Contribution of passive forces in flexor and extensor muscles of 5th instar and adult locusts to hind limb movement

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Abstract

Movement of insects is simple owing to their body morphology; however, several details of the forces involved in their movement are unknown. Among insects, locusts demonstrate several specialised behaviours such as jumping, grooming, walking, and scratching, all of which involve the femur, tibia, femoro-tibial joint, flexor muscle, and extensor muscle. Several investigations have revealed that active contractions and passive forces generated in the flexor and extensor muscle are responsible for locust movement. This study aimed to analyse if there was any difference in the passive forces generated within these muscles between the juvenile and adult locust stages. In order to accomplish this, data of resting joint angles following release from flexion and extension in different conditions of hind limbs, such as intact, flexor-ablated, extensor-ablated, and both flexor and extensor-ablated hind limbs of 5th instar and adult locusts were meta-analysed and forest plots were generated. Using this data, box plots were generated, and paired t-tests were carried out. The results revealed that passive forces leading to extension were much larger in adult locusts as compared to 5th instar locusts, whereas forces leading to flexion were similar at both stages. However, when the flexor muscle was ablated, the mean difference between the resting joint angles of 5th instar and adult stageswere much higher for angles following release from both flexion and extension indicating that forces generated in this muscle may play a role in controlling the degree of hind limb movement. In contrast, ablation of the extensor muscle resulted in a mean difference similar to that of intact legs. Removal of both muscles restricted movement in the hind legs of both the 5th instar and adult locusts. In light of these results, future studies need to further analyse the role of the flexor and extensor muscles in the movement of hind limbs in locusts.

1.Introduction

1.1 Background

Although insects are comparatively simpler than vertebrates possessing unsophisticated and simple nervous systems, there are still a lot of aspects of insect locomotion that have not yet been completely deciphered, and therefore, the interest of biological and biorobotics scientists in this field is immense (Ritzmann, 2010). In reality, insect brains are highly sophisticated, and they give rise to adaptive movements by interacting with reflex systems in thoracic ganglia. These movements are also influenced by sensors present on the head that signal the presence of predators or other harmful materials in their surroundings (Ritzmann, 2010). Several insects possess specialized pairs of limbs to meet specific functions, for example, locusts possess jumping legs, praying mantids possess raptorial legs, and mole crickets possess digging legs. Apart from this, most insects also possess two pairs of walking legs that are responsible for locomotion (Durr et al., 2018). Therefore, the ability of insects to use their various pairs of limbs for specialized movement types requires adaptive modification as well as context-dependent activation of their limbs (Durr et al., 2018).

This section provides an overview of limb movement in locusts by describing the structure of the locust hind limb followed by how this structure contributes to movement, discusses the active and passive forces that lead to movement in the hind limbs, and reflects on the differences in how these forces influence hind limb movement as the locust progresses through its life cycle. The chapter ends with the aim, research question, and objectives of this project work.

1.2 Structure of hind limb in locusts

In the hind legs of locusts, the tibia and femur segments are joined via a hinge joint or a femorotibial (FT) joint which can rotate in a single plane. Around the joint are present extensor and flexor tibiae muscles, both pinnate in structure, with the extensor muscle having an 88% larger cross-sectional area and 5 times greater mass than the flexor muscle. The contractions of the extensor and flexor tibiae muscles allow the flexion and extension of the tibia around the joint (Bennet-Clark, 1975). The larger cross-sectional area and greater mass of the extensor muscle indicates that the peak active force at the extensor muscle is 17 to 33 greater than that of the flexor muscle. The position of extensor muscle tendons around the tibia is such that the forces transmitted from the muscles to the tibia are non-linear and asymmetric. The flexor muscle tendon is so located that it further contributes to non-linearity in movement at the joint. Therefore, this contributes to the weaker and smaller flexor muscle having a mechanical advantage over the stronger extensor muscle (Heitler, 1974).

1.3 Movement of hind legs in locusts

Limb movements are important for several insect behaviours such as prey capture, stepping, reaching, and grooming, the dynamics of which change throughout the development of the insect. The neuronal pathways that are responsible for these movements need to adapt to changes in the insect's body during development. This is brought about by sensory-motor plasticity that helps maintain critical insect behaviours across developmental stages (Easter, 1983). The underlying neuronal and biomechanical changes are largely responsible for adaptive behaviours at different stages of an insect's development, and an understanding of these changes can give insights into how hind limb movement varies in juvenile and adult locusts.

There are two types of forces that lead to movement of hind limbs in locusts – active and passive forces. Active forces are those that are driven by muscle contractions, whereas passive forces originate in muscles, tendons, and limb joints. In the resting state, passive muscle forces contribute to certain history-dependent positions of the extensor and flexor muscles, which may be against gravitational forces. This is in contrast to the resting state of limbs of vertebrates which, due to their large size, always come to rest in a gravity-dependent position.During movements such as walking, reciprocal actions are demonstrated by the extensor and flexor muscles (Page et al., 2008). When activation is provided through the fast extensor tibiae motor neuron, it leads to a shift in the resting angle, and the extent of movement of the extensor muscle depends on the parameters of the activation. This also determines the strength of passive stretch of the flexor muscle (Ache, 2010). Similarly, when flexor muscles are activated through stimulation by its innervating motor neurons, it leads to flexion to a major extent and extension to a smaller extent brought about by passive forces resulting in movement (Ache and Matheson, 2012). Signals for contraction and relaxation are provided by excitatory and inhibitory neurons that innervate the extensor and flexor muscles. When the extensor muscle receives a signal to contract, the tibia extends and when the flexor muscle receives a signal to contract, the tibia flexes. The location of the extensor and flexor muscles and the movement produced through the contraction of these muscles is shown in Figure 1.

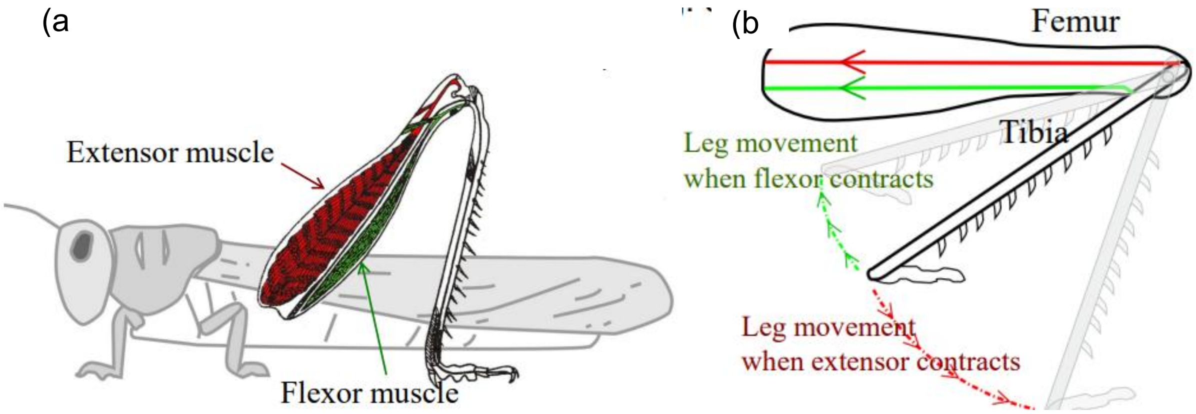


Figure 1: Structure and movement of the hind limb of locusts – (a) Location of the extensor and flexor muscles in the locust, (b) Movement produced by contraction of the extensor and flexor muscles (Source: Wilson et al., 2013)

1.4 Active and passive forces that facilitate hind limb movement

While neuronal pathways are largely responsible for various movement behaviours in locusts, biomechanical features of the insect's body also play a very important role in contributing to movement. Insects, which typically have a low body weight, are majorly influenced by passive forces which can be quite large as compared to gravitational forces (Hooper et al., 2009). These internal passive forces allow insects to take up a gravity-independent resting position. In locusts, the passive forces can be as large as the active forces and several types of limb movements can be driven by these forces (Page et al., 2008). These residual passive forces in the muscles can elicit active extensions in subsequent stages of movement (Ache and Matheson, 2012).

Movement of hind limbs in locusts is usually a combination of a wide range of forces. These forces may be active in the form of muscle contractions, passive in the form of tendon recoils, explosive recoils when skeletal elements are bent, viscosity, and gravitational forces. The key is to understand the interaction between these different forces that ultimately lead to movement in the locust (Sutton, 2013). Active forces that are responsible for hind limb movement in locusts include contraction of the extensor muscle that causes the limb to extend and contraction of the flexor muscle that causes the limb to flex. These movements are brought about by signals transmitted by nerves that innervate these muscles, and these signals are generated in response to various stimuli present in the insect’s vicinity (Heitler, 1977).

It has been shown that when the extensor muscle receives signals, it contracts, leading to the extension of the joint. When the signal stops, the joint moves from the extended to the flexed position. Therefore, there is a force that acts opposite to the contraction force in the extensor muscle and causes flexion in the joint when the extensor muscle ceases to contract (Ache and Matheson, 2012). This may be because when the extensor muscle contracts, the flexor muscle is stretched, and passive recoil in this muscle flexes it once the contraction signal ends (Ache and Matheson, 2012). Therefore, in locusts, active forces generate extensions and passive forces generate flexions. This is in contrast to the stick insect where active forces generate flexions and passive forces generate extensions, owing to the fact that the flexor muscle is three times stronger than the extensor muscle. As a result, active forces generated in the flexor muscle are strong enough to cause flexion in the joint and passive forces bring about extension in the joint (Ache and Matheson, 2012).

1.5 Differences in flexion and extension through the life cycle

Hind limbs in locusts are responsible not only for walking or jumping, but also for scratching, grooming, stepping, reaching, and prey capture. Therefore, as the body morphology changes through the life cycle, the structure of hind limbs also needs to adapt such that it remains functional for all its required behaviours (Easter, 1983). It has been observed that adult locusts can walk across bigger distances as compared to young ones, which suggests age-dependent recalibration (Ben-Nun et al., 2013). Growth of an insect causes changes in the lengths of the hind limbs along with its musculoskeletal properties, which, combined with the underlying neuronal pathways, results in changes in movement of the hind limbs (Patel and Matheson, 2019).

Locusts are hemimetabolous insects, meaning that they do not go through a pupal stage while transitioning from a larva to an adult. Such insects typically maintain the same body structure throughout their lives with an abrupt enlargement at each developmental stage (Uvarov, 1966). At the adult stage of the locust, there is an increase in wing length which is disproportionate to the length of the body and legs. As a result, the tips of the wings are more posterior in adults as compared to 5th instar locusts. Development of the locust also results in two rotations of the wings morphologically, which brings about major changes in movements arising from the tactile stimulation of the wings such as scratching (Khattar, 1972).

Differences in the parameters of hind limb movements in the 5th instar (or developmental) stage and the adult stage of the locust depend on the purpose for which they occur. For example, young locusts usually move to escape from predators and adult locusts move to reach the required take-off velocity for flight (Gabriel, 1985). Furthermore, as the body size and mass of the adult locust stage is larger than that of the 5th instar stage, the energy availability for leg movement in adults is more, which gives a greater jump range for adults when compared to 5th instar locusts (Bennet-Clark and Lucey, 1967).

Changes in body morphology between the 5th instar and adult stages greatly affect movement of hind limbs between these two stages. For instance, there is a disproportionate increase in the dorsal-to-ventral ratio of the tibia as the locust develops into an adult, which leads to lesser bending demonstrated by the tibia (Currey, 1967). However, the overall ratio of the femoro-tibial joint is retained in both the 5th instar and the adult stages, and so, extension and flexion of muscles follow a similar pattern (Heitler, 1974).

It has been shown that when compared to juveniles, adult locusts can easily walk over large gaps, using visual cues to judge the distance of the gap. The size of the gap that can be crossed increases with the developmental stage; however, it is not explicitly expressed in the younger locusts but becomes apparent after interacting with the external environment, pointing towards age-dependent recalibration (Ben-Nun et al., 2013). Jumping is another specialized movement in locusts with adult locusts being capable of jumping considerable distances to escape from predators and launch into flight. In order to facilitate jumping, the flexor tibiae muscle contracts first resulting in flexion of the femoro-tibial joint with the tibia coming up against the femur (Heitler and Burrows, 1977). Following this, a cocontraction phase occurs when both the flexor and extensor tibiae muscles undergo simultaneous contraction. During this time, there is a build-up of potential energy within the muscles. After the contraction phase, the flexor muscles relax resulting in extension of the tibia, and the stored energy strongly and rapidly launches the locust in flight (Heitler and Burrows, 1977).

When considering limb movements in locusts for grooming purposes, it should be noted that differential growth of the limbs and body can lead to changes in the spatial relationship between the limb and the grooming target. Changes in the sensory system of the insect can also lead to differences in the representation of the external environment as well as the body surface (Chiba et al., 1988). During development, there occur changes in the length of limbs as well as their musculoskeletal properties. If there were no corresponding changes in the underlying motor patterns, the movement trajectories of insects would be inappropriate. Therefore, plasticity of the underlying neuronal networks needs to be in sync with the developmental and biomechanical changes in the insects (Murphey et al., 1980).

1.6 Aims and objectives

The main aim of this project is to identify and evaluate the contribution of passive forces in the maximum flexion and extension angles in the hind limbs of 5th instar and adult stages.

The central question of this study is, “To what extent does passive forces affect movement in hind limbs of locusts, and how does this vary in the 5th instar and adult stages?”

Questions subsequent to the central question are:

1. Is there a significant difference between the flexion angles and extension angles of intact hind limbs in the 5th instar and adult stages?
2. Is there a significant difference between the flexion angles and extension angles of flexor-ablated hind limbs in the 5th instar and adult stages?
3. Is there a significant difference between the flexion angles and extension angles of extensor-ablated hind limbs in the 5th instar and adult stages?
4. Is there a significant difference between the flexion angles and extension angles of both-ablated hind limbs in the 5th instar and adult stages?
5. How does the presence of passive forces influence movement of hind limbs in the 5th instar and adult stages?

3. Methods and Materials

3.1 Data Acquisition for Meta-Analysis

Four datasets were used for the meta-analysis obtained through experiments performed by students in previous years. These four datasets were: [Student-2018-A], [Student-2018-B], [Student-2019-B], and [Student-2019-C]. Data of flexion and extension angles of hind legs of desert locusts in the 5th instar and adult stages were used for the meta-analysis that were collected under four different conditions – resting joint angles in intact hind legs (Appendix Table 1.1), resting joint angles in flexor ablated hind legs (Appendix Table 1.2), resting joint angles in extensor ablated hind legs (Appendix Table 1.3), and resting joint angles in flexor and extensor ablated hind legs (Appendix Table 1.4). As can be seen in Appendix Tables 1.1 to 1.4, the mean and standard deviation of all flexion and extension angles for each stage was computed and the total number of animals used for the measurements was also noted from the available datasets for all four conditions. The literature was also searched using the PubMed search engine and different combinations of the keywords [locusts, hind legs, flexion angle, extension angle, passive joint forces] were used to search for published data. However, no suitable dataset was obtained that could be used for the meta-analysis.

3.2 Meta-Analysis

The data was analysed using Review Manager (RevMan) version 5.3 (The Cochrane Collaboration, 2014). As it has been shown that extension is brought about to a large extent by active forces and to a small extent by passive forces, and flexion is brought about to a large extent by passive forces and to a small extent by active forces in the locust, separate meta-analyses were carried out to determine possible statistically significant differences in the resting joint angles following release from flexion and extension of the 5th instar and adult locust stages separately under each of the four conditions, namely intact hind legs, flexor ablated hind legs, extensor ablated hind legs, and flexor and extensor ablated hind legs. The objective of this approach was to see if there was a difference in the resting joint angles following release from extension and flexion of the hind limb between the two developmental stages, and to determine if passive joint forces that bring about flexion followed by extension brought about by active forces vary between 5th instar and adult locusts.

Mean and standard deviation values of resting joint angles following release from flexion and extension of the 5th instar and adult stages for each of the four experimental conditions were used to acquire the overall mean difference (MD) for analysing the difference in resting joint angles between the 5th instar and adult stages using 95% Confidence Intervals (CIs) and the continuous method of analysis. Calculation of mean difference provides a convenient measure of the difference between two properties in a given population. In this study, the two properties are flexion angles in 5th instar and adult locust stages, and extension angles in 5th instar and adult locust stages.

Eight different meta-analyses were performed. For each of the four conditions, one assessed the resting joint angles following release from flexion between the 5th instar and adult locust stages, and the other assessed resting joint angles following release from extension between the 5th instar and adult locust stages. Forest plots and box plots were generated for each analysis and the mean difference, Chi^2 value, and the strength of overall effect was calculated for each plot. Heterogeneity among the datasets was reported using Chi^2 value, P value, and I^2 statistic. The mean difference for all the individual meta-analyses provided a difference in measure of the strength of flexion and extension angles between the two developmental stages.

3.3 Box plots and statistical analyses

The values of mean differences obtained from the four datasets in each of the eight meta-analyses were used to generate box plots to provide a graphical representation of the complete data obtained from all the meta-analyses. Using the values of mean differences, the minimum value, quartile 1, median, quartile 3, and maximum values were computed, the data summary of which is provided in Appendix 2.1. This data was then used to generate box plots using the BoxPlotR tool developed by Spitzer et al. (2014). Statistical analyses were carried out using paired t-tests, wherein the flexion and extension angles of the intact hind legs were compared to each of the ablated conditions for the 5th instar and adult developmental stages separately. The Bonferroni correction was applied to derive the adjusted p-value using the formula:

Adjusted p value = Original p value / n,

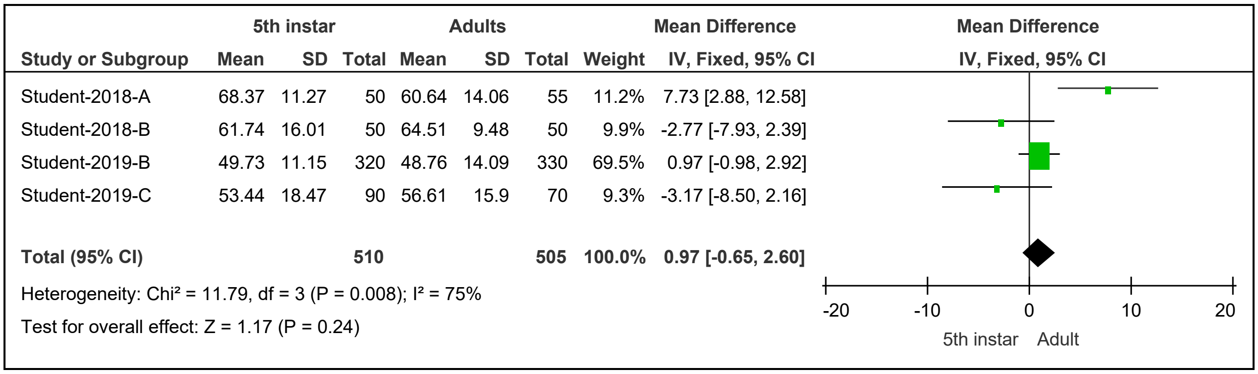
where n is the number of analyses that were carried out (here, 3).

4. Results

4.1 Analysis of resting joint angles following release from flexion in intact hind limbs of locusts in the 5th instar and adult stages

The data of resting joint angles following release from flexion were obtained from the 4 datasets for the 5th instar and adult stages and were meta-analysed to identify the mean difference in joint angles following release from flexion in the intact hind limbs of 5th instar and adult locusts. The values of mean, standard deviation, and total sample number for each dataset were used for the meta-analysis.

The raw data that were extracted from the datasets were used to generate a forest plot using the RevMan software (Figure 4.1). This plot provides a measure of the difference in resting joint angles following release from flexion in the intact hind limbs of 5th instar and adult locusts. The mean difference for the data was 0.97 degrees with a range of [-0.65, 2.60] and a confidence interval of 95% was generated. The chi^2 test for heterogeneity yielded a value of 11.79 and the I^2 value was 75% indicating considerable heterogeneity in the results. This indicates that in the flexed position in intact hind legs, there is no difference in the resting joint angles of the 5th instar and adult locusts.



	5th instar			Adults				Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Fixed, 95% CI**
Student-2018-A	68.37	11.27	50	60.64	14.06	55	11.2%	7.73 [2.88, 12.58]
Student-2018-B	61.74	16.01	50	64.51	9.48	50	9.9%	-2.77 [-7.93, 2.39]
Student-2019-B	49.73	11.15	320	48.76	14.09	330	69.5%	0.97 [-0.98, 2.92]
Student-2019-C	53.44	18.47	90	56.61	15.9	70	9.3%	-3.17 [-8.50, 2.16]
Total (95% CI)			**510**			**505**	**100.0%**	**0.97 [-0.65, 2.60]**

Heterogeneity: Chi² = 11.79, df = 3 (P = 0.008); I² = 75%

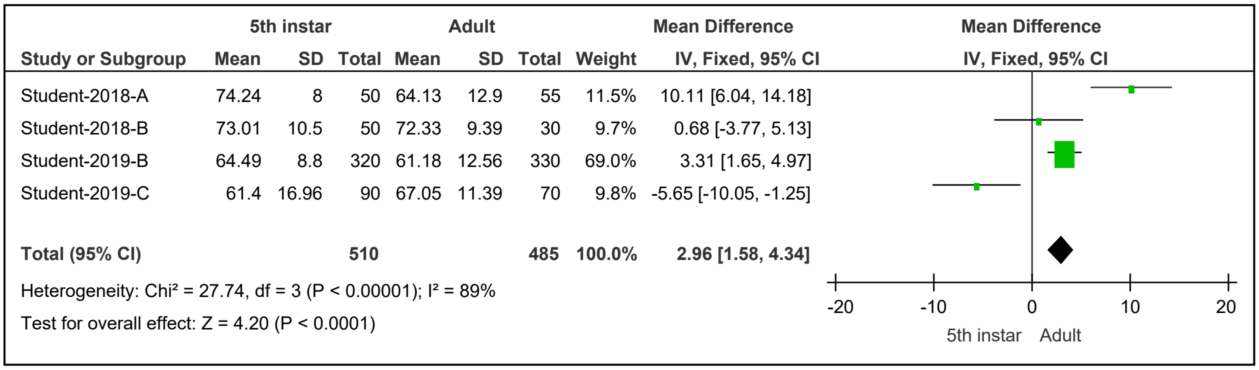
Test for overall effect: Z = 1.17 (P = 0.24)

Figure 4.1: Forest plot showing the mean difference in resting joint angles following release from flexion in intact hind legs of 5th instar and adult locusts. The horizontal axis represents the mean difference and the vertical axis is the line of null effect. Each green box represents its corresponding dataset, and the size of the box represents the weight of the dataset in terms of number of values. The black diamond represents the point estimate of the average of all individual studies included in the meta-analysis.

4.2 Analysis of resting joint angles following release from extension in intact hind limbs of locusts in the 5th instar and adult stages

The data of resting joint angles following release from extension were obtained from the 4 datasets for the 5th instar and adult stages and were meta-analysed to identify the mean difference in joint angles following release from extension in the intact hind limbs of 5th instar and adult locusts. The values of mean, standard deviation, and total sample number for each dataset were used for the meta-analysis.

The raw data that were extracted from the datasets were used to generate a forest plot using the RevMan software (Figure 4.2). This plot provides a measure of the difference in resting joint angles following release from extension in the intact hind limbs of 5th instar and adult locusts. The mean difference for the data was 2.96 degrees with a range of [1.58, 4.34] and a confidence interval of 95% was generated. The chi^2 test for heterogeneity yielded a value of 27.74 and the I^2 value was 89% indicating considerable heterogeneity in the results. This indicates that in the extended position in intact hind legs, the resting joint angle of adult locusts is considerably higher than the resting joint angle of the 5th instar locusts.



	5th instar			Adult				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI
Student-2018-A	74.24	8	50	64.13	12.9	55	11.5%	10.11 [6.04, 14.18]
Student-2018-B	73.01	10.5	50	72.33	9.39	30	9.7%	0.68 [-3.77, 5.13]
Student-2019-B	64.49	8.8	320	61.18	12.56	330	69.0%	3.31 [1.65, 4.97]
Student-2019-C	61.4	16.96	90	67.05	11.39	70	9.8%	-5.65 [-10.05, -1.25]
Total (95% CI)			**510**			**485**	**100.0%**	**2.96 [1.58, 4.34]**

Heterogeneity: Chi² = 27.74, df = 3 (P < 0.00001); I² = 89%

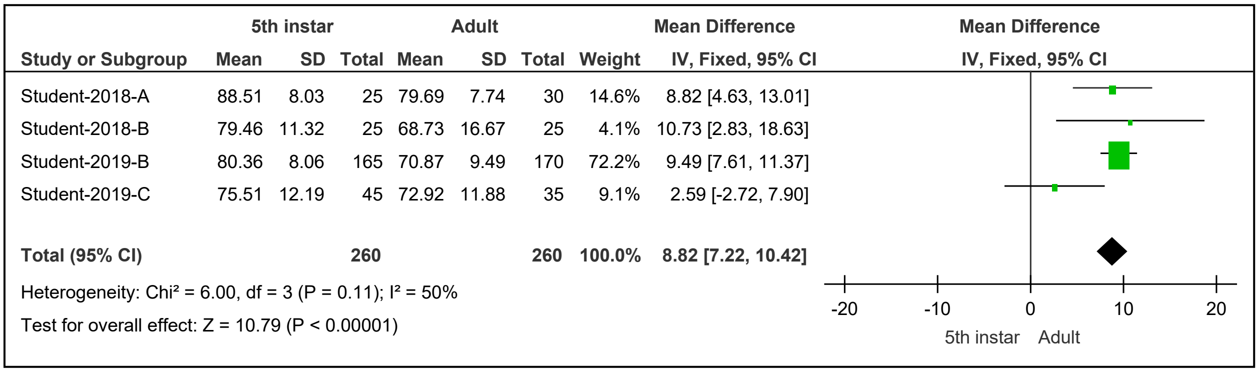
Test for overall effect: Z = 4.20 (P < 0.0001)

Figure 4.2: Forest plot showing the mean difference in resting joint angles following release from extension in intact hind legs of 5th instar and adult locusts. The horizontal axis represents the mean difference and the vertical axis is the line of null effect. Each green box represents its corresponding dataset, and the size of the box represents the weight of the dataset in terms of number of values. The black diamond represents the point estimate of the average of all individual studies included in the meta-analysis.

4.3 Analysis of resting joint angles following release from flexion in flexor-ablated hind limbs of locusts in the 5th instar and adult stages

The data of resting joint angles following release from flexion were obtained from the 4 datasets for the 5th instar and adult stages and were meta-analysed to identify the mean difference in joint angles following release from flexion in the flexor-ablated hind limbs of 5th instar and adult locusts. The values of mean, standard deviation, and total sample number for each dataset were used for the meta-analysis.

The raw data that were extracted from the datasets were used to generate a forest plot using the RevMan software (Figure 4.3). This plot provides a measure of the difference in resting joint angles following release from flexion in the flexor-ablated hind limbs of 5th instar and adult locusts. The mean difference for the data was 8.82 degrees with a range of [7.22, 10.42] and a confidence interval of 95% was generated. The chi^2 test for heterogeneity yielded a value of 6 and the I^2 value was 50% indicating substantial heterogeneity in the results. This indicates that in the absence of the flexor muscle, the resting joint angle of the adult locusts at flexion is considerably higher than the 5th instar locusts.



	5th instar			Adult				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI
Student-2018-A	88.51	8.03	25	79.69	7.74	30	14.6%	8.82 [4.63, 13.01]
Student-2018-B	79.46	11.32	25	68.73	16.67	25	4.1%	10.73 [2.83, 18.63]
Student-2019-B	80.36	8.06	165	70.87	9.49	170	72.2%	9.49 [7.61, 11.37]
Student-2019-C	75.51	12.19	45	72.92	11.88	35	9.1%	2.59 [-2.72, 7.90]
Total (95% CI)			**260**			**260**	**100.0%**	**8.82 [7.22, 10.42]**

Heterogeneity: Chi² = 6.00, df = 3 (P = 0.11); I² = 50%

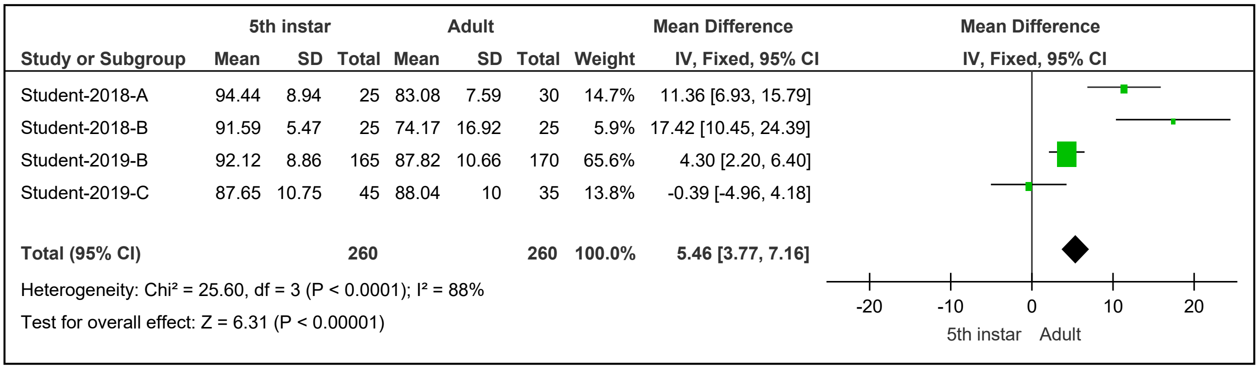
Test for overall effect: Z = 10.79 (P < 0.00001)

Figure 4.3: Forest plot showing the mean difference in resting joint angles following release from flexion in flexor-ablated hind legs of 5th instar and adult locusts. The horizontal axis represents the mean difference and the vertical axis is the line of null effect. Each green box represents its corresponding dataset, and the size of the box represents the weight of the dataset in terms of number of values. The black diamond represents the point estimate of the average of all individual studies included in the meta-analysis.

4.4 Analysis of resting joint angles following release from extension in flexor-ablated hind limbs of locusts in the 5th instar and adult stages

The data of resting joint angles following release from extension were obtained from the 4 datasets for the 5th instar and adult stages and were meta-analysed to identify the mean difference in joint angles following release from extension in the flexor-ablated hind limbs of 5th instar and adult locusts. The values of mean, standard deviation, and total sample number for each dataset were used for the meta-analysis.

The raw data that were extracted from the datasets were used to generate a forest plot using the RevMan software (Figure 4.4). This plot provides a measure of the difference in resting joint angles following release from extension in the flexor-ablated hind limbs of 5th instar and adult locusts. The mean difference for the data was 5.46 degrees with a range of [3.77, 7.16] and a confidence interval of 95% was generated. The chi^2 test for heterogeneity yielded a value of 25.6 and the I^2 value was 88% indicating considerable heterogeneity in the results. This indicates that in the extended position in flexor-ablated hind legs, the resting joint angle of adult locusts is considerably higher than the resting joint angle of the 5th instar locusts.



	5th instar			**Adult**				**Mean Difference**
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Fixed, 95% CI**
Student-2018-A	94.44	8.94	25	83.08	7.59	30	14.7%	11.36 [6.93, 15.79]
Student-2018-B	91.59	5.47	25	74.17	16.92	25	5.9%	17.42 [10.45, 24.39]
Student-2019-B	92.12	8.86	165	87.82	10.66	170	65.6%	4.30 [2.20, 6.40]
Student-2019-C	87.65	10.75	45	88.04	10	35	13.8%	-0.39 [-4.96, 4.18]
Total (95% CI)			**260**			**260**	**100.0%**	**5.46 [3.77, 7.16]**

Heterogeneity: Chi² = 25.60, df = 3 (P < 0.0001); I² = 88%

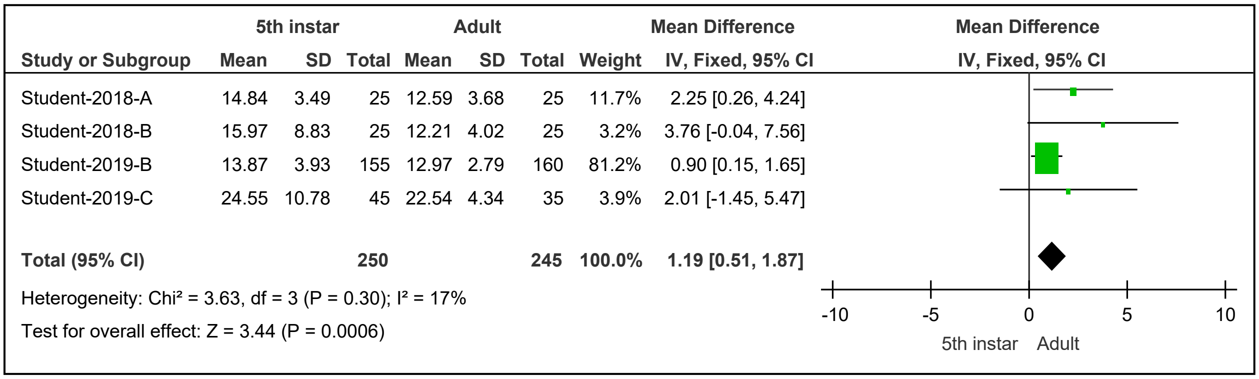
Test for overall effect: Z = 6.31 (P < 0.00001)

Figure 4.4: Forest plot showing the mean difference in resting joint angles following release from extension in flexor-ablated hind legs of 5th instar and adult locusts. The horizontal axis represents the mean difference and the vertical axis is the line of null effect. Each green box represents its corresponding dataset, and the size of the box represents the weight of the dataset in terms of number of values. The black diamond represents the point estimate of the average of all individual studies included in the meta-analysis.

4.5 Analysis of resting joint angles following release from flexion in extensor-ablated hind limbs of locusts in the 5th instar and adult stages

The data of resting joint angles following release from flexion were obtained from the 4 datasets for the 5th instar and adult stages and were meta-analysed to identify the mean difference in joint angles following release from flexion in the extensor-ablated hind limbs of 5th instar and adult locusts. The values of mean, standard deviation, and total sample number for each dataset were used for the meta-analysis.

The raw data that were extracted from the datasets were used to generate a forest plot using the RevMan software (Figure 4.5). This plot provides a measure of the difference in resting joint angles following release from flexion in the extensor-ablated hind limbs of 5th instar and adult locusts. The mean difference for the data was 1.19 degrees with a range of [0.51, 1.87] and a confidence interval of 95% was generated. The chi^2 test for heterogeneity yielded a value of 3.63 and the I^2 value was 17% indicating low heterogeneity in the results. This indicates that in the absence of the extensor muscle, the resting joint angle of the adult locusts at flexion is higher than the 5th instar locusts.



	5th instar			Adult				Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Fixed, 95% CI**
Student-2018-A	14.84	3.49	25	12.59	3.68	25	11.7%	2.25 [0.26, 4.24]
Student-2018-B	15.97	8.83	25	12.21	4.02	25	3.2%	3.76 [-0.04, 7.56]
Student-2019-B	13.87	3.93	155	12.97	2.79	160	81.2%	0.90 [0.15, 1.65]
Student-2019-C	24.55	10.78	45	22.54	4.34	35	3.9%	2.01 [-1.45, 5.47]
Total (95% CI)			**250**			**245**	**100.0%**	**1.19 [0.51, 1.87]**

Heterogeneity: Chi² = 3.63, df = 3 (P = 0.30); I² = 17%

Test for overall effect: Z = 3.44 (P = 0.0006)

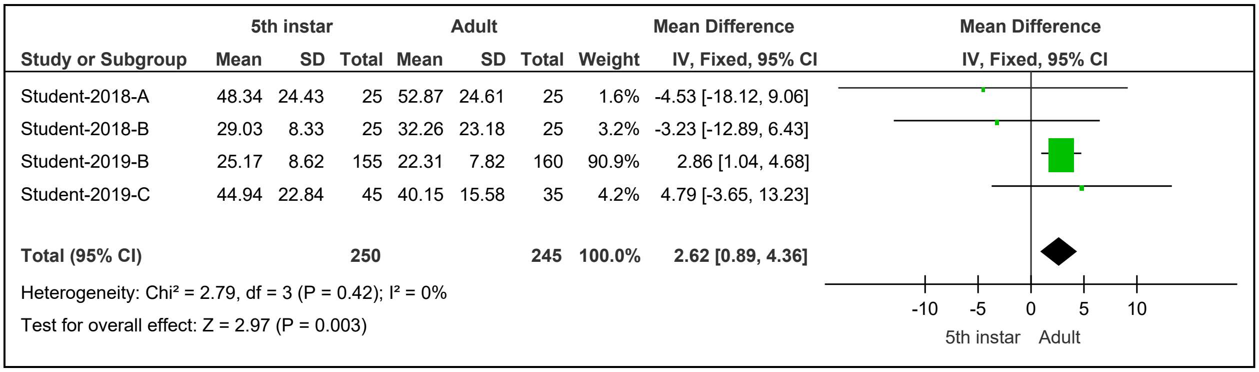
Figure 4.5: Forest plot showing the mean difference in resting joint angles following release from flexion in extensor-ablated hind legs of 5th instar and adult locusts. The horizontal axis represents the mean difference and the vertical axis is the line of null effect. Each green box represents its corresponding dataset, and the size of the box represents the weight of the dataset in terms of number of values. The black diamond

represents the point estimate of the average of all individual studies included in the meta-analysis.

4.6 Analysis of resting joint angles following release from extension in extensor-ablated hind limbs of locusts in the 5th instar and adult stages

The data of resting joint angles following release from extension were obtained from the 4 datasets for the 5th instar and adult stages and were meta-analysed to identify the mean difference in joint angles following release from extension in the extensor-ablated hind limbs of 5th instar and adult locusts. The values of mean, standard deviation, and total sample number for each dataset were used for the meta-analysis.

The raw data that were extracted from the datasets were used to generate a forest plot using the RevMan software (Figure 4.6). This plot provides a measure of the difference in resting joint angles following release from extension in the extensor-ablated hind limbs of 5th instar and adult locusts. The mean difference for the data was 2.62 degrees with a range of [0.89, 4.36] and a confidence interval of 95% was generated. The chi^2 test for heterogeneity yielded a value of 2.79 and the I^2 value was 0% indicating negligible heterogeneity in the results. This indicates that in the extended position in extensor-ablated hind legs, the resting joint angle of adult locusts is higher than the resting joint angle of the 5th instar locusts.



	5th instar			Adult				Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Fixed, 95% CI**
Student-2018-A	48.34	24.43	25	52.87	24.61	25	1.6%	-4.53 [-18.12, 9.06]
Student-2018-B	29.03	8.33	25	32.26	23.18	25	3.2%	-3.23 [-12.89, 6.43]
Student-2019-B	25.17	8.62	155	22.31	7.82	160	90.9%	2.86 [1.04, 4.68]
Student-2019-C	44.94	22.84	45	40.15	15.58	35	4.2%	4.79 [-3.65, 13.23]
Total (95% CI)			**250**			**245**	**100.0%**	**2.62 [0.89, 4.36]**

Heterogeneity: Chi² = 2.79, df = 3 (P = 0.42); I² = 0%
Test for overall effect: Z = 2.97 (P = 0.003)

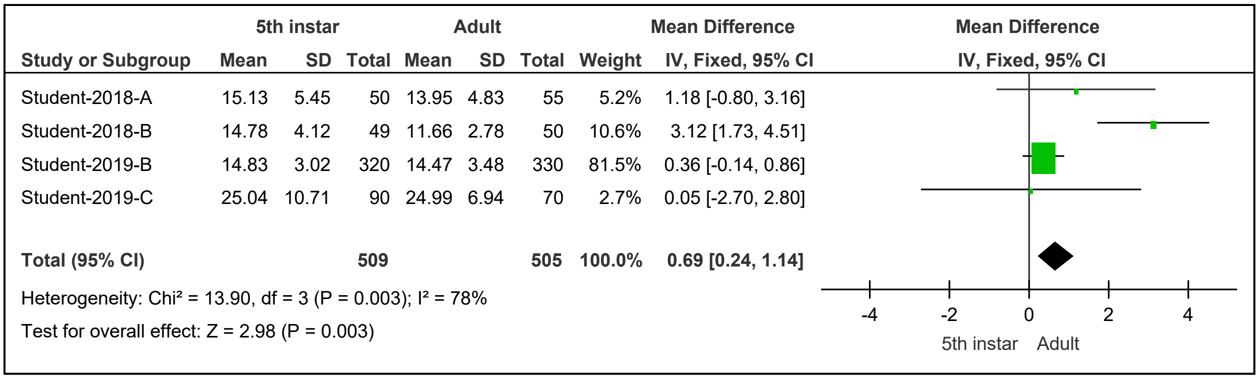
Figure 4.6: Forest plot showing the mean difference in resting joint angles following release from extension in extensor-ablated hind legs of 5th instar and adult locusts. The horizontal axis represents the mean difference and the vertical axis is the line of null

effect. Each green box represents its corresponding dataset, and the size of the box represents the weight of the dataset in terms of number of values. The black diamond represents the point estimate of the average of all individual studies included in the meta-analysis.

4.7 Analysis of resting joint angles following release from flexion in both flexor and extensor-ablated hind limbs of locusts in the 5th instar and adult stages

The data of resting joint angles following release from flexion were obtained from the 4 datasets for the 5th instar and adult stages and were meta-analysed to identify the mean difference in joint angles following release from flexion in both flexor and extensor-ablated hind limbs of 5th instar and adult locusts. The values of mean, standard deviation, and total sample number for each dataset were used for the meta-analysis.

The raw data that were extracted from the datasets were used to generate a forest plot using the RevMan software (Figure 4.7). This plot provides a measure of the difference in resting joint angles following release from flexion in both flexor and extensor-ablated hind limbs of 5th instar and adult locusts. The mean difference for the data was 0.69 degrees with a range of [0.24, 1.14] and a confidence interval of 95% was generated. The chi^2 test for heterogeneity yielded a value of 13.9 and the I^2 value was 78% indicating considerable heterogeneity in the results. This indicates that in the absence of both the flexor and extensor muscles, the resting joint angle of the adult locusts at flexion is considerably lower than the 5th instar locusts.



Study or Subgroup	5th instar Mean	5th instar SD	5th instar Total	Adult Mean	Adult SD	Adult Total	Weight	Mean Difference IV, Fixed, 95% CI
Student-2018-A	15.13	5.45	50	13.95	4.83	55	5.2%	1.18 [-0.80, 3.16]
Student-2018-B	14.78	4.12	49	11.66	2.78	50	10.6%	3.12 [1.73, 4.51]
Student-2019-B	14.83	3.02	320	14.47	3.48	330	81.5%	0.36 [-0.14, 0.86]
Student-2019-C	25.04	10.71	90	24.99	6.94	70	2.7%	0.05 [-2.70, 2.80]
Total (95% CI)			**509**			**505**	**100.0%**	**0.69 [0.24, 1.14]**

Heterogeneity: Chi² = 13.90, df = 3 (P = 0.003); I² = 78%

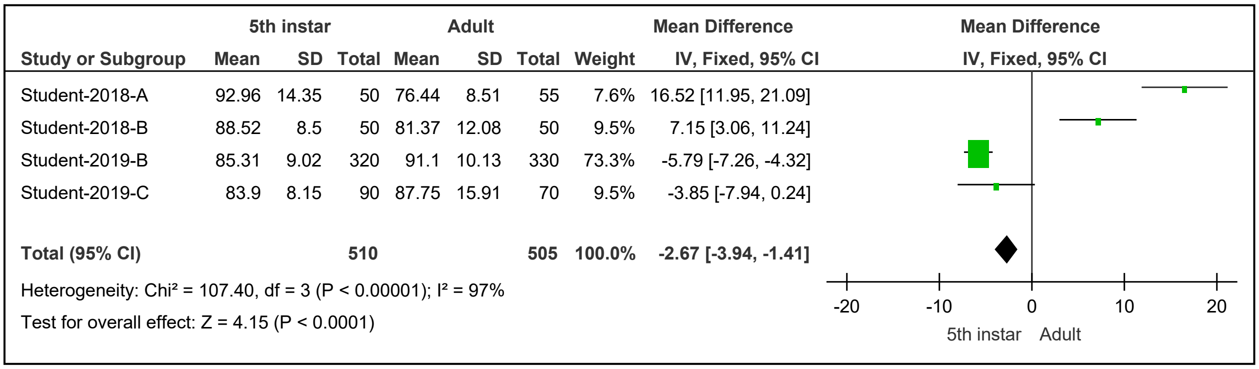
Test for overall effect: Z = 2.98 (P = 0.003)

Figure 4.7: Forest plot showing the mean difference in resting joint angles following release from flexion in both flexor and extensor-ablated hind legs of 5th instar and adult locusts. The horizontal axis represents the mean difference and the vertical axis is the line of null effect. Each green box represents its corresponding dataset, and the size of the box represents the weight of the dataset in terms of number of values. The black diamond represents the point estimate of the average of all individual studies included in the meta-analysis.

4.8 Analysis of resting joint angles following release from extension in both flexor and extensor-ablated hind limbs of locusts in the 5th instar and adult stages

The data of resting joint angles following release from extension were obtained from the 4 datasets for the 5th instar and adult stages and were meta-analysed to identify the mean difference in joint angles following release from extension in both the flexor and extensor-ablated hind limbs of 5th instar and adult locusts. The values of mean, standard deviation, and total sample number for each dataset were used for the meta-analysis.

The raw data that were extracted from the datasets were used to generate a forest plot using the RevMan software (Figure 4.8). This plot provides a measure of the difference in resting joint angles following release from extension in both flexor and extensor-ablated hind limbs of 5th instar and adult locusts. The mean difference for the data was -2.67 degrees with a range of [-3.94, -1.41] and a confidence interval of 95% was generated. The chi^2 test for heterogeneity yielded a value of 107.4 and the I^2 value was 97% indicating considerable heterogeneity in the results. This indicates that in the extended position in both flexor and extensor-ablated hind legs, the resting joint angle of adult locusts is considerably lower than the resting joint angle of the 5th instar locusts.



Study or Subgroup	5th instar Mean	SD	Total	Adult Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Student-2018-A	92.96	14.35	50	76.44	8.51	55	7.6%	16.52 [11.95, 21.09]
Student-2018-B	88.52	8.5	50	81.37	12.08	50	9.5%	7.15 [3.06, 11.24]
Student-2019-B	85.31	9.02	320	91.1	10.13	330	73.3%	-5.79 [-7.26, -4.32]
Student-2019-C	83.9	8.15	90	87.75	15.91	70	9.5%	-3.85 [-7.94, 0.24]
Total (95% CI)			**510**			**505**	**100.0%**	**-2.67 [-3.94, -1.41]**

Heterogeneity: Chi² = 107.40, df = 3 (P < 0.00001); I² = 97%

Test for overall effect: Z = 4.15 (P < 0.0001)

Figure 4.8: Forest plot showing the mean difference in resting joint angles following release from extension in both flexor and extensor-ablated hind legs of 5th instar and adult locusts. The horizontal axis represents the mean difference and the vertical axis is the line of null effect. Each green box represents its corresponding dataset, and the size of the box represents the weight of the dataset in terms of number of values. The black diamond represents the point estimate of the average of all individual studies included in the meta-analysis.

4.9 Analysis of cumulative data using box plots

The data of 4 mean difference values for each of the eight meta-analyses were used for making box plots using BoxPlotR (Spitzer et al., 2014) to provide a graphical comparison between the resting joint angles following release from flexion and extension in the two developmental stages of 5th instar and adult locusts under different conditions of hind limbs as shown in Figure 4.9.

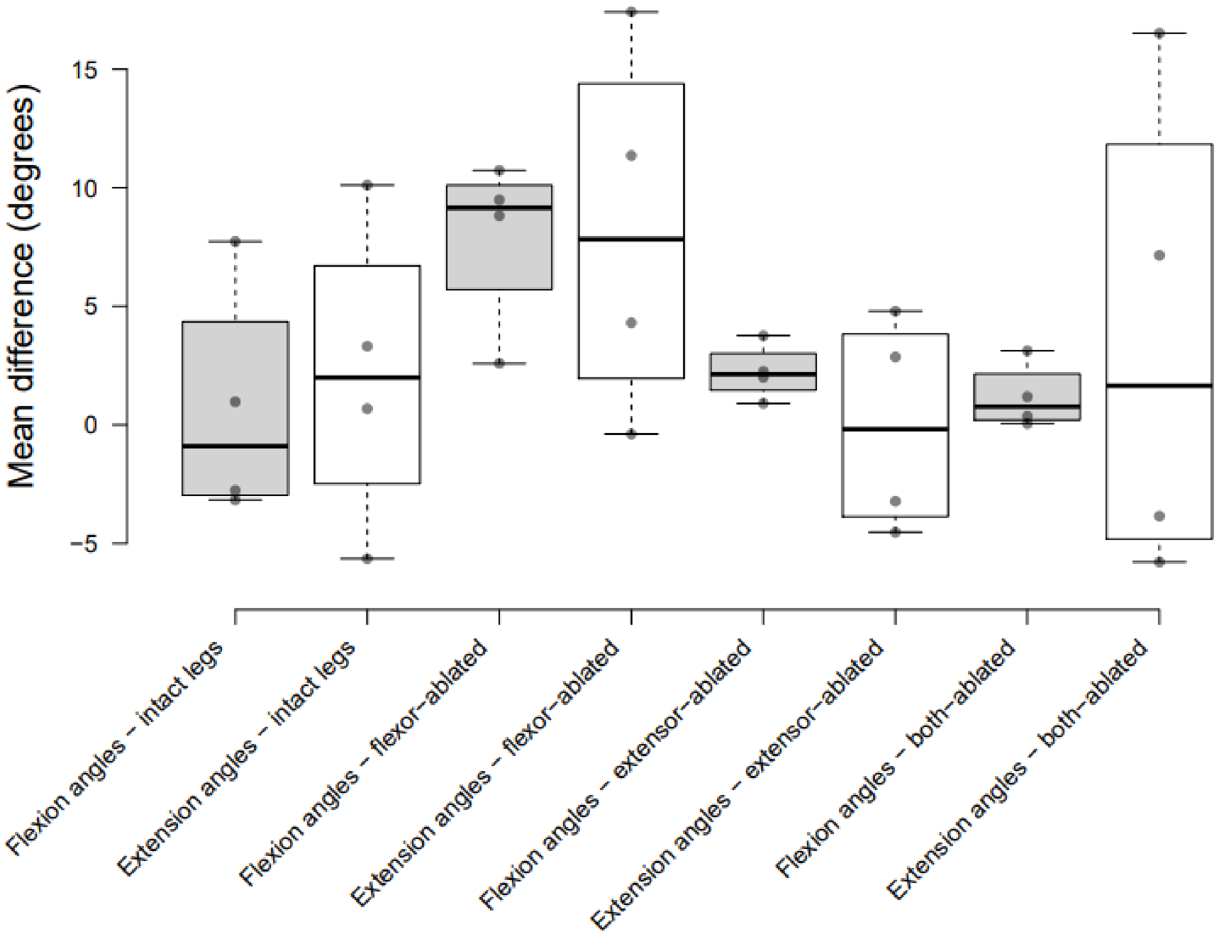


Figure 4.9: Box plots representing the mean difference in resting joint angles at flexion and extension under different conditions of hind limbs of 5th instar and adult locusts. The black lines in the centre of each box represent the median values, the small dots represent each of the 4 data points for each dataset, and the limits of the box represent the 25th and 75th percentile of the data range.

4.10 Statistical analyses using paired t-tests

The results of paired t-tests using the mean differences in the datasets are shown in Table 4.1.

Table 4.1: Results of statistical analyses using paired t-tests. The P-value was considered significant if the value was less than 0.05, and the adjusted P-value was considered significant if the value was less than 0.0018 after applying Bonferroni correction.

Analysis	**Mean difference**	**t-value**	**P-value**	**Adjusted P-value after Bonferroni correction**	**Significance**
5th instar stage					
Resting joint angles at flexion in intact and flexor-ablated legs	-22.64	8.0625	0.0040	0.00133	Very statistically significant
Resting joint angles at flexion in intact and extensor-ablated legs	41.0125	7.5636	0.0048	0.0016	Very statistically significant
Resting joint angles at flexion in intact and both-ablated legs	40.875	7.2518	0.0054	0.0018	Very statistically significant
Resting joint angles at extension in intact and flexor-ablated legs	-23.165	10.4236	0.0019	0.00063	Very statistically significant
Resting joint angles at extension in intact and extensor-ablated legs	31.415	4.9962	0.0154	0.00513	Statistically significant
Resting joint angles at extension in intact and both-ablated legs	-19.3875	12.8726	0.001	0.00033	Very statistically significant
Adult stage					

Resting joint angles at flexion in intact and flexor-ablated legs	-15.4225	3.9368	0.0292	0.00973	Statistically significant
Resting joint angles at flexion in intact and extensor-ablated legs	42.5525	9.4577	0.0025	0.00083	Very statistically significant
Resting joint angles at flexion in intact and both-ablated legs	41.3625	8.2006	0.0038	0.00127	Very statistically significant
Resting joint angles at extension in intact and flexor-ablated legs	-17.105	3.202	0.0493	0.01643	Statistically significant
Resting joint angles at extension in intact and extensor-ablated legs	29.275	4.369	0.0222	0.0074	Statistically significant
Resting joint angles at extension in intact and both-ablated legs	-17.9925	3.8504	0.0309	0.0103	Statistically significant

4. Discussion

This study was undertaken to understand the changes in passive joint forces through the life cycle of locusts, and the effect of these changes on hind limb movements. The analysis was carried out using resting joint angles on intact hind legs, flexor-ablated hind legs, extensor-ablated hind legs, and both flexor and extensor-ablated hind legs, to understand the roles of forces generated within the flexor and extensor muscles on the resting joint angles of locust hind legs following release after flexion and extension. The results of meta-analysis were represented using forest plots and box plots, and statistical analysis was carried out using paired t-tests.

The forest plots revealed the mean differences in the resting joint angles following release from flexion and extension in the 5th instar and adult stages. In intact hind legs, the mean difference in resting joint angles following release from flexion is 0.97 and following release from extension is 2.96 between the two stages. This indicates that the flexion angle is almost the same in both 5th instar and adult stages, whereas the angle at extension is slightly larger in the adult stage as compared to the 5th instar stage. This is in line with the findings of Scott (2005) who carried out an in-depth laboratory analysis on the jumping characteristics of adult locusts. Adult locusts demonstrate an escape jump to distance themselves from predators and launch their body into flight, for which they jump considerable distances at a time resulting in much larger extensions as compared to 5th instar locusts (Scott, 2005). Also, the extensor muscle is 21 times stronger and 5 times heavier than the flexor muscle, allowing the generation of large forces for hind limb movement (Burrows, 1996).

When the flexor muscle is removed, the mean difference in resting joint angle following release from flexion is 8.82 and the mean difference in resting joint angle following release from extension is 5.46 between the two locust stages. This shows that removal of the flexor muscle results in a higher mean difference in the joint angles following release after both flexion and extension to a considerable degree as compared to the mean difference for intact legs. Also, the increased mean difference in flexor-ablated legs for resting joint angles following release from both flexion and extension indicate that adult locusts are more severely affected by the removal of the flexor muscle than the 5th instar locusts. According to Ache and Matheson (2013), passive joint forces lead to passive movement of the hind legs by counteracting the effects of active movement of the antagonistic muscle and supporting the weaker muscle, allowing the leg to come to rest following active flexions and extensions. Here, we show that removal of the flexor muscle results in a much larger flexion and extension angle as compared to intact legs in both 5th instar and adult locusts, indicating that the presence of the flexor muscle may have a role in controlling the passive flexion and extension angles of the locusts beyond a certain range. However, in line with the results of Ache and Matheson (2013), our results show that flexion takes place even in the absence of flexor muscle, indicating that passive forces arising in the joint are responsible for flexion of hind limbs in locusts.

The removal of the extensor muscle leads to a mean difference in joint angle following release from flexion of 1.19 and following release from extension of 2.62 between the two developmental stages. These values are almost similar to the values obtained for intact legs, thereby indicating that removal of extensor muscle may not have a significant impact on the resting joint angles following release from flexion and extension of 5th instar and adult locusts. This might suggest that there may exist forces outside the extensor muscle that may result in flexion and extension of the locust hind legs, suggesting passive forces arising in the FT joint. According to Ache and Matheson (2013), all tibial movements are a result of forces arising in either the flexor or extensor muscle. As a result, in the absence of the extensor muscle, there must be active and/or passive forces in the flexor muscle and femoro-tibial joint that must lead to flexion and extension in the locusts.

In the absence of both the flexor and extensor muscles, the mean difference in the joint angles following release from flexion is 0.69 and following release from extension is -2.67 between the two locust stages, indicating that these two muscles are responsible for hind leg movement and their absence prevents movement in the locust hind limbs.

Box plots representing the data from the meta-analyses were generated which demonstrate that removal of flexor muscle leads to an increase in the mean difference in resting joint angles following release from flexion and extension between the two developmental stages, indicating that the role of the flexor muscle increases with the developmental stage for both flexions and extensions. As seen in the plot, the median value for mean difference in joint angle following release from extension is higher in intact legs as compared to the mean difference in joint angle following release from flexion. This shows that forces leading to flexion in 5th instar and adult locusts are almost similar, whereas forces leading to extension are slightly higher in adults as compared to 5th instar locusts. As shown by Scott (2005), adult locusts demonstrate a much larger extension in order to fulfil the requirements of their escape jump. Ache and Matheson (2013) showed that the larger the extension, the larger are the passive forces generated to bring back the extended muscles to the resting phase. Therefore, these passive forces must be larger in the adult locust as compared to the 5th instar locust due to the demands of movement in adult locusts as evident in this study. Comparing the mean difference in resting joint angles after release from both flexion and extension in flexor-ablated legs is considerably higher than the values for intact legs, indicating that removal of the flexor muscle affects the adult stage to a much larger extent than the 5th instar stage. However, this is not true for extensor-ablated legs and both flexor and extensor-ablated legs where the mean difference between the resting joint angles are lower as compared to the values of flexor-ablated legs. This indicates that removal of the extensor muscle and both the flexor and extensor muscles have equal effect on the 5th instar and adult stages.

Paired t-tests were conducted to assess the statistical significance of the data of mean difference between the resting joint angles at flexion and extension of intact legs and one or more ablated legs for the 5th instar and adult locust stages. All comparisons were found to be statistically significant as evident from the low P values indicating that forces generated in both the flexor and extensor muscles are important for resulting in movement in the hind limbs of locusts.

To the best of my knowledge, there is very limited research in the changes in passive forces from the 5th instar to the adult locust stage on resting joint angles at flexion and extension. Corroborating my findings, both the flexor and extensor muscles are responsible for hind leg movement in the 5thinstar and adult locusts. Forces resulting in extension are much larger in adult locusts as compared to 5th instar locusts because of their requirements of escape movement. As a result, the passive forces that bring the tibia from the extended stage back to its resting stage are also larger in adult locusts when compared to 5th instar locusts. Removal of the flexor muscle has an immense impact on both flexion and extension in both the 5th instar and adult stages indicating that passive forces generated in this muscle may affect hind limb movement in locusts. In contrast, however, removal of the extensor muscle has no effect on the difference in joint angles at flexion and extension between the 5th instar and adult locusts indicating the possibility of another underlying mechanism for resulting in flexion and extension in the absence of this muscle.

As indicated by the I^2 and chi^2 values, the heterogeneity for data for most of the forest plots was quite high. The sample sizes were also variable among the 4 datasets, and, as evident by the data points on the box plots, the range across which the data was distributed was large. Therefore, this study was limited by a low number of datasets coupled with large variations in values within each dataset. In order to overcome this limitation, several statistical tests were carried out to assess the significance of the results; however, it cannot be negated that a larger number of datasets could have provided deeper insights into the uncovered mechanisms. Excluding the outliers would also have resulted in a very small amount of data making it difficult to draw any meaningful conclusion. However, despite the limitations, the findings of this study need to be further investigated based on their consistent signifcance across the different statistical tests.

5. Conclusion

This study was one of the first of its kind where a meta-analysis of the mean difference between the joint angles following release from flexion and extension between the 5th instar and adult stages was carried out with the objective of understanding the differences in passive forces that lead to hind limb movement in locusts at different developmental stages. Insights from this study point towards a greater involvement of passive forces generated in the flexor muscle as compared to those generated in the extensor muscle in hind limb movement of locusts. Furthermore, these forces are found to be larger at the adult stage, possibly owing to a larger force leading to extension of hind limbs in adult locusts. Future studies are needed to further analyse the forces generated in the flexor and extensor muscles and their effect in bringing about movement in the locust hind limbs. Further research needs to be done regarding the movement changes that occur in the absence of either the flexor muscle or the extensor muscle in order to elucidate their roles in locust hind limb movement.

This report has served to further our understanding of the differences in forces between the 5th instar and adult stages, which is affected more when the flexor muscle is ablated. However, contrary to my expectations, the difference between the forces when the extensor muscle is ablated is similar to the difference in forces in intact legs, which points towards a compensatory mechanism that may be present in adult locusts. This compensatory mechanism needs to be further investigated in future studies as it could provide insights into the forces that control and maintain hind limb movement in locusts. Furthermore, the FT joint of the hind limb of locusts could also be restricted to understand the role of passive joint forces in helping the limb achieve its resting angle following release from flexion and extension. Restricting the joint along with removal of either the flexor or the extensor muscle could provide insights about the possible compensatory mechanism that may be involved in hind limb movement of locusts.

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Appendix

Table A1.1: Resting joint angles following release from flexion and extension of intact hind limbs of 5th instar and adult locusts

Dataset	Flexion angle			Extension angle		
	Mean	**SD**	**Total**	**Mean**	**SD**	**Total**
5th instar stage						
Student-2018-A	68.37	11.27	50	74.24	8	50
Student-2018-B	61.74	16.01	50	73.01	10.5	50
Student-2019-B	49.73	11.15	320	64.49	8.8	320
Student-2019-C	53.44	18.47	90	61.4	16.96	90
Adult stage						
Student-2018-A	60.64	14.06	55	64.13	12.9	55
Student-2018-B	64.51	9.48	50	72.33	9.39	50
Student-2019-B	48.76	14.09	330	61.18	12.56	330
Student-2019-C	56.61	15.9	70	67.05	11.39	70

Table A1.2: Resting joint angles following release from flexion and extension of flexor-ablated hind limbs of 5th instar and adult locusts

Dataset	Flexion angle			Extension angle		
	Mean	**SD**	**Total**	**Mean**	**SD**	**Total**
5th instar stage						
Student-2018-A	88.51	8.03	25	94.44	8.94	25
Student-2018-B	79.46	11.32	25	91.59	5.47	25
Student-2019-B	80.36	8.06	165	92.12	8.86	165
Student-2019-C	75.51	12.19	45	87.65	10.75	45
Adult stage						
Student-2018-A	79.69	7.74	30	83.08	7.59	30
Student-2018-B	68.73	16.67	25	74.17	16.92	25
Student-2019-B	70.87	9.49	170	87.82	10.66	170
Student-2019-C	72.92	11.88	35	88.04	10	35

Table A1.3: Resting joint angles following release from flexion and extension of extensor-ablated hind limbs of 5th instar and adult locusts

Dataset	Flexion angle			Extension angle		
	Mean	**SD**	**Total**	**Mean**	**SD**	**Total**
5th instar stage						
Student-2018-A	14.84	3.49	25	48.34	24.43	25
Student-2018-B	15.97	8.83	25	29.03	8.33	25
Student-2019-B	13.87	3.93	155	25.17	8.62	155
Student-2019-C	24.55	10.78	45	44.94	22.84	45
Adult stage						
Student-2018-A	12.59	3.68	25	52.87	24.61	25
Student-2018-B	12.21	4.02	25	32.26	23.18	25
Student-2019-B	12.97	2.79	160	22.31	7.82	160
Student-2019-C	22.54	4.34	35	40.15	15.58	35

Table A1.4: Resting joint angles following release from flexion and extension of both-ablated hind limbs of 5th instar and adult locusts

Dataset	Flexion angle			Extension angle		
	Mean	**SD**	**Total**	**Mean**	**SD**	**Total**
5th instar stage						
Student-2018-A	15.13	5.45	50	92.96	14.35	50
Student-2018-B	14.78	4.12	49	88.52	8.5	50
Student-2019-B	14.83	3.02	320	85.31	9.02	320
Student-2019-C	25.04	10.71	90	83.9	8.15	90
Adult stage						
Student-2018-A	13.95	4.83	55	76.44	8.51	55
Student-2018-B	11.66	2.78	50	81.37	12.08	50
Student-2019-B	14.47	3.48	330	91.1	10.13	330
Student-2019-C	24.99	6.94	70	87.75	15.91	70

Table A2.1: Box plot statistics used for generating the box and whisker plot

	Flexion angles – intact legs	**Extension angles – intact legs**	**Flexion angles – flexor-ablated legs**	**Extension angles – flexor-ablated legs**	**Flexion angles – extensor-ablated legs**	**Extension angles – extensor-ablated legs**	**Flexion angles – both-ablated legs**	**Extension angles – both-ablated legs**
Upper whisker	7.73	10.11	10.73	17.42	3.76	4.79	3.12	16.52
3rd quartile	4.35	6.71	10.11	14.39	3	3.83	2.15	11.84
Median	-0.9	2	9.16	7.83	2.13	-0.19	0.77	1.65
1st quartile	-2.97	-2.49	5.71	1.95	1.45	-3.88	0.2	-4.82
Lower whisker	-3.17	-5.65	2.59	-0.39	0.9	-4.53	0.05	-5.79
Number of data points	4	4	4	4	4	4	4	4