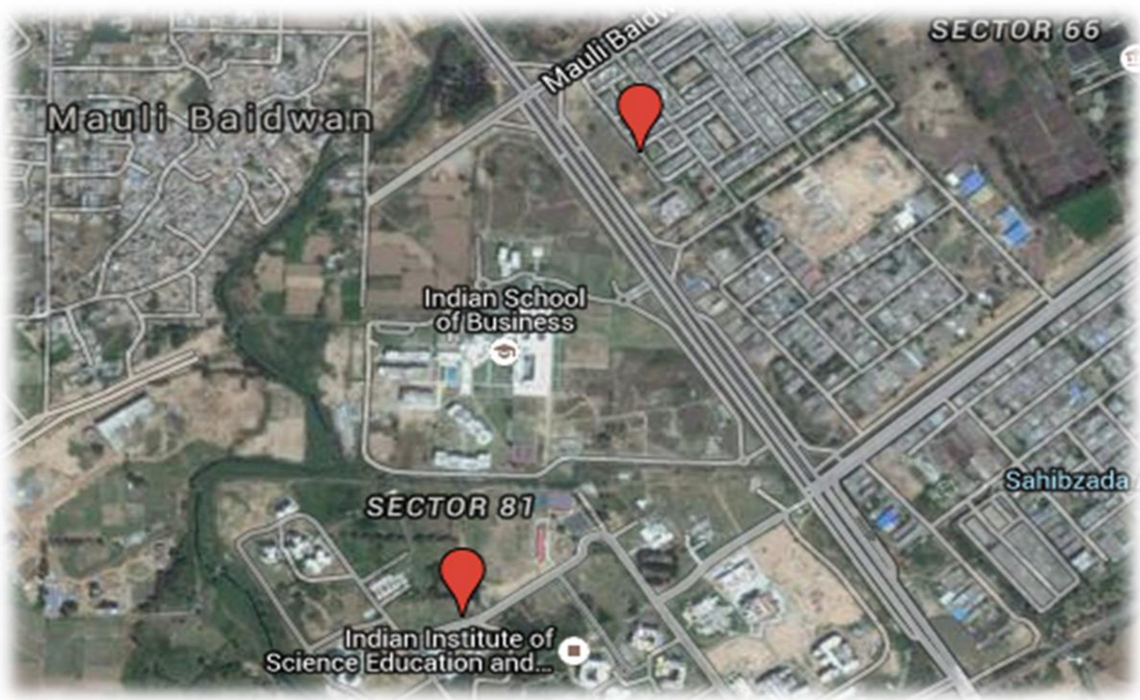


Figure 2.1: Satellite image depicting both sampling sites

All the collected individuals were kept in isolation in clear round plastic containers of radius 6.2 cm to eliminate the effect of prior physical contact from other conspecifics. Note that these isolated individuals could still potentially hear, see and smell other individuals. They were maintained on a 12 hour day and night cycle in a temperature and humidity controlled Memmert incubator (T = 24 degrees, Humidity = 40% rh). Food was provided in the form of pedigree pellets, crushed calcium tablets and water was supplied through soaked cotton balls. All individuals were kept in isolation for at least 4 days in lab conditions before subjecting them to any experimental trials so as to eliminate the effects of any prior experience.

2.1.2 Pilot experiments

Pilot experiments were conducted on 14 different pairs of *Acanthogryllus asiaticus* using 13 individuals (many individuals were reused for these pilot experiments) to optimize the arena size, the maximum duration of observation and to observe the baseline behaviours of crickets seen during agnostic interactions. The optimized details extracted from these set of pilot experiments which helped in establishing the experimental protocol are presented in the subsequent sections.

2.1.3 Experimental setup

A cardboard box of dimension (length= 25cm, width= 15 cm and height= 30 cm) was used to make a fighting arena whose base was covered with soil. A removable cardboard block was placed at a distance of 12 cm from one side of the box making (12*15) cm as an effective fighting area. This block served the purpose of an escape route for crickets which was lifted only after a clear winner and a clear loser emerged from the combat. The walls of the cardboard were covered with transparencies (hard transparent plastic sheets) to reduce friction so that crickets are not able to climb on the walls. Each trial was video recorded using Sony cybershot^{TM} DSC-HX-400V (Sony corp., Japan) and also, audio recordings of the entire duel was done using TASCAM handheld recorder DR-07 MKII (TEAC Corp, US) (fig.2.2)

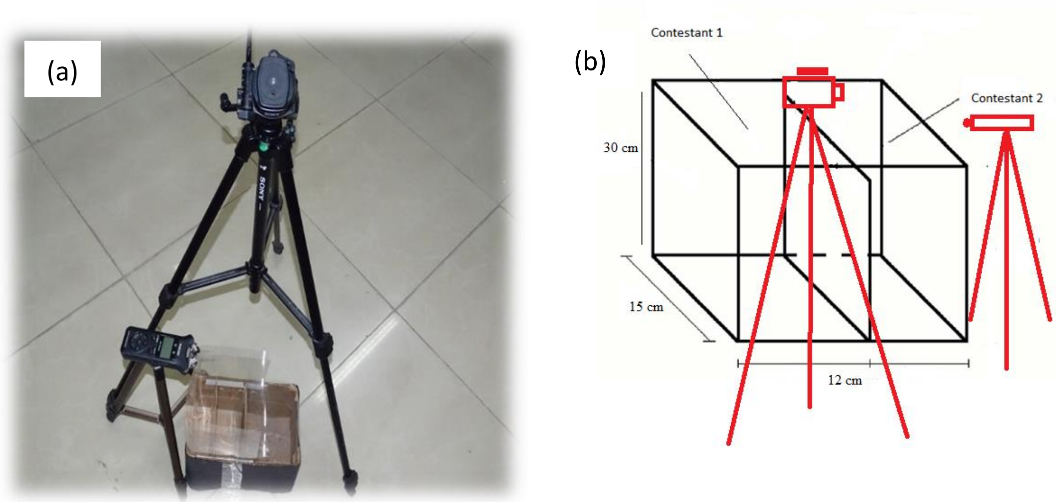


Figure 2.2: (a) Image of experimental set (b) Pictorial representation of setup

2.1.4 Experimental protocol

All the experiments were conducted at night between 8pm- 12pm (during which this species of crickets are most active) for a maximum of 10 min per trial so as to not confound the subsequent fights with physical exhaustion/ energy depletion. No incentive was given to the crickets since it is their innate quality to fight when found in proximity.

On the day of trial, four naïve individuals (those who have never been in physical contact post collection from field) were selected randomly for each set of winner/loser effect. Out of those four, two individuals were selected at least 6 hours prior to the experiment and marked with non-toxic acrylic color on their pronotum to allow identification during contests. Just before the trial started, arena was setup as described above, soil was shuffled after every trial and the walls of the arena was wiped using 70% ethanol to eliminate the effect of any possible pheromones left by crickets. Individuals were introduced in the arena 4 min after ethanol cleaning. At the start of each contest, an opaque partitioning was used in the center and each individual was introduced on either side of the separation simultaneously. 1 min of acclimatization time was given to all individuals and then the video camera and audio recorder were switched on simultaneously before the trial was started. Trials were started by removing the opaque separation. The contests were terminated by providing an escape route so as to allow individuals to run away from the arena once a clear winner and a clear loser emerged. In case no decision was made, the contest was terminated in 10 min.

2.1.5 Ethogram

Since all the contests were video recorded, I was able to characterize every behaviour that was seen during the agnostic interaction. In fact, the behavioural differences seen before the contest, during the contest, and after the contest formed the basis of the decision on winner and loser. Before the interaction, contestants were mainly engaged in exploring the surround. The beginning of an interaction was marked by either antennal contact of both the contestants or aggressive calls made by one of them which further led to an escalated physical combat in most of the cases. The physical combat consists of highly stereotyped sequences which are detailed below. Most of the categories are taken from previous studies on cricket aggression (Adamo & Hoy, 1995; Simmons, 1986) and others have been named and described by me (table 2.1).

The end of the contest was generally marked by a characteristic victory call by the winner and sometimes body jerks and a chase by the winner behind the losing individual. The loser was marked by retreat and avoidance of another confrontation.

Behavioural category	Description
Antennal fencing	Opponents lashing on each other with their respective antenna
Mandible extension	Extending one's mandible
Grapple	Interlocking mandibles or head to head collision in order to push or pull opponent during a fight
Mandible spar	Attacking the opponent by using extended mandibles on any part of the body
Fore punch	Using forelegs to push or pull opponents
Chase	Running after an opponent
Aggressive call	Production of high intensity continuous calls during an aggressive encounter
Back attack	Attacking the opponent from behind
Body jerks	Continuous jerky movements made by the contestants
Slam dunk	A forceful move that throws away it's opponent

Table 2.1: Description of aggressive behaviours shown by *Acanthogryllus asiaticus*

2.2 OBJECTIVE 1: Are morphological characteristics of field cricket, *Acanthogryllus asiaticus* indicators of male-male aggressive outcomes?

The goal of this experiment was to use (a) body weight and morphological characters of *Acanthogryllys asiaticus* such as (b) body length, (c) hind leg length, (d) forewing length and (e) pronotum width as a proxy of RHP, and test if these characteristics have a role to play in determining the agonistic contest outcome. 43 pairs of naïve individuals were fought against each to address this question. 2 naïve individuals were randomly selected, each was marked with acrylic color for identification and then pitched against each other in the fighting arena using a protocol mentioned in the previous section. Winner and loser of each fight was noted. Some of the individuals were used again after their recent experience in lab but only after atleast 10 days of isolation since previous studies on other crickets have shown that after 24 hours memory of prior experience diminishes and individuals act as naïve males (Adamo & Hoy, 1995).

After all the trials were completed over a period of 2 months, their body weight and other morphometric measurements were taken. Body length, hind leg length, forewing length and pronotum width was measured using a Leica stereozoom microscope M205C loaded with a leica application suite, version 4.6.0 on a 5mm scale (N= 99) (Fig 2.3). NOTE: The scale of the microscope was not calibrated at that time and hence our measurements of absolute size are not accurate. But since I am only interested in relative sizes, this disparity between actual size and measured size is not of much significance for our analysis. For body weight, many individuals were dead till the completion of experiments and were preserved as wet samples and hence, a protocol was established to take the dry weights (weights taken after drying the dead individuals). Body weight was taken only when all the individuals were dead, removed from alcohol and kept for drying. After 24 hours, their weights were taken and individuals with missing body parts were discarded from analysis (Total number of individuals whose weight were taken =100, number of discarded individuals=10).

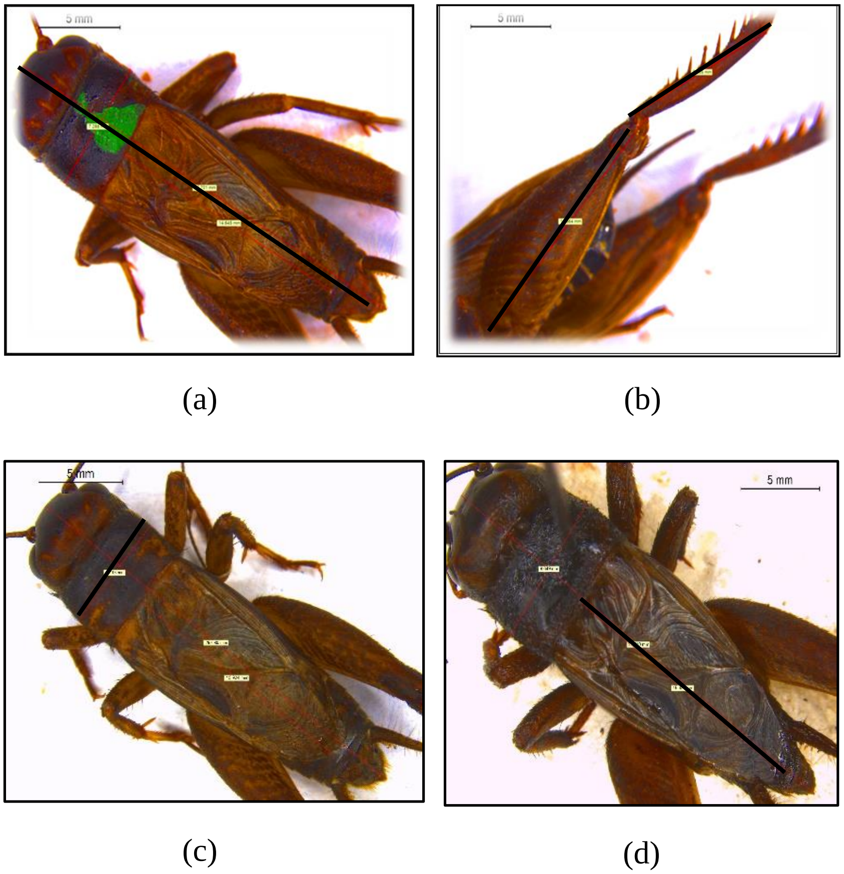


Figure 2.3: Black line in all the images depicts the measured parameter where (a) body length (b) hind leg length (c) pronotum width and (d) forewing length

2.2.1 Statistical analysis

Winner and loser of each contest were noted and their corresponding morphometric measurements were recorded. Since the winner and loser come from the same contest, a paired sample t-test was performed to check for any significant differences in the above-mentioned characters between the two dependent groups i.e. winners and losers. One assumption of paired sample t-test is that the difference between the two groups should follow a normal distribution. To check for that, differences in each of the character: body weight, body length, hind leg length, forewing length and pronotum width for paired winner and loser was checked for normality using Kolmogorov-Smirnov test and Lilliefor test and also, a Q-Q plot was made to further validate the assumption of normally distributed data since Q-Q plot graphically tells us whether our data follows assumed distribution (David Scott). All the analysis was done using Statistica 64, version 12.7.207.0 software.

2.2.2 Results

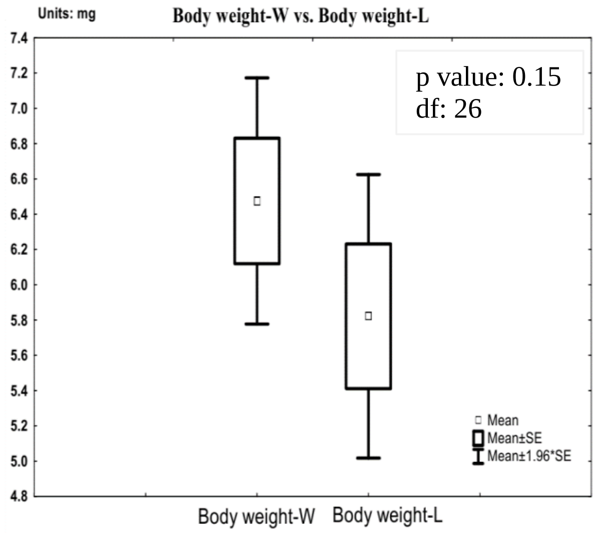
All the morphological characters showed a normal distribution. The following table depicts the p values using Kolmogorov-Smirnov and Lilliefors normality tests (Refer to Appendix A for individual Q-Q plot of every character):

Variable	N	Max D	K-S p-value	Lilliefors p-value
Difference in body weight	27	0.121	$p > 0.20$	$p > 0.20$
Difference in body length	33	0.079	$p > 0.20$	$p > 0.20$
Difference in forewing length	31	0.123	$p > 0.20$	$p > 0.20$
Difference in hind leg length	30	0.154	$p > 0.20$	$p < 0.10$
Difference in pronotum width	33	0.090	$p > 0.20$	$p > 0.20$

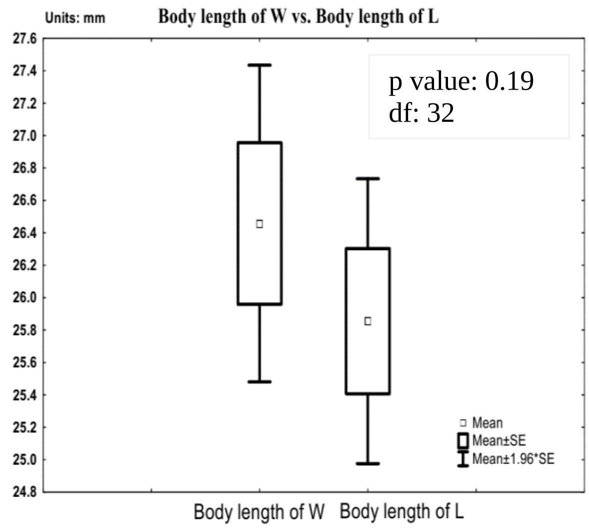
Table 2.2: Results of Kolmogorov-Smirnov test and Lilliefors normality test for all the measured variables

This means that the assumption of the paired sample t test: The difference in the measured parameters of winners and losers come from a normal distribution is correct.

Paired sample t-test showed no significant difference in the body weight of winners and losers (fig 2.4a) (6.47 ± 1.85 vs 5.82 ± 2.13 mg, respectively: $t= 1.49$, $df= 26$, $p= 0.15$); no significant difference in body length of winners and losers (fig 2.4b) (26.46 ± 2.86 vs 25.85 ± 2.58 mm, respectively: $t= 1.32$, $df= 32$, $p= 0.19$); no significant difference in forewing length of winners and losers (fig 2.4c) (14.56 ± 1.21 vs 14.36 ± 1.02 mm, respectively: $t= 1.10$, $df= 30$, $p= 0.28$) and no significant difference in hind leg length of winners and losers (fig 2.4d) (23.08 ± 2.15 v 22.58 ± 2.61 mm, respectively: $t= 1.16$, $df= 29$, $p= 0.25$). Although, statistical significance was seen in the pronotum width of winners and losers (fig 2.4e) (7.49 ± 0.67 vs 7.27 ± 0.63 mm, respectively; $t= 2.03$, $df= 32$, $p= 0.050$) but because it is a weak statistical significance, inferences based on this is not advisable.



(a)



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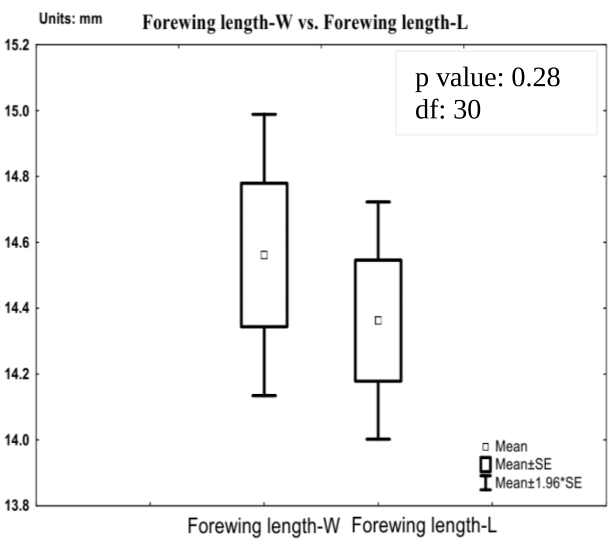
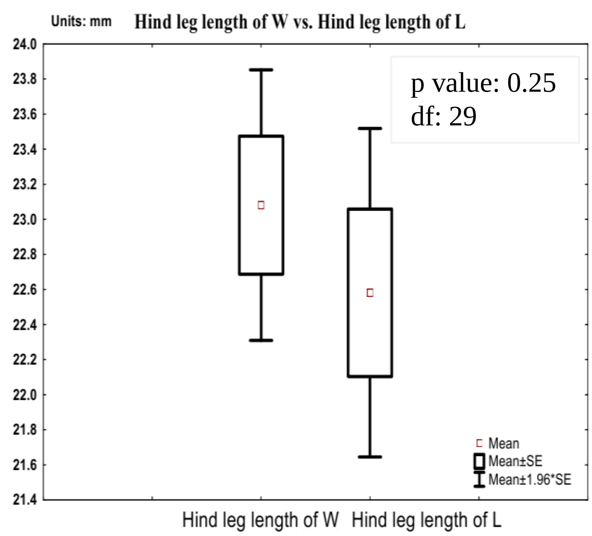


(c)



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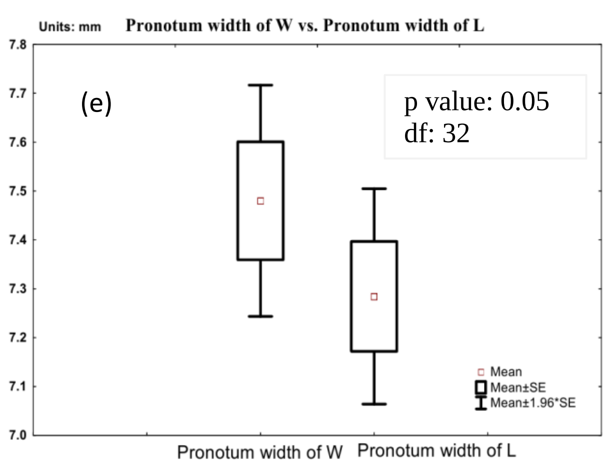


Figure 2.4: Box plots depicting asymmetries between winner and losers of naïve-naïve contests for (a) body weight (b) body length (c) forewing length (d) hindleg length and (e) pronotum width

2.2.3 Conclusions

Our results strongly suggest that body size, body weight and other morphological characters are not indicators of fight outcome in our model species.

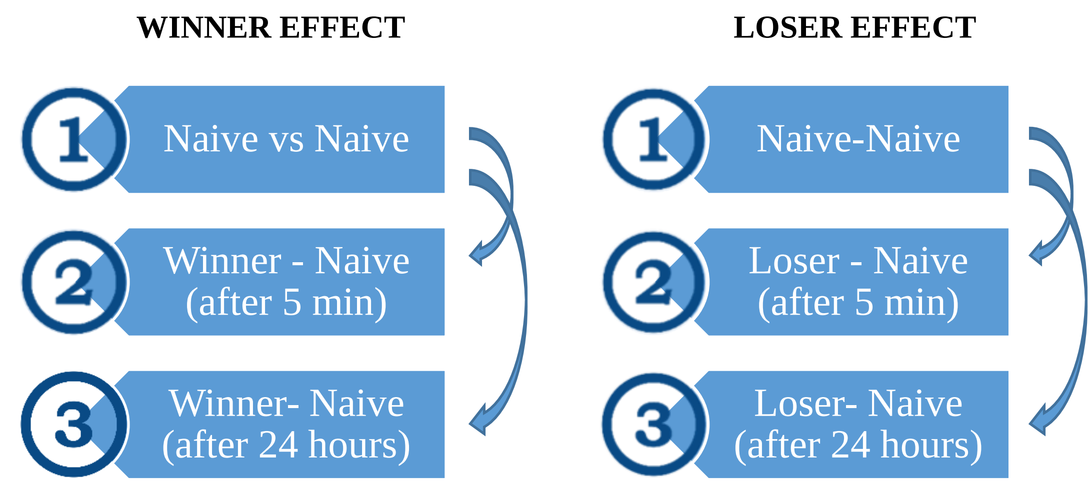
2.3 OBJECTIVE 2: Does winning/losing experience affect future aggression in male *Acanthogryllus asiaticus*?

My goal here was to test the influence of recent experience on the future aggressive behaviour and contest outcome in field cricket, *Acanthogryllus asiaticus.* I was also interested in identifying the time window for which these effects persist if it all they are present.

There are two widely used methodologies to stage contests in order to study experience effects. First is **random selection procedure** where the dominance and subordinate experience to the naïve individuals is decided 'a priory' based on either asymmetries (ex- size, weight, prior resident) or by pairing them with habitual winner and loser (Hsu et al., 2006). The second method is **self-selection procedure** where fights between equivalent opponents (individuals matched on the basis of size, weight etc.) are staged and dominance and subordinates are selected 'ex-post facto'. Since my previous results indicate that neither body weight nor other morphological features have any effect on contest outcome, two random individuals were considered as being equivalent and self-selection procedure was adopted since it is a more natural method of acquiring experience in the field (Bégin et al., 1996). However, this method potentially confounds past experiences with the intrinsic fighting ability of individuals since winning/losing of an individual may be attributed to something inherent like high/low fighting ability, the effect of hormones or high/low motivation. A theoretical study has revealed that when self-selection procedure is to be followed, one must test the effect of prior experience with a null hypothesis of winning/losing probability of 0.67, a much more stringent and conservative threshold as opposed to 0.5 in order to control for any confounding effects (Bégin et al., 1996).

To examine the existence of winner and loser effect independently (since we have no idea about the proximate mechanisms governing these effects in our model system, the presence of winner and loser effects was decided to be checked independently), 24 sets of trials were staged to test the effect of winning experience [5 sets were performed by a summer project student:

Ms. Lata Kalra] and 19 entirely independent set of trials were staged to test the effect of losing experience where each set consisted of 3 contests in the following manner:



Individuals were paired randomly in all the contests. The first contest was staged between two marked naïve individuals in the same manner as experiment 1. The marked winner/loser of first contest was then pitched against unmarked naïve individual (selected randomly) after an interval of 5 min and 24 hours from the first fight to check if this effect exist after 5 min and lasts at least for 24 hours. All the contests were video and audio recorded. Winner and loser were noted for each trial. (Note: Some of the individuals used for winner set were again used for loser set and vice versa but only after a min of 10 days of isolation after their recent fighting experience).

Information about timing of first approach, timing and individual who made the first call, individual who initiated the fight, duration of fight, frequency of calls by winner and loser and behaviours observed during fight were extracted from the video recordings (Details about the observations made from the videos are given in Appendix B)

2.3.1 Statistical analysis

Firstly, the existence of winner/loser effects after 5 min of prior experience was tested. Only those contests were included in the analysis where we had a clear winner and loser. As a result, we were left with a sample size of n=23 for winner effect and n=17 for loser effect. The frequency of winners winning a subsequent contest (WW) and losers losing a subsequent contest (LL) out of total staged contests were counted. WW:WL and LL:LW were checked for a null hypothesis of 0.67. Chi-square goodness of fit test was performed to determine the effect of prior experience on contest outcome.

To investigate the effects of prior experience on future contest behaviour, frequency of winner/loser and naïve males initiating a subsequent contest through first call and physical interaction was taken was analysed using chi-square test. The frequency of aggressive calls by winner and loser in naïve-naïve contests vs winner/loser-naïve contests were compared using Wilcoxon paired sign test.

2.3.2 Results

- Effect of past experiences on future contest outcome

Out of 23 consecutive fights, winners won 12 subsequent fights (WW) but lost 11 fights (WL). This clearly demonstrates that winning experience does not have a significant effect on future contest outcome (chi-square= 2.28, df= 1, p= 0.13). In contrast, losers lost 14 subsequent fights out of 17 fights and won only 3 fights. Yet, contrary to the expectation, contest outcome was not influenced by losing experience also (chi-square= 1.81, df=1, p= 0.18) (fig 2.5).

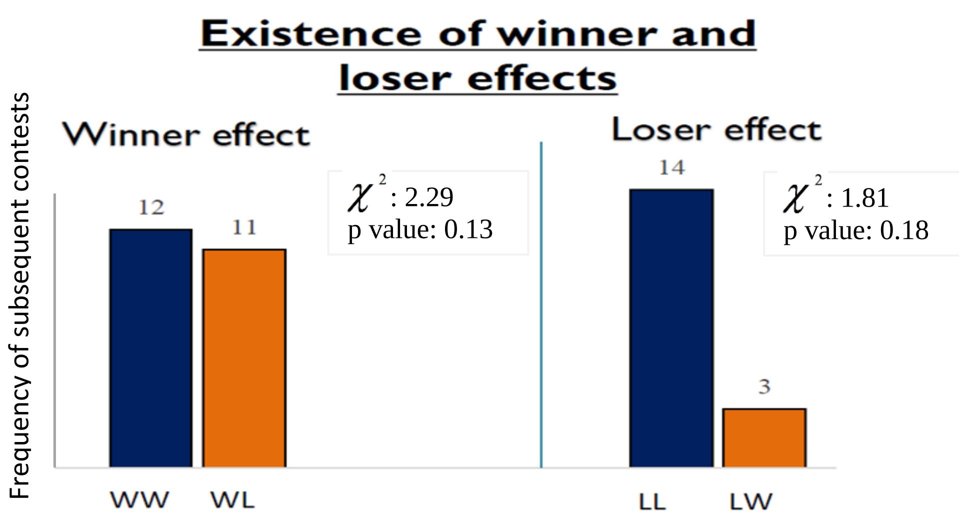
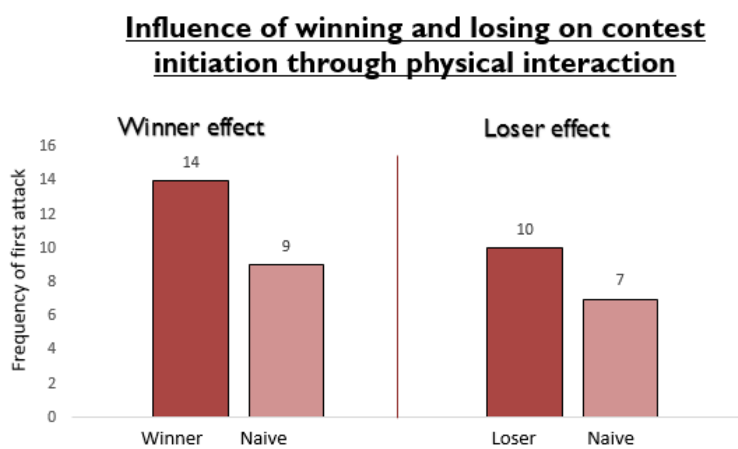


Figure 2.5: Blue bars indicate the frequency of consecutive wins/loss by prior winner/loser, respectively, while orange bars indicate frequency of a different outcome in subsequent fight as compared to prior experience.

- Effect of past experience on future contest behaviour
  - Individuals with prior winning experience did not show any significant difference in the tendency to attack first when compared to naïve individuals (chi-square= 1.08, df= 1, p= 0.29) (fig 2.6a). Similarly, losers are also as likely to make a first attack on the opponent as naïve males (chi-square= 0.81, df= 1, p= 0.36) (fig 2.6b).

(a)



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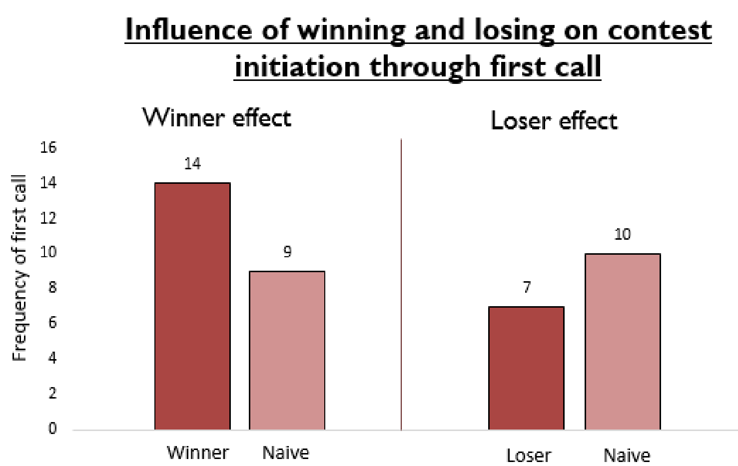

Figure 2.6: (a) Frequency of fights initiated by means of physical attack after 5 min of prior experience by winners/losers as compared to naïve males; (b) Frequency of fights where winners/loser of previous fight initiated a subsequent contest as compared to naïve males

  - The frequency of calls by an individual was not affected by their prior experiences. Winners and losers did not give significantly higher/lower number of calls after winning and losing experiences, respectively (Wilcoxon paired test: N=17, p= 0.23 for winners and N= 7, p= 0.50 for losers) (fig. 2.7).

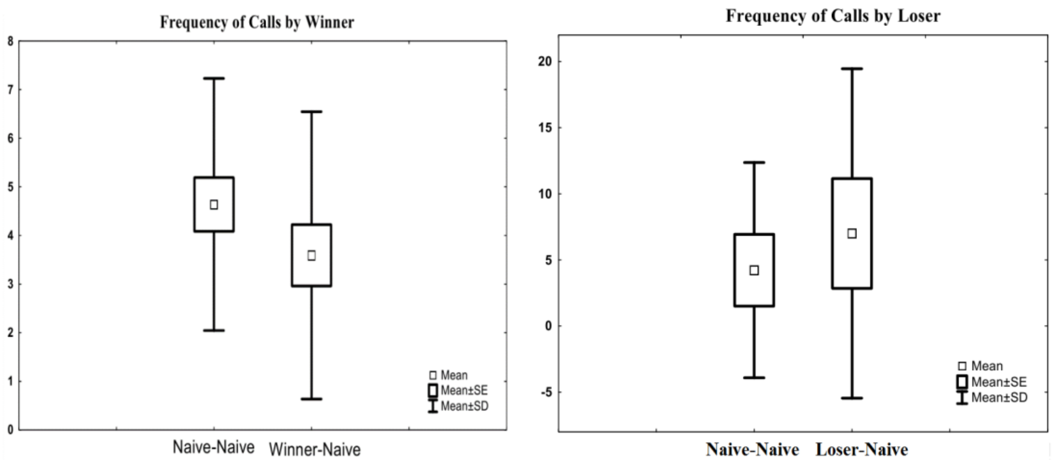

Figure 2.7: Box plots depicting frequency of calls by winner/loser before and after a contest experience

In summary, I did not find any significant difference between winner/loser in any of the behavioural parameters measured.

2.3.3 Conclusions

Our results demonstrate that there is no winner or loser effect in *Acanthogryllus asiaticus* males. Also, prior experiences does not seem to influence the future agonistic behavior of winner/loser.

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CHAPTER 3

Characterizing long distance mating calls and victory calls

3.1 Sound production mechanism in crickets

Male crickets have specialized structures on their forewings known as file and plectrum which are responsible for the production of sound through stridulation. The underside of the top wing have teeth like structures known as file and the bottom wing has a blade like plectrum (fig 3.1) which are scrapped together during the closing of vertically lifted wings, producing a pulse of sound known as a syllable. Series of opening and closing of raised wings gives burst of pulses known as chirp (Kavanagh, 1987). The sound produced is then amplified by resonant structures on the wing such as a mirror and harp (Bennet-Clark, 1999).

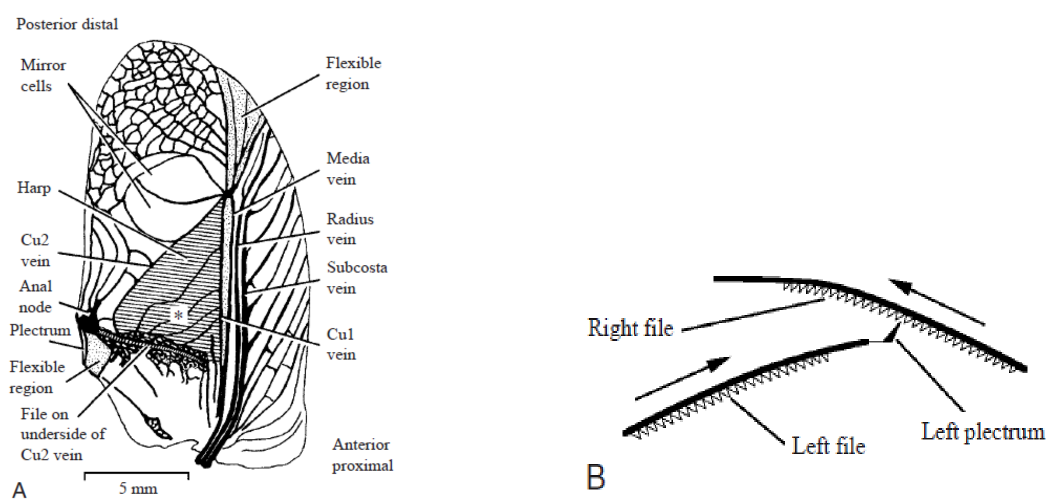


Figure 3.1: (A) Diagram of underside of cricket forewing (B) Diagram of a section of right and left forewings showing the interaction between both the wings while sound production

Reference: (Bennet-Clark et al., 2002)

3.2 Types of cricket sound

Mainly four types of cricket calls have been identified (Richard D . Alexander, 1962)

- Long distance mating call (LDMC): These type of calls are highly species specific and produced by males from their territory to attract sexually receptive females.
- Courtship song: This call is produced by males in anticipation of copulation when females are in proximity
- Aggressive sound: These calls are produced by males when they are involved in fighting with a conspecific male
- Post-copulatory song: These calls are produced by males after transferring sperms in order to keep a watch of females and prevent them from removing the spermatophore.

However, a recent study on post-conflict display has categorized post-conflict aggressive calls by winners as victory calls which was quantified by higher rates of songs as compared to aggressive calls which are produced during a conflict (Bertram et al., 2010).

3.3 Features of cricket songs:

There are mainly two components of cricket song which helps in distinguishing different call types-

- Temporal component: Temporal pattern of a call carry acoustic information of a particular call in the form of chirp period, chirp duration, syllable period, syllable duration etc. (Hennig et al., 2014). These parameters are known to be most variable features of a cricket call.
- Spectral component: Cricket calls are generally pure tones with one dominant frequency that corresponds to harmonic frequencies. Different call types differ in their dominant frequencies which are an attribute of resonant structures of wing (Desutter-Grandcolas et al., 2004)

A combination of the temporal and spectral features give each species of cricket a stereotypic, species-specific mating call. Intraspecies variation in calls do exist and may reflect in one or more of the above mentioned acoustic features.

3.4 OBJECTIVE: Characterization and comparison of long distance mating calls and victory calls of *Acanthogryllus asiaticus.*

Acoustic features of *Acanthogryllus asiaticus* are unknown till now. My work on aggression further sparked my interest in the studying the function of victory calls. For my thesis, I only took the first step to study these calls by characterizing them and identifying the features which are different from normal calling song.

3.4.1 Materials and Methods

Call recordings

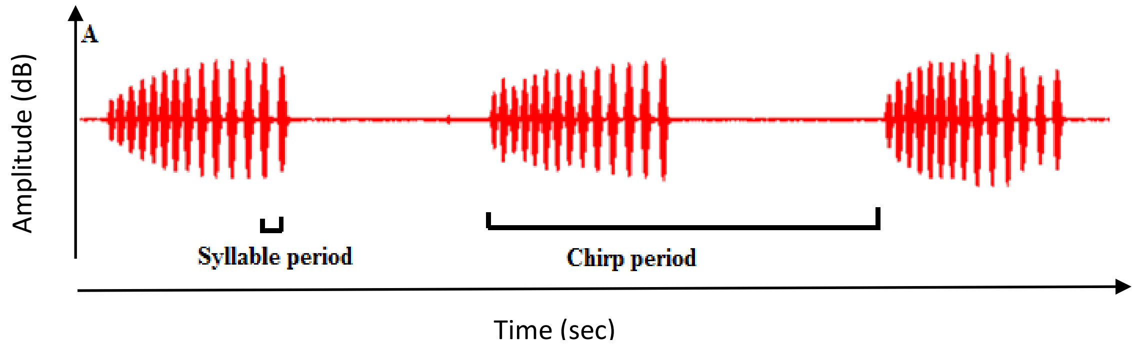
Recording of victory calls were obtained from the audio recordings of naïve-naïve contests (N=29) (refer to experiment 1 and experiment 2) while long-distance mating calls were obtained by recording calling songs of some of the individuals collected for experiment 2 (discussed in the previous chapter) in lab conditions at a constant temperature of 24 degrees (N= 18) using TASCAM handheld recorder DR-07 MKII (TEAC Corp, US). [Lab recordings were made with the help of a PhD student – Ms. Soniya Yambem]

Analysis of calls

All the obtained recordings were filtered by taking noise profile and removing all the waveforms that had a frequency of <2000 Hz using Audacity 2.0.5 (Free Software foundation Inc., USA). Filtered calls were used to measure following key temporal and spectral acoustic parameters for both the call types (fig 3.2):

- Chirp period: Onset of one chirp to onset on another chirp
- Syllable period: Onset of one syllable to onset of another syllable
- Number of syllables: Number of full syllables in a chirp
- Peak frequency: The frequency at which max power lies.

Note: Mostly all the chirps of victory calls were used for acoustic analysis since these calls lasted for only 1 to 3 sec while a random 10-second sequence was taken for acoustic analysis of LDMC. Finally, averaged values were used for analysis.



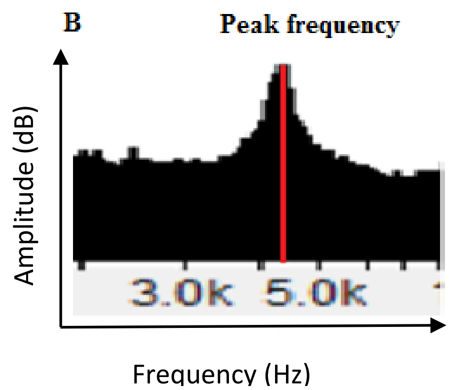


Figure 3.2: (A) Oscillogram of cricket call depicting temporal call features. (B) Power spectrum depicting the peak frequency of a call.

3.4.2 Statistical analysis

Average values of chirp period, syllable period, number of syllables and peak frequency per individual were noted to characterize victory call and long distance mating call. Since the call parameters were not following a normal distribution, a non-parametric test: Mann-Whitney U test was performed to compare each of the call features for the two call types.

3.4.3 Results

Characterization of LDMC (fig 3.3a)

N = 18

Chirp period	**Syllable period**	**No. of syllables/chirp**	**Peak frequency**
1.014 ± 0.132 sec	0.026 ± 0.001 sec	12.34 ± 1.733	5.11 ± 0.178 KHz

Characterization of Victory call (fig. 3.3b)

N = 29

Chirp period	Syllable period	No. of syllables/chirp	Peak frequency
0.734 ± 0.126 sec	0.028 ± 0.003 sec	11.25 ± 1.493	5.28 ± 0.142 KHz

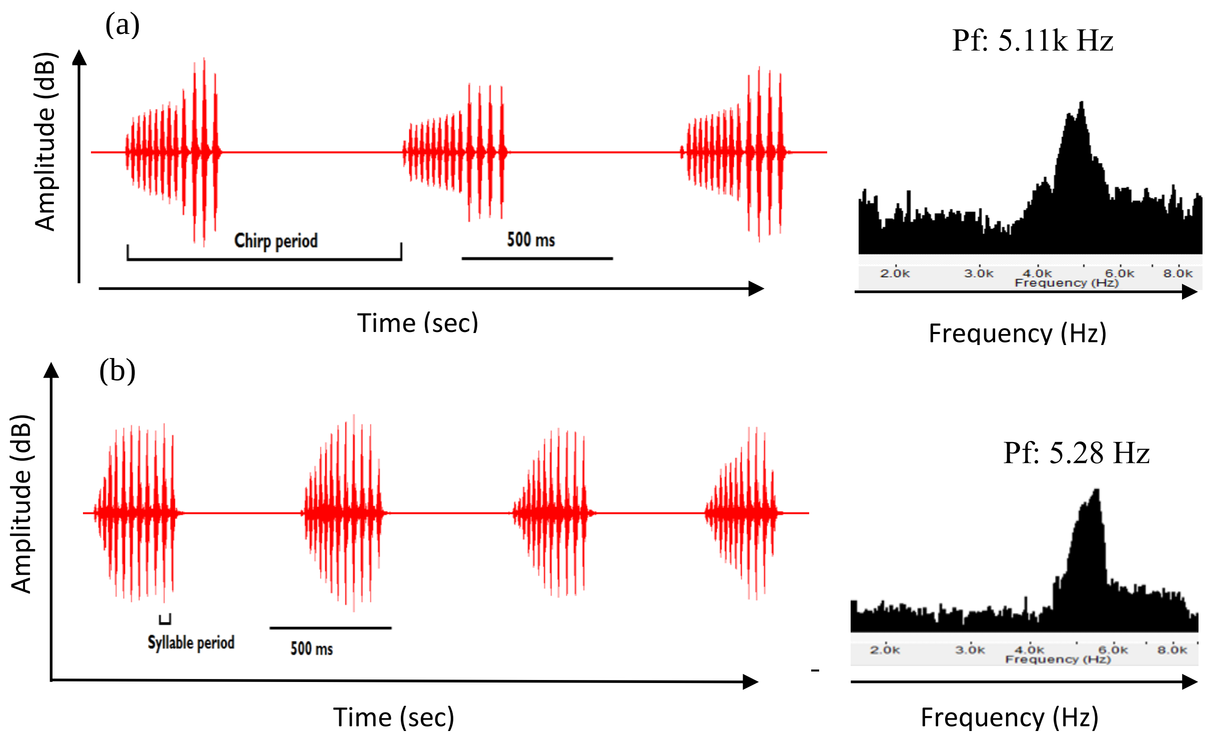


Figure 3.3: Temporal and spectral representation of (a) LDMC (b) Victory call

LDMC VS Victory call

Chirp period and number of syllables of LDMC are significantly higher than chirp period and no of syllables of VC (U: 23, N1= 18, N2=25, p= 0.000001 and U: 164.50, N1=18, N2= 29, p= 0.035, respectively). While, syllable period and peak frequency of LDMC is significantly lower than that of VC (U: 158, N1=18, N2=29, p=0.025 and U: 136, N1=18, N2=29, p= 0.006, respectively) (fig 3.4).

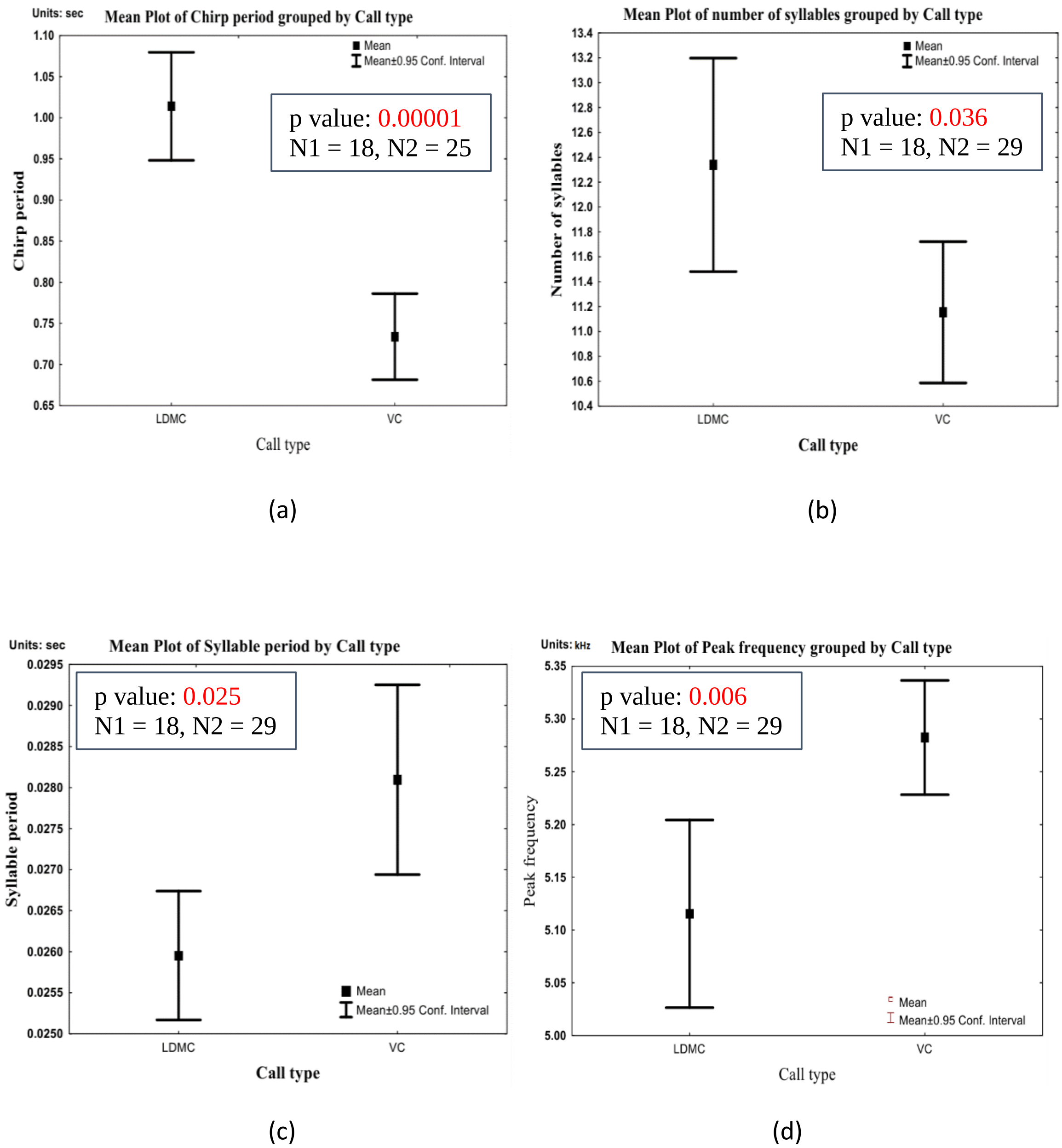


Figure 3.4: Plots representing the differences in means ± sd of (a) chirp period (b) no of syllables (c) syllable period and (d) peak frequency between long distance mating calls and victory calls of *Acanthogryllus asiaticus*

3.4.4 Conclusion

Calls produced by males after winning a fight differs significantly from long distance mating calls.

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CHAPTER 4

Discussion

4.1 Body weight and morphological characteristics are not indicators of fighting success

Assessment of intrinsic male attributes of individuals involved in aggressive contests reveals that features such as body size, body weight and other morphological characteristics are not indicators of fight outcome in *Acanthogryllus asiaticus.* This result is in contrast to the vast amount of extant literature which support the importance of size/weight for fighting success in crickets (Brown et al., 2006; Hack, 1997; Hofmann et al, 2001; Simmons, 1986). Although these parameters are the most commonly used proxies for RHP, there are many more components of RHP such as weaponry, muscular mass, songs, age etc. that might be able to shed some light on the importance of intrinsic male factors for determining fight outcomes (Brown et al., 2006; Judge & Bonanno, 2008). Nevertheless, RHP is not the only factor affecting fighting success and other factors like motivation, past experiences and residency ownership have also been shown to influence aggression in crickets (Adamo et al., 1995; Brown et al., 2006; Khazraïe et al., 1999).

4.2 Past experiences do not affect future aggressive outcome and behaviour

My study on the influence of past experiences on future aggression in *Acanthogryllus asiaticus* casts doubt on the apparent role of recent fighting experience in fight outcome and future aggression. Results indicate that winning and losing experiences alone are not sufficient to alter the probability of winners winning and losers losing subsequent contests, demonstrating no winner and loser effects in my model species. Recent winners and naïve males both are equally likely to win a subsequent contest but in contrast, the tendency of losers to lose again was much higher than a naïve male but not significant enough to attribute this tendency to prior experience since inherent factors of individuals were a confounding factor in our study. Apart from the confounding factor of the inherent ability of individuals which was taken care through the null hypothesis, the main caveat of my study comes from the age and experience of the individuals. Since all the crickets were wild caught, I was not able to control for the experience accumulated by individuals in the field and age of the collected individuals. Also, the housing facility of crickets did not provide true isolation, since males could still have passive interactions with other males by means of olfactory and acoustic cues. All these factors can potentially impact the fighting ability of individuals irrespective of size and recent fighting experience.

Past experiences are also known to affect the fighting ability of individuals which can be examined through changes in aggressive behaviour of animals, mainly the tendency to escalate a fight which in turn affects the outcome of fight (Hsu et al., 2006). Hence, the second line of evidence for arbitrary contest outcome of experienced individuals comes from our behaviour analysis which tells us that there are no evident behavioural differences in terms of escalation of fight in the focal males after winning/losing experience. Winning/losing mechanisms should ideally evolve in species that frequency interact in order to acquire resources but the large spatial distribution between males will make them less likely to come in frequent contact with each other and hence, natural selection would not favour the evolution of such effects. *Acanthogryllus asiaticus* males are also quite sparsely distributed in nature with an average inter-male distance of ~4.75m in a chorus (Singh et al., unpublished data), providing a possible explanation the results.

Further investigations should be made strictly after controlling for age and experience either by taking lab-bred populations or by catching sub-adults from the field (in order to track their age and eliminate any experiences from the field). Also, better experimental designs with ‘random selection procedures’ or conservative size matched contests would be more favourable to study the effects of past experiences.

4.3 Victory calls are different from long-distance mating calls

My analysis on song features of victory calls and long distance mating calls have shown that the temporal features (chirp period and syllable period), number of syllables produced and peak frequencies of both the call types are very different from each other. This is a very small piece of work and still there is a lot of scope for future work. Many questions need to be addressed in order to explore the role of acoustics on cricket aggression. It would be interesting to measure the differences in song parameters between aggressive and victory calls. One could also test the role of victory calls in terms of the phonotaxis response given by naïve males as well as females by subjecting them to manipulative playback experiments. Other than that, long distance mating calls of habitual winners and habitual losers can be recorded and female preference can be seen. If some preference does exist in nature, then their call parameters can be compared to see the features that are mostly preferred by female crickets in nature. Further studies on victory calls in relation to its effect on the opponent as well as on audience are also needed in order to establish the function of these calls.

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Appendices

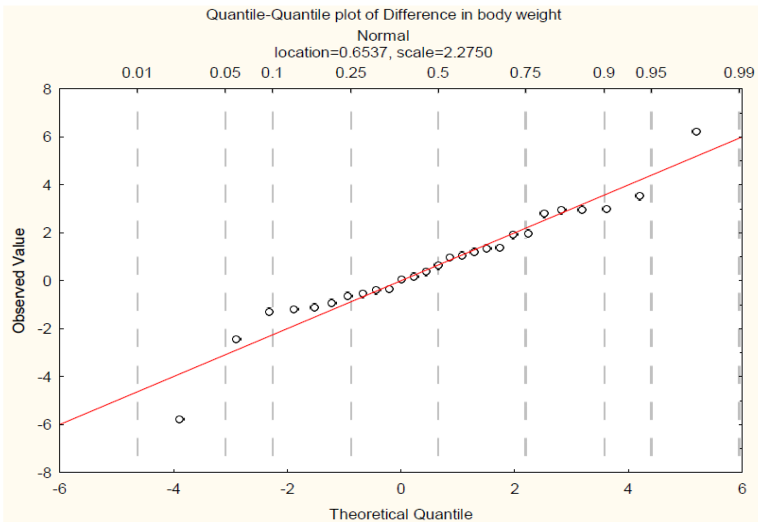
Appendix A

Q-Q Plots

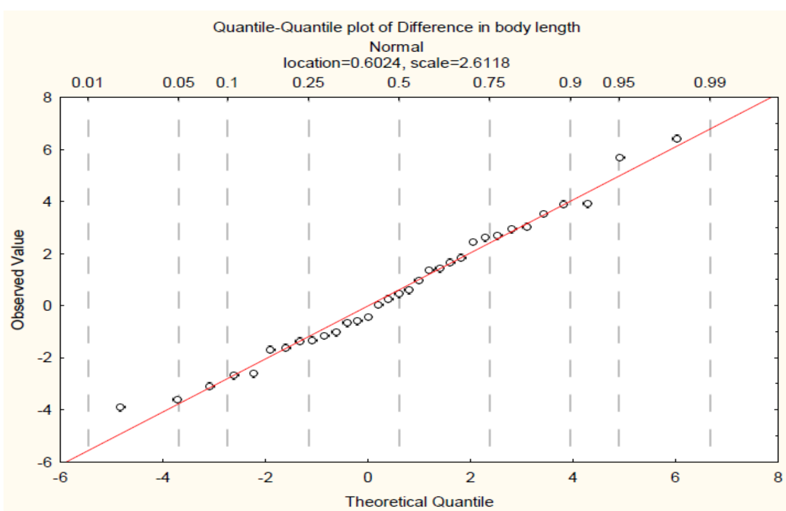
Following are the Q-Q plots for differences in body weight and other morphological characters between winners and losers.

The data was plotted against a theoretical distribution: Normal distribution

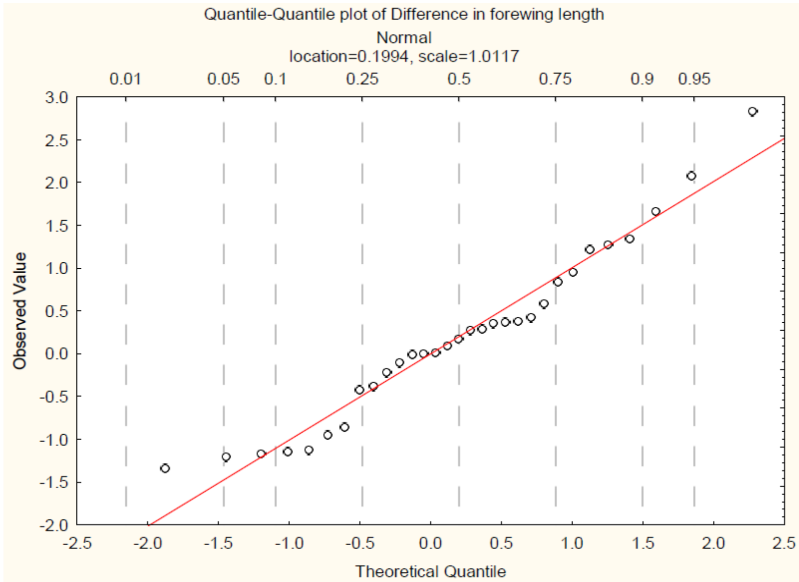
1) Body weight



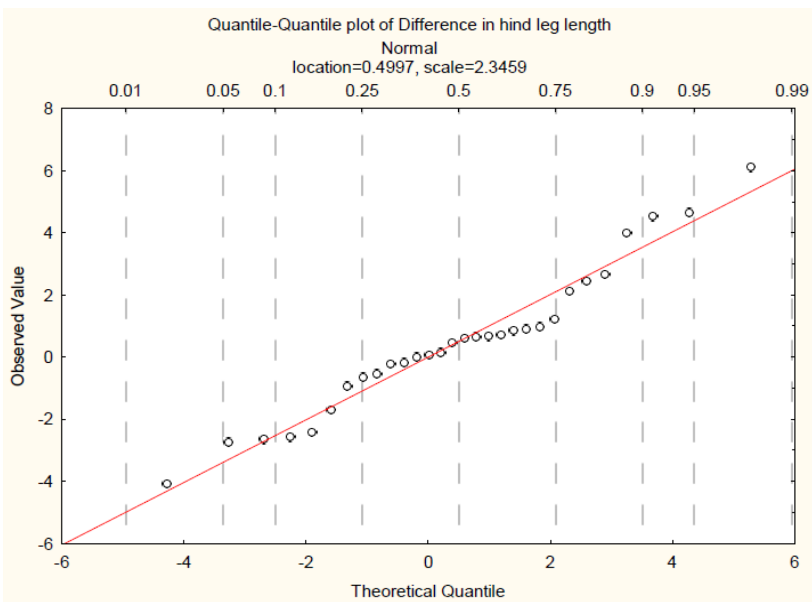
2) Body length



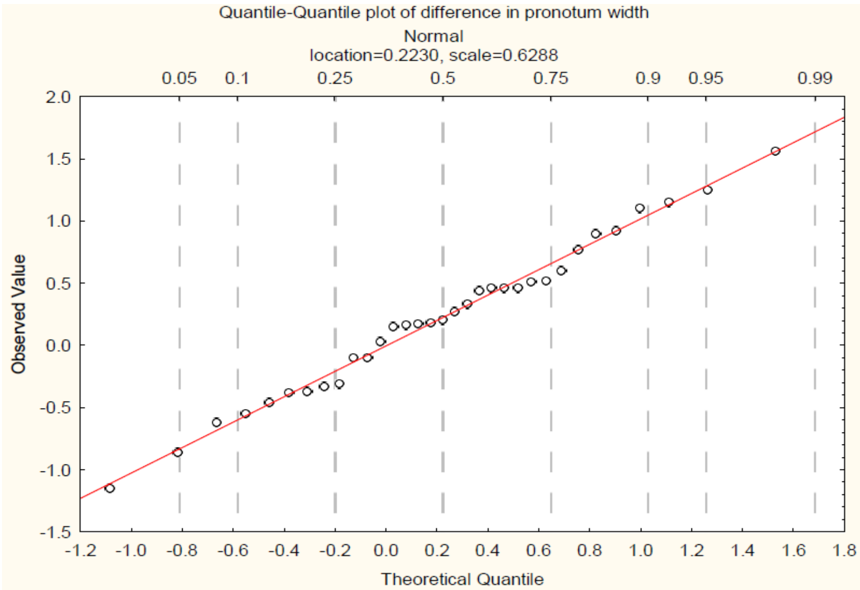
3) Forewing length



4) Hind leg length



5) Pronotum width



Appendix B

Details of video analysis

Following are the detailed description of the behaviours extracted from video of each contest:

- First approach: The individual who approached towards its opponent in order to initiate an interaction was noted
- First call: The individual who produced the first aggressive call during the entire contest and the time at which it produced the call after the start of experiment was noted.
- Contest initiation: The individual who initiated the physical combat and the time of initiation of this behaviour was noted.
- Contest end: The time at which the winner and loser were decided
- Contest duration: Total duration of physical combat starting from antennal fencing to onset of victory calls
- Frequency of calls by winner: Total number of aggressive calls by winners after start of physical combat till emergence of winner + 2 victory calls (since the number of victory calls differed in each case, depending on the termination of trial, I used a baseline of 2 calls which was produced almost after winning experience)
- Behaviours seen during the contest: All the behaviours observed during physical combat were noted according to the ethogram (refer to chapter 2)

Note: The start of the trial was marked with removal of separation and hence, all the time measurements were taken accordingly.

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