

The effect of weight gain on the neuromechanics of locust jumps
(*Schistocerca americana*)

By

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Abstract

During the adult reproductive stage, female locusts (*Schistocerca americana*) experience multiple ovigerous cycles during which they undergo significant weight gain. The magnitude of their weight change is thought to negatively impact the kinematics of the locust jump during the gravid phase. We hypothesized that this decrease in performance would result in an evolutionary pressure for female locusts to develop an intrinsic means to detect increases in body mass and respond with a modulation in their movement. Based on this hypothesis, we predicted that the female locusts would display a consistent jump performance in response to weight change, facilitated by a change in the contraction time of their extensor tibiae muscles. Conversely, this type of control is not expected in male locusts since they do not experience the same cycles of weight. These predictions were tested using an artificial weight gain system to induce weight gains similar to natural changes.

Simultaneous high-speed video capture (1500 frames per second) and bilateral electromyogram (EMG) recordings were obtained from nine female and five male locusts during evoked escape jumps. Multiple jumps were recorded for each individual, corresponding to different artificial weight gain conditions. The peak angular velocity of the jumping legs was calculated using position tracking software (DeepLabCut) and subsequent kinematic analysis of the leg rotation using custom scripts in R. Contraction time was calculated from the extensor tibiae muscle EMG recordings. Female and male locusts maintained consistent peak angular velocity during extensions of their jumping legs in response to weight gain, indicating that both sexes modulate jump performance for different body masses. Furthermore, both sexes displayed increased contraction times for small weight increases, but contraction times for large weight increases were comparable to those of their natural body mass.

Introduction

For many organisms, survival necessitates performing movements that go beyond the constraints of their physical anatomy. This is especially true for smaller organisms that must overcome decreased muscle and limb size. For these organisms, enacting rapid movements necessitates using additional force-generating systems. One such system of movement that a wide range of smaller organisms utilize is latch-mediated spring actuated (LaMSA), which generates speed and power that cannot be achieved through muscle action alone (Longo et al., 2019). LaMSA systems use spring actuation to convert elastic energy stored in flexible body components into kinetic energy for movement (Longo et al., 2019). Equally, LaMSA systems use latch mediation to modulate how energy is stored in the spring system and how this energy is released during movement (Longo et al., 2019). Using LaMSA systems, organisms benefit from increased energy conservation, a more stable acceleration of movement, and a prominent amplification of power output (Longo et al., 2019). Ultimately, the increased movement performance enabled by LaMSA systems is especially beneficial for smaller organisms with reduced muscle and limb size and, thus, limited performance output.

While LaMSA movements are essential for overcoming reduced muscle size to produce fast, powerful movements, they can also compensate for lack of muscle, as is the case with the bunchberry dogwood (Edwards et al., 2005). The bunchberry dogwood is a species of low-lying forest ground cover dependent on pollen dispersal for reproduction, a conundrum given its reduced height (Edwards et al., 2005; Montana Field Guide, 2017). To compensate for its reduced stature, the bunchberry dogwood uses a LaMSA mechanism to catapult pollen granules into the air, thereby increasing its dispersal range (Edwards et al., 2005). The bunchberry dogwood's flowers grow with their stamens bent inward, maintaining a high elastic force;

disturbance of the flower releases this stored energy, which allows the stamens to unfurl, sending pollen into the air (Edwards et al., 2005). While the bunchberry dogwood utilizes LaMSA movements to facilitate reproduction, other organisms, such as the trap-jaw ant, utilize these facilitated movements to increase evasion success (Gibson et al., 2018). The trap-jaw ant can lock its mandibles open using a latching mechanism, and elastic energy produced by the contraction of its mandible abductor muscles stores energy in its flexible head capsule (Gibson et al., 2018). The energy produced by this system enables the trap-jaw ant to produce a force capable of propelling itself into the air, away from a predator, or pushing back an adversary (Patek et al., 2011). Using LaMSA systems, various organisms can enact movement that would otherwise be impossible, given their physical constraints.

Jumping insects are some of the most common and diversified groups of organisms to use LaMSA systems to achieve their impressive jumps, making the locust an important model for investigation. Locusts belong to the order Orthoptera, which contains other species of jumping insects, such as grasshoppers, crickets, and katydids (Gurney, 2017). As is common with jumping insects, the locust displays an impressive jump, reaching vertical heights of 45 cm and horizontal distances of over 90 cm, equating to 10 times their body height and 20 times their body length (Hoyle, 1958). Male locusts typically weigh around 1.5-2.0g, while females are larger, weighing between 2.5 and 3.5g (Burrows, 2012). In addition, female locusts have been shown to undergo a roughly 40% increase in body mass during gravidity, and it is common for multiple gravidity phases to occur throughout the lifetime of a mature adult female (Queathem, 1990; Squitier & Capinera, 1996).

The prominent, oversized third pair of legs on a locust enable jumps on the high end of movement speed observed in biological systems (Burrows, 2012). The locust's jump can be

separated into three phases, which together generate the power needed to perform these characteristic jumps (Sutton & Burrows, 2008). The first phase, the cocking phase, primarily involves the locust orienting its femur and tibia to prepare for the jump (Sutton & Burrows, 2008). The locust uses its flexor tibiae muscle to fully flex its tibia, entering a crouched position in preparation for the jump (Burrows, 2012).

During the second phase, co-contraction of the flexor and extensor tibiae muscles occurs to engage the latch and charge the spring, generating sufficient force for the jump while inhibiting movement initiation (Sutton & Burrows, 2008). The significantly larger extensor tibiae muscle is innervated by two excitatory motor neurons, one slow and one fast, and is also innervated by a branch of inhibitory interneurons (Hoyle, 1978). While the cross-sectional area of the flexor muscle is only about 12% of the extensor tibiae muscle, it can sufficiently prevent the extension of the tibia during the co-contraction phase due to its mechanical advantage over the extensor tibiae when the tibia is fully flexed (Burrows, 2012; Burrows & Morris, 2003). In addition, the locust possesses a flexor apodeme lock, an internal ridge of the exoskeleton in the joint connecting the femur and tibia. When the tibia is fully flexed, this structure latches the tibia in the flexed position just under the femur (Burrows, 2012). Furthermore, the flexor apodeme is distorted during co-contraction to store elastic energy (Burrows, 2012). Supplementing the elastic energy stored in the flexor apodeme, the femoral-tibial joint contains a pair of semilunar processes, which are dark flexible cuticles that are distorted to store elastic energy (Burrows, 2012). These semilunar processes are externally composed of hard, durable cuticles, while the inside is lined with flexible resilin, which together forms a composite material (Burrows & Sutton, 2012). The flexible cuticles in the locust's femoral-tibiae joint store the potential energy

generated by the contraction of the extensor tibiae muscle until it is converted to kinetic energy when the latch is released (Burrows, 2012).

During the third phase of the locust's jump, the triggering phase, the excitatory motor neurons innervating the flexor tibiae muscle are inhibited, and the inhibitory neurons innervating the tibiae muscle are activated (Sutton & Burrows, 2008; Burrows, 2012). This inhibition of the flexor tibiae muscle releases the flexor apodeme latch (Burrows, 2012). As the latch is inhibited, the flexor tibiae muscle is also inhibited; however, the extensor tibiae muscle continues to receive excitatory signals (Burrows, 2012). The release of the apodeme latch enables the potential energy stored in the locust's spring-like structures to be converted to kinetic energy (Burrows, 2012). This motor pattern by the locust implements a latch-mediated spring actuated system that ultimately results in their impressive jumps.

The locust's use of a LaMSA system to increase the speed and leg forces for jumping comes with limits to movement modification (Kagaya & Patek, 2016). Once released, the speed of the jump does not allow time for neural feedback to monitor or modify the movement (Kagaya & Patek, 2016). The locust's jump is therefore termed ballistic, which means all control signals must be set prior to the movement since it cannot be modified once initiated (Khai-Chung & Hoffman, 1988). Some organisms that use LaMSA systems to power their movements exhibit the ability to modulate their movement through feed-forward control, which involves modifying the muscle activity controlling the latch release to vary movement performance (Kagaya & Patek, 2016). Although locusts are not known to exhibit variable control of their jump performance, the capacity for movement modification has yet to be investigated.

Studies characterizing mantis shrimp (*Neogonodactylus bredini*) strike patterns revealed what appears to be a linear relationship between co-contraction phase duration and strike angular

velocity (Kagaya & Patek, 2016). This relationship would allow mantis shrimp to vary their strike speed by modulating the release of their latch mechanism. This is congruent with the understanding that movement in mantis shrimp is initiated through sensory receptor input; the same is true of locusts, indicating that the co-contraction phase of their force generation can be prolonged until sufficient stimuli are presented to provoke movement (Kagaya & Patek, 2016; Heitler & Burrows, 1977). In a similar model to the locust, the bush cricket (*Pholidoptera griseoptera*) is a jumping insect that is known to adjust the kinematics of its jump and does so by varying the neural input of the excitatory motor neurons innervating the extensor muscles of the jumping legs (Burrows & Morris, 2003). This neuromechanical jump control suggests a possible method through which locusts can modify their jumping output. LaMSA systems leverage latch and spring structures to enhance the force generation of their muscles, and the feed-forward modification of muscle activity has been identified as a source of movement modulation for several organisms.

This experiment investigated the capacity of the American locusts (*Schistocerca americana*) to alter their jump performance in response to artificial weight gain. Since female locusts experience variability in body mass due to multiple gravidity cycles, the ability to pre-plan movement to maintain an optimal jump performance would be advantageous. (Squitier & Capinera, 1996). Therefore, it was hypothesized that the evolutionary pressure to maintain optimal jump performance led female locusts to evolve a neuromechanical mechanism that modulates jump force output at different body weight conditions. Given this hypothesis, it is first predicted that female locusts would respond to an artificial weight gain similar to a perceived natural gain in mass when gravid. Based on this assumption, we predicted that artificial weight changes in female locusts would result in the maintenance of a consistent maximum angular

velocity of their leg extension across all weight conditions in a naturalistic range. Consistent angular velocity of leg extension is predicted to be produced by a longer contraction time of the extensor tibiae muscle at heavier weights. Finally, male locusts were not predicted to produce similar responses to artificial weight gain since the evolutionary pressures associated with gravidity cycles are absent. To investigate the degree of control locusts exhibit over their jump performance, we recorded simultaneous high-speed video (HSV) and electromyogram activity of locusts jumping under randomized treatments of no weight change and artificial weight gains ranging from 16-44% (Bead mass: 0.28-1.3g) increases in baseline body mass. These weight increases are within the natural range experienced by females during gravidity cycles. We analyzed the maximum angular velocity of the tibia rotation from the femur in response to these weighted conditions. Investigating the maximum angular velocity of the locust's leg extension provides information regarding the performance output of the locust LaMSA before variables such as gravity, wind resistance, and body rotation impact the kinematic outputs of the locust's jump trajectory; thus, it is an optimal gauge of performance output. In addition to the jumping leg kinematics, we also analyzed the contraction time of the extensor tibiae muscle in response to weight gain. Together, this information provides evidence that locusts modulate their jump performance in response to weight change and provides a potential mechanism for this regulation.

Materials and Methods

Experimental Setup

A harness and weight system was developed to modify the total body mass between jump trials. The harness was created from a single open loop of a paperclip affixed to the dorsal thorax using a cyanoacrylate adhesive (Loctite). Magnetic beads were created using modeling clay (Fimo) integrated with 1/32" circular magnets (K&J Magnetics) at one end to help secure the beads onto the harness. Twenty-two clay beads were created to mirror a natural range of weight gained during female gravid cycles; females were exposed to clay beads ranging from 0.43g to 1.3g. The range of male weights was scaled down to reflect a similar percentage change in body mass to that of gravid female locusts; the clay beads weighed between 0.28g and 0.70g.

To record and gather simultaneous electromyogram (EMG) recordings from the extensor tibiae muscle of each jumping leg custom, two-channel differential tether electrode leads were created for each locust for permanent surgical implant. The length of the lead was composed primarily of 0.0045" diameter insulated copper wire (Remington). Strands of five copper wires were braided together to form a single tether of two differential electrodes and a ground. The copper wire was soldered to a five-prong female PCB pin component, and the soldering points were covered with liquid tape (Garden Bender) to isolate each circuit. The free ends of the leads were stripped of insulation and soldered to short, stainless steel wires (A-M Systems) with an insulated diameter of 0.0055" and a bare diameter of 0.003". Stainless steel was used for the implantable end of the electrode since copper can induce toxic side effects to biological tissue. The soldering points between the steel and copper wire were also insulated using liquid tape. Connections for each of the five wire electrodes were checked using a voltmeter. The female PCB pin component is attached to a male PCB plug on a universal multi-electrode connection

cable plugged directly into the extracellular amplifier (Model 1700 Differential AC Amplifier, A-M Systems).

Custom holding containers were created to isolate each trial locust after surgery and between weight treatment trials. This holding container also served as the platform from which trial jumps were recorded. Clear plastic Frappichio cups (Starbucks) were weighted with aquarium sand and topped with sandpaper to give the locusts extra traction for jumping. The locusts were housed atop the sandpaper and covered with a domed lid. The electrode tethers were fed out of the top of the lid, which was then plugged with a piece of foam to secure the leads and allow airflow. Locusts were given a piece of lettuce daily, and all holding containers were stored in a warm, humidified chamber.

Locusts were provided from a colony maintained by the Union College Biology Department. A random selection of nine female locusts and five male locusts were chosen, ranging between one and three months of age. Prior to surgery, the locusts were anesthetized in a 4°C refrigerator. During surgery, the paper clip harness was first attached using cyanoacrylate and an accelerant (Zip Kicker). The ground and a single pair of differential electrodes were trimmed so only the ends were exposed and inserted as a bundle into the left extensor tibiae muscle through a single hole cut into the cuticle. The wire electrodes were inserted at a depth of one to three mm into the femur, and the incision was patched using cyanoacrylate and accelerant. Additional cyanoacrylate and accelerant were used to secure the wires at an adjacent point on the femurs and at two points on the thorax to reduce any extra slack in the wires. The same procedure was mirrored on the right side of the locusts for the right differential electrodes. Although analysis of jump kinematics and extensor muscle activity was recorded from one leg of the locust, bilateral implantation allowed for the control of any impact the surgery had on jump

performance. Following surgery, each locust was sexed and massed with its entire implant. After surgery, locusts were assigned a random order of treatment weight conditions, and if the locust's first condition included added weight, their corresponding clay bead was added. All locusts were given a minimum of 24 hours to recover from surgery.

Experimental Procedure

Locusts were jumped under three randomized conditions: no increase in weight, a small increase in weight, and a large increase in weight. The clay beads added for the small increase condition averaged 0.56g, resulting in an average increase in body mass of 24%, while the large increase condition averaged 0.84g and 38%, respectively. Five jumps were recorded for each treatment condition, since after five jumps, locusts are known to reduce jump performance due to fatigue (Bennet-Clark, 1975). Following the first set of experimental jumps, the locusts were again massed and then affixed with their next weight condition bead, or left bare, and then left overnight to recover. The same procedure was followed for the second set of experimental jumps and preparation for the third condition. After the third set of jumps, the locusts were massed for a final time and then left in a freezer for euthanization.

EMG extensor tibiae muscle activity during each jump was detected and amplified on an extracellular amplifier, digitized using a Power lab (PowerLab 26T), and recorded using Labchart software (Labchart v8.1.21, AD Instruments). Simultaneous high-speed video recordings (1500 fps) were also collected for each jump using a Photron Fastcam Mini camera (WX100). The EMG and HSV recordings were synchronized using a TTL pulse trigger to stop the high-speed video and save the last five seconds of rolling footage while marking the trigger point on the EMG recording file.

At the start of each condition, the trial locust to be jumped was brought to the experimental area in its custom holding container. The electrode tether was connected to the EMG system, and both recording systems were started. The domed top of the holding container and any uneaten lettuce were removed. The container was positioned approximately 30 cm from a mesh screen which the locusts jumped towards. Locusts always jumped perpendicular to the camera, with their left side in view. Locusts either jumped of their own accord or were stimulated to do so by lightly brushing their abdomen with a makeup blush brush. Following each jump, the recording files were saved, and the locust was manually returned to its container from the mesh screen. After completing all five jumps, the locust was massed, affixed with its next clay weight if its next condition was weighted, and provided with fresh lettuce in their humidified isolation chamber.

Kinematic Analysis

The HSV recordings of the locust's jumps were evaluated to rule out extraneous jumps; locust legs slipping and missing during a jump frequently excluded a video. Data analysis of the maximum angular velocity of the locusts' jumping legs was performed using DeepLabCut (<https://github.com/DeepLabCut/DeepLabCut>), an animal pose estimation software, and custom R scripts (Version 4.2.2) implemented with R Studio (Version 2022.12.0). The high-speed jump recordings were processed in DeepLabCut to label two points along the femur and two points on the tibia. Output files of the location of these points throughout the videos were subsequently processed using R to convert the points into vectors for the femur and tibia. The change in the angle formed between these two vectors was plotted vs. time and fit to a 4-10 order polynomial curve. The peak angular velocity of each jump was subsequently determined by taking the maximum value of the first derivative of the fit curve. For each animal, values of the maximum

angular velocity of their leg extension were averaged for each weight change condition. A mixed model analysis was performed (JMP Pro 16) on each sex to determine any significant differences between the maximum angular velocity values between the different weight gain conditions. The variables of sex and body mass were assigned as fixed, while each locust, nested within its given sex, was assigned as a random variable.

EMG Analysis

Data analysis of the contraction time of the femoral extensor muscle leading up to each jump was performed using the EMG software (Labchart v8.1.21). Synchronization of the high-speed videos and EMG recordings allowed for identifying when movement was initiated in EMG traces based on the video frame number in the HSV file. Contraction time was defined as the time between when leg movement started and the moment increased spike activity was observed in the EMG recordings before that moment. Thus, the time between an increase in spike activity and the initiation of the jump movement was recorded as contraction time for a given jump. Figures were created using a combination of JMP Pro 16, Adobe Illustrator, and Adobe Photoshop.

Results

Maximum Angular Velocity of Jumping Legs

Beginning with the analysis of the jumps performed by the female locusts: no significant difference was found between the averaged maximum angular velocity of the extension of the jumping leg across the different conditions of weight gain. This supports the prediction that the average maximum angular velocity of the jumping legs of the female locusts is consistent across conditions (Figure 1). The kinematics of 123 jumps were analyzed from nine female locusts. The average maximum angular velocity of the jumping legs for the no-weight increase condition was 5,641 °/sec (degrees per second). The average weight of the clay beads added to the locust was 0.69g for the small weight increase condition, resulting in an average increase in baseline body mass of 26.2%. The average maximum angular velocity of the jumping legs for the small increase condition was 4,324 °/sec. Lastly, clay beads averaging 1.07g were added to the locusts for the large weight increase condition, resulting in an average increase in body mass of 39.8%. The average maximum angular velocity of the jumping legs for the largest weight increase condition was 5,512 °/sec. A mixed model analysis was performed on the data, and no significant difference was found among the treatment conditions ($p=0.3812$; Figure 1).

Male locusts were similar to females in that we found no significant changes in the average maximum angular velocity of their jumping leg rotation across different weight gain conditions; this was in contrast to our prediction that male locusts would not have the ability to modify jumps (Figure 1). These results were derived from 67 jumps from five male locusts. The average maximum angular velocity of the jumping legs for the no-weight increase condition was 5,964 °/sec. For the small weight increase condition, the average weight of the clay beads was 0.328g, resulting in a 20.2% average increase in body mass. An average maximum angular

velocity of 6,173 °/sec was measured for the small increase condition. In addition, clay beads averaging 0.544g were added to the locusts for the large increase in weight condition, resulting in an average increase in baseline body mass of 34.6%. The average maximum angular velocity of the jumping legs for the large increase condition was 6,635 °/sec. Just as in the females, a mixed model analysis was performed on the data, and no significant difference was found between the treatment conditions ($p=0.5173$) (Figure 1).

Extensor Tibiae Muscle Contraction Time

Analysis of female extensor tibiae muscle contraction time in response to weight gain conditions revealed an increase in contraction time for only a portion of the weight gain conditions; this is in partial support of the prediction that contraction time would increase proportionally to an increase in added weight in female locusts. Two female locusts were analyzed for changes in contraction time; the female depicted by a green-trace in Figure 2 plot weighed roughly 2.8g, while the blue-trace female weighed about 3.0g. The green-trace female experienced a roughly 190% increase in extensor tibiae muscle contraction duration for the small increase in weight condition, which resulted in a 24% increase in body mass (Figure 2). However, the contraction time for the large increase in weight condition was comparable to that of the no weight condition, with both times being around 0.15 seconds (Figure 2). Alternatively, the heavier female (Figure 2, blue-trace) displayed an inverse relationship between increased weight and contraction time duration (Figure 2). While the two female's weight change conditions influenced similar percent changes in weight, the blue-trace female's contraction times were consistently longer than the green-trace female's (Figure 2).

An analysis of the male locust's femoral extensor muscle contraction time across weight gain conditions revealed that, just as in the females, an increase in contraction time was observed

for only the small increase in weight condition; this is in contrast to the prediction that contraction time would vary across weight conditions in the male locusts (Figure 2). The two male locusts analyzed weighed roughly 1.7g and 1.6 grams, depicted as red and purple traces in Figure 2, respectively. For the first male, shown in red, a roughly 66% increase in contraction time duration was observed between the no weight gain condition and the small weight gain condition, which was a 16% increase in body mass (Figure 2). The contraction time decreased between the small and large increase in weight conditions but did not quite reach that of the no weight gain condition (Figure 2). Similarly, the purple locust experienced a 17% increase in body mass from the no weight to small weight gain condition, which resulted in a roughly 100% increase in contraction time duration (Figure 2). For the large increase in weight condition, there was a decrease in contraction time back to a duration comparable to the control condition (Figure 2). The male locusts displayed the ability to increase their contraction times in response to weight gain up to a roughly 15% increase in weight (Figure 2). This response appears lower than that of the green female locust, which was able to increase its contraction time in response to weight gains up to a 25% increase (Figure 2). Furthermore, although the contraction times for the no-weight condition were similar between the males and green female, the degree to which the female increased its contraction time far surpassed the males' (Figure 2).

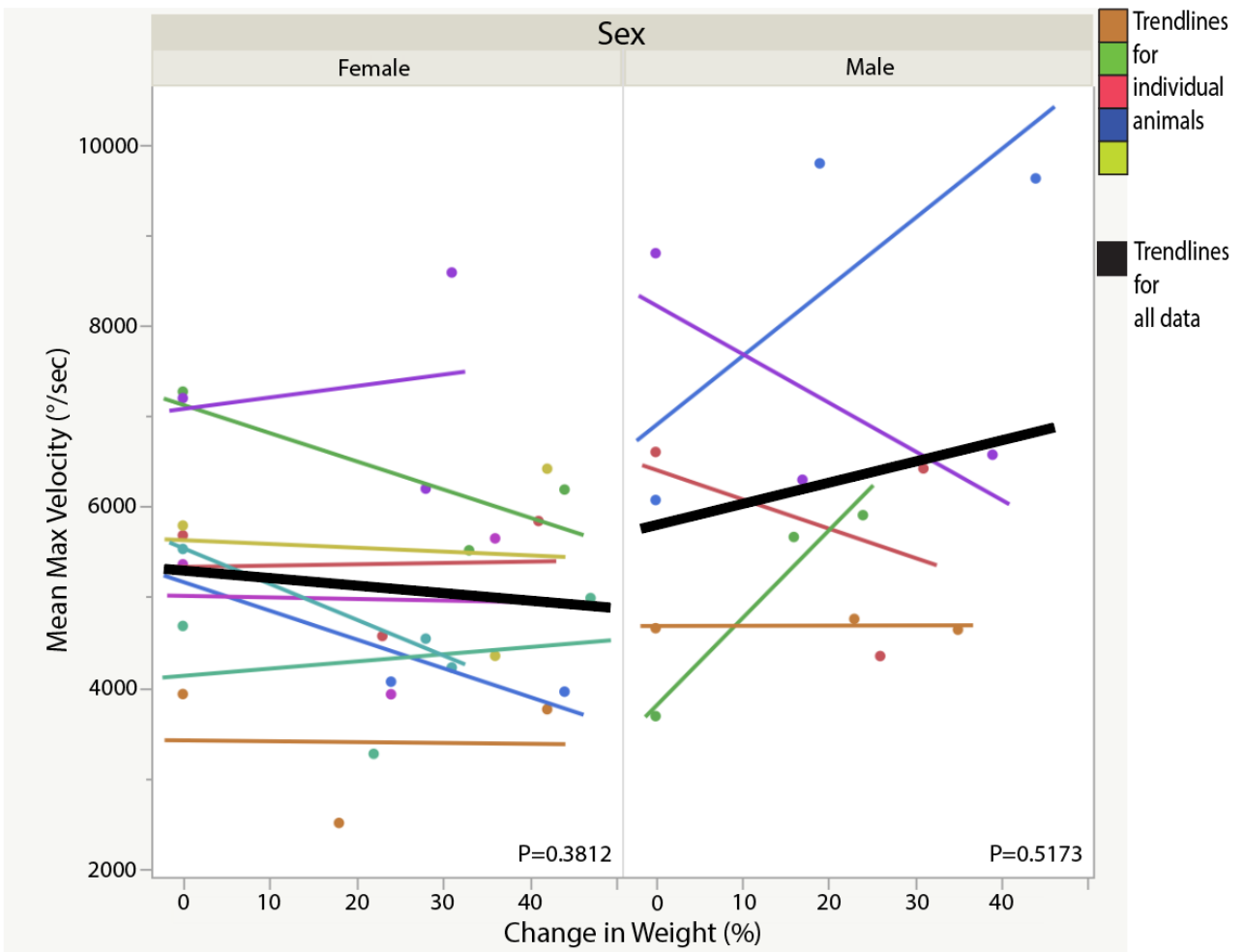


Figure 1. The effect of weight change on average maximum angular velocity of locust (*Schistocerca americana*) jumping legs. Animals were jumped under conditions of no increased weight, a slight increase in weight, and a large increase in weight which were calculated as % changes in weight. Mean max velocity determined from kinematics of five jumps recorded for each condition. Results of a mixed model analysis reported in the lower right hand corner. Colored lines indicate best fit trend lines for individual animals, while the bold black line is a best fit trend line for each sex. Female N = 9; Male N = 5.

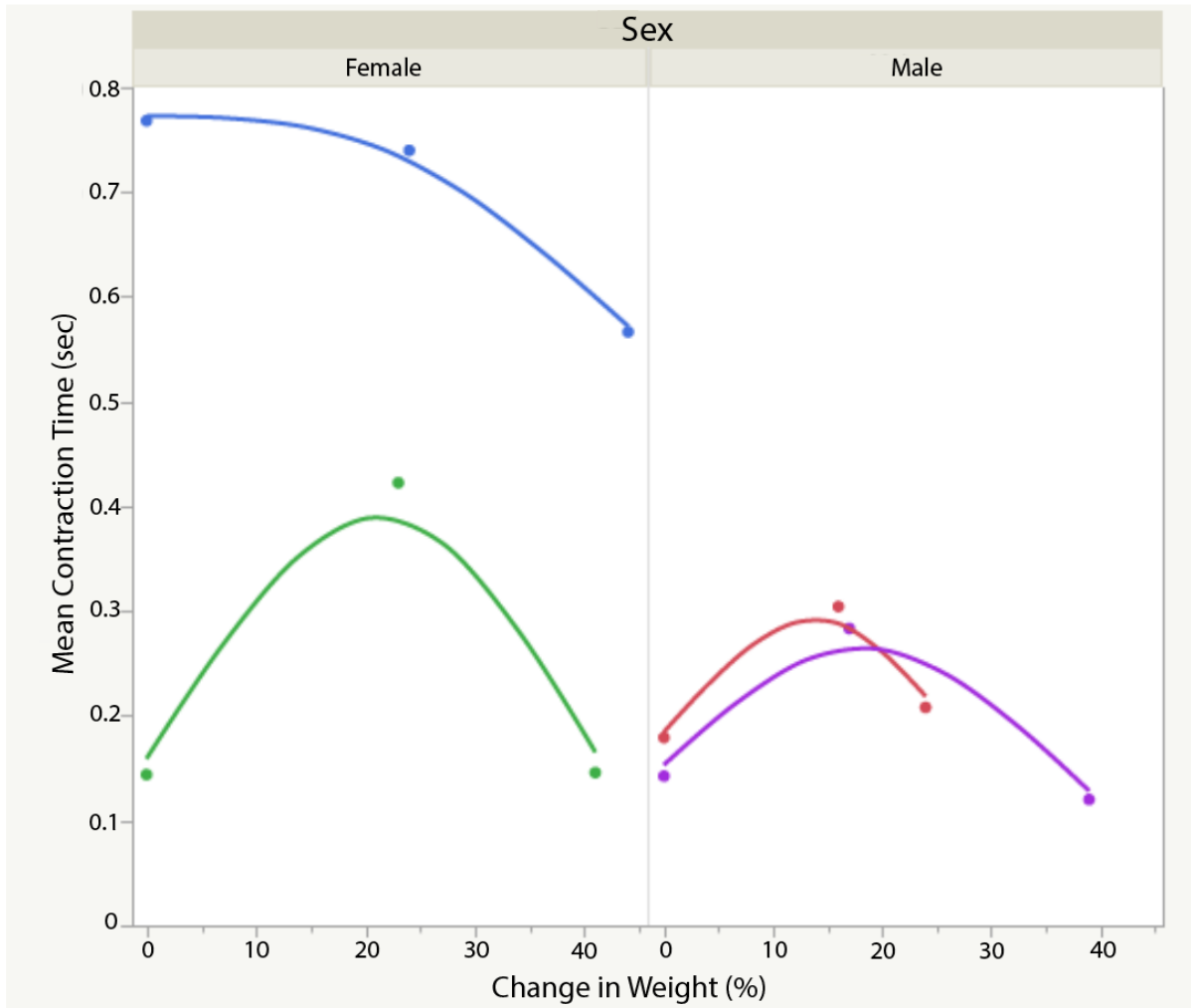


Figure 2. The effect of weight change on average extensor tibiae muscle contraction time. Sets of jumps performed by two female and two male locusts (*Schistocerca americana*) were analyzed to determine femoral extensor muscle contraction time leading up to a jump. Female contraction times were overall, greater than males. Females responded to weight gain up to 25% with increased contraction time. Males responded to weight gain up to 15% before returning to unweighted response.

Discussion

Locusts of both sexes displayed consistent maximum angular velocity of their jumping legs across all weight conditions, supporting the hypothesis that locusts possess feed-forward modulation of their ballistic jumps. This conclusion is bolstered by the evidence that locusts increase the contraction time of their extensor tibiae muscle to generate an increased force in response to increased body mass. These findings are congruent with evidence of preemptive planning performed by other organisms, such as the bush cricket mentioned earlier, in modifying ballistic movement based on variable sensory input, refuting the idea that locusts jump with a single parameter of neuromechanical output for optimal jump performance. Such a change in our understanding of the neuromechanical controls of locusts' jumps provides new avenues of research related to sensori-motor processing in locusts and how they interface with control systems for LaMSA movements.

We initially predicted female locusts would exhibit consistency in jump performance during escape jumps evoked under different conditions of weight gain. Analyses of the jumps performed by female locusts supported this prediction, with no statistically significant differences found in the maximum angular velocity of their leg extensions between the different weight conditions. These results provide evidence in support of our hypothesis that the evolutionary pressure exerted on female locusts to maintain optimal jump performance selected for the extant ability to modulate jump output in response to weight gain. With female locusts repeatedly undergoing conditions of significant weight gain and loss, the ability to maintain a consistent jump performance is advantageous for the survival of both the gravid female itself as well as the future generations each female produces (Squitier & Capinera, 1996; Queathem, 1990).

Contrasting the expectations that female locusts maintain a consistent leg extension velocity, male locusts were predicted to display variability in the maximum angular velocity of their leg movements across all artificial weight gain conditions. To our surprise, males and females yielded similar results: there was no significant difference in jump performance across weight gain conditions. This suggests that the mechanism of feed-forward jump modulation is conserved in both sexes, despite gravidity only impacting females (Squitier & Capinera, 1996). Although gravid cycles of weight fluctuation are exclusive to female locusts, locusts of both sex undergo the linear process of weight gain during maturation (Burrows, 2012). During maturation, locusts progress through a series of five to six molts, during which both sexes experience a consistent increase in body mass between and within each stage. Such weight gain within a stage with a minimal increase in leg muscle mass would be detrimental to jump performance (Burrows, 2012). This may provide the evolutionary pressure in both male and female locusts to maintain jump performance within a range of weight gains.

In addition to measuring the effect of weight gain on locust jump performance, this study also sought to test a possible mechanism responsible for jump modulation. Moving a greater mass requires more power than moving less mass the same distance. Given this idea, we predicted female locusts would increase the contraction time of their extensor tibiae muscles to overcome an increase in mass. Such a mechanism would result in the storage of more elastic energy in their spring-like cuticles and proportionally more power. As consistent jump performance was not expected in male locusts, it was predicted that the contraction time of the male locust's extensor tibiae muscles would be constant across conditions of weight change. Ultimately, results indicated that both male and female locusts increase their contraction times to

facilitate a consistent jump performance. This supports the findings that male locusts maintain a consistent maximum angular velocity of their leg extensions across conditions of weight change.

Although both sexes displayed similar response trends to conditions of weight gain, female locusts appear to increase their contraction times for a larger range of weight gain than males. While the greatest extensor tibiae contraction times for male locusts were observed for 15% increases in weight, the female locusts displayed the greatest contraction time during a 25% increase in weight. In addition, the female locusts generally displayed a greater increase in contraction time when compared to male locusts with similar control condition contraction times. Although the male and female locusts display similar responses to gains in weight, females display a greater response over a larger weight range than males. This suggests that while jump modulation is conserved among both sexes, the added pressure of gravid-associated weight fluctuations leads to a larger response in females.

The extensor tibiae muscle activity is crucial for generating the energy storage needed to achieve a locust jump. Though this experiment investigated extensor tibiae contraction times, factors such as spike frequency or the number of motor neurons recruited in response to different weight conditions might also influence the energy stored in the LaMSA system. The bush cricket generates faster tibial extension velocity through an increase in the frequency of spikes received by their extensor tibiae muscle (Burrows & Morris, 2003). This increased activation of the extensor tibiae muscle is sufficient to promote an increase in the force produced by the muscle and in turn a faster, more powerful movement. Future analysis of the EMG data generated in this study should specifically investigate the relationship between weight gain and spike frequency in locusts. Additionally, the locust's extensor tibiae muscle is innervated by two excitatory motor neurons, and these two neurons innervate a differing number of muscle fibers (Hoyle, 1978). One

neuron innervates 76% of extensor muscle fibers, while the other neuron innervates roughly 8.5%; the remaining 15.5% of muscle fibers are innervated by both neurons (Hoyle, 1978). The recruitment of different motor neurons will produce different degrees of muscle activation, providing another potential mechanism by which the locust varies jump performance output. The largest weight gain condition may thus recruit additional motor neurons to increase the number of muscle fibers stimulated. If this is the case, it would explain the decrease in contraction time observed in all animals during the heaviest condition. This could be tested by adding spike sorting analyses to the current dataset to test how many units are active in each weight treatment. Ultimately, the contraction time seems to only partially support our hypothesis. Further analysis of the EMG recording data is required to test these additional proposed mechanisms.

Although both male and female locusts had similar responses to weight gain treatments, there were subtle differences between the sexes. First, a longer contraction time was observed for the small increase in weight condition for the female locust, with a shorter contraction time observed in the small increase in weight condition for the male locusts. There was, however, a notable difference between the resulting percent weight changes for the small weight gain conditions between the two locusts. More similar results might have been viewed if these resulting weight changes were more aligned. That being said, one of the male locust's large increases in weight condition resulted in a comparable percent change in weight to that of the small increase in weight condition for the female locust; a 20-25% increase in body mass. Interestingly, the female locust increased the contraction time of its extensor tibiae muscle at this weight condition. In contrast, the male locust's contraction time is comparable to its no change in weight condition. This could indicate that the ability of female locusts have a greater range of ability to respond to weight increases than males. Expanding the number of weight conditions to

include greater weight gains outside of the natural range would reveal the upper range of what is acceptable to maintain jump performance, both for the kinematic performance and the contraction time response.

One of the females used in the analysis of contraction times exhibited a contraction time response curve that was noticeably different from the other locusts investigated. For this female, the contraction time of their extensor tibiae muscle decreased minimally between the no weight change condition and the small increase in weight condition, and again a decrease in contraction time was observed between the small increase and the large increase in weight conditions. In addition, the contraction times leading up to the jumps of this locust were dramatically higher than that of the other locusts investigated. One possible explanation for this anomaly is that this female might have been fully gravid during the course of the experiment. If this locust was already experiencing the maximum range of a natural increase in its body mass due to gravity, the added artificial weight might have far surpassed its ability to compensate with a change in contraction time. Controlling for gravity could have provided an answer to these questions and should be considered for future research. Ultimately, time constraints permitted the analysis of contraction time for only two locusts of each sex, but a complete evaluation of the EMG data should establish firmer trends in contraction time given different natural starting weights.

This experiment is also limited by how weight was artificially adjusted for the locusts. *In vivo*, female locusts experience a gradual change in weight during egg mass development, which contrasts with the instantaneous weight changes that were induced in this experiment. Rather than investigating the effect of artificial weight gain on jump performance, it could be pertinent to study the change in performance over a complete cycle of egg development. Beginning with locusts just finishing their final instar, the natural development of egg mass would provide a

more accurate gradient of weight change and a gradual increase in weight that is more in line with *in vivo* development.

The findings of this study open the door for future research concerning the circuits between proprioceptive detection of weight gain and jumping motor performance. Sensory systems such as the lump receptor and campaniform sensilla are proposed to modulate jump performance, indicating that the same or similar mechanisms might exist for detecting weight change (Burrows & Morris, 2001; Burrows & Pflüger, 1988). The lump receptor comprises two multipolar neurons that are activated when the tibia is fully flexed and monitor the amount of force stored in the flexible jumping leg cuticles to ensure that the force stored does not irreversibly warp the cuticles (Burrows & Morris, 2001). The locust's campaniform sensilla, another type of receptor that responds to mechanical stress, provide additional feedback against resistance met when preparing for a jump (Burrows & Pflüger, 1988). Both sensory systems serve to inform the locust's jump and should be considered in further understanding how locusts detect and respond to weight change, building on the work exhibited here with extensor muscle contraction time.

The results of this study are informative to ongoing research in other organisms with LaMSA movements. For the locust, this study suggests that there is a conserved neuromechanical mechanism for maintaining an optimal angular velocity of their leg extension in response to artificial changes in weight. Given the range of organisms that use LaMSA movements, such a mechanism may exist beyond the locust lineage. Other invertebrates using LaMSA movement (such as crickets, mantis shrimp, and trap-jaw ants) may also control LaMSA output in response to different stimuli. Comparative studies of LaMSA neuromechanics may reveal some of the common algorithms for controlling ballistic movement. Such research also

potentially impacts the design of abiotic systems using LaMSA mechanics. In contrast to the electrochemical energy storage that takes place in systems like batteries, elastic systems can store energy in a simpler, less polluting manner with just as much reliability (Shiwei et al., 2022).

Evaluating LaMSA efficiency and mutability in invertebrate models improves our understanding of how both biotic and abiotic systems leverage the elastic storage of energy.

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