An insect-computer hybrid walking robot

Cao, Feng

2018

Cao, F. (2018). An insect-computer hybrid walking robot. Doctoral thesis, Nanyang Technological University, Singapore.

<http://hdl.handle.net/10356/74213>

<https://doi.org/10.32657/10356/74213>

NANYANG
TECHNOLOGICAL
UNIVERSITY

**AN INSECT-COMPUTER HYBRID WALKING
ROBOT**

CAO FENG

**SCHOOL OF MECHANICAL AND AEROSPACE
ENGINEERING**

2018

AN INSECT-COMPUTER HYBRID WALKING ROBOT

CAO FENG

School of Mechanical and Aerospace Engineering

A thesis submitted to the Nanyang Technological University in partial
fulfilment of the requirement for the degree of Doctor of Philosophy

2018

Abstract

This study presents the development of an insect-computer hybrid walking system. Anatomy of a beetle (*Mecynorrhina torquata*) was first done by the author to locate target muscles for motion control. Experiments were done by the author to confirm the magnitude, wave form, and frequency of the stimulation signal required to elicit desired leg motions. A micro biological actuator was built by the author by stimulating the leg muscles to control the corresponding leg motions. Power consumption of leg motion control via muscle stimulation was measured and proved to be very low (on the order of 100 μ W to a few milliwatts). Graded and closed-loop control of the leg motion magnitude was achieved by using a proportional controller. Sequential muscle stimulation protocol was developed by studying the natural walking gait of the beetle. Existing insect-computer hybrid robots in literature lack the control of walking gait, step frequency, and speed. By altering the stimulation sequences and adjusting the muscle stimulation durations in the control protocol developed in this study, the insect's walking gait, step frequency and walking speed became controllable by users. A wireless control "backpack" was developed by the author for the beetle and this enabled the insect-computer hybrid system to be remotely controlled by users. The contact mechanism between the beetle's leg and the walking substrate was investigated and the beetle's natural leg spines were found to be anisotropic that only increase the foot traction in forward walking. As such, I designed an isotropic artificial leg spine to enhance the walking performance in both forward and backward direction.

Acknowledgement

First of all, I would like to express my gratitude to my supervisor, Asst. Prof. Hirotaka Sato for his invaluable supports, encouragements and advices throughout the research.

I would like to thank all of my lab mates and friends, Mr. Huynh Ngoc Anh, Mr. Poon Kee Chun, Mr. Desmond Tan, Mr. Ferdinandus, Mr. Li Yao, Ms. Zhan Jing, and Dr. Vo Doan Tat Thang for their help and valuable discussions.

I would like to appreciate Mr. Chew Hock See, Mr. Tan Kiat Seng, Ms. Kerh Geok Hong, Wendy, and Mr. Edwin Lam for their help and support during my experiment.

I would like to express my thankfulness to my parents and my wife for their supports.

Contents

Abstract	i
Acknowledgement	ii
Figure list	vi
Table list.....	xviii
Abbreviation list.....	xix
Chapter 1: Introduction	1
1.1 Background of insect-computer hybrid robots.....	1
1.2 Objectives and scopes	3
Chapter 2: Literature review	6
2.1 Anatomy of insect legs.....	6
2.2 Motion control methods	8
2.2.1 Motion control of cockroach.....	9
2.2.2 Motion control of moth	12
2.2.3 Motion control of spider.....	14
2.2.4 Motion control of beetle.....	15
2.3 Walking gaits and leg motion control during walking	19
2.3.1 Insect walking gaits.....	19
2.3.2 Neuromuscular firing in insect walking	22
2.4 Sensors integrated onto insect-computer hybrid robot.....	24
2.5 Biomechanical properties of insect muscles	26
2.6 Evaluation of current research achievements.....	30
Chapter 3: Materials and methods.....	35
3.1 Study animal	35
3.2 Anatomy study	35
3.3 Electrode implantation into muscles	36
3.4 Natural muscle EMG recording	38
3.5 Motion capturing techniques.....	40
3.6 Printed circuit board for remote walking control	42

Chapter 4: A biological actuator	44
4.1 Graded single leg motion control.....	44
4.1.1 Insect anatomy	44
4.1.2 Threshold stimulation voltage to elicit significant leg motion.....	45
4.1.3 The leg motion amplitude can be graded by adjusting stimulation frequency.....	49
4.1.4 Comparison between leg motions elicited by electrical stimulation and leg motion elicited by intrinsic neural input.....	54
4.2 Closed-loop control of the leg's angular displacement.....	58
4.2.1 Techniques designed for closed-loop leg motion control	58
4.2.2 Experimental results of closed-loop leg motion control	60
4.3 Power consumption of the muscle stimulator	63
4.4 Repeatability of leg motion elicitation.....	65
4.4.1 Techniques used for repeatability test.....	66
4.4.2 Repeatability test results	66
Chapter 5: Insect-computer hybrid walking machine	71
5.1 Beetle's natural walking gait.....	71
5.1.1 Experimental setup for walking gait study.....	71
5.1.2 Beetle's walking gait study results.....	72
5.2 Design of sequential muscle stimulation protocol for walking control.....	77
5.2.1 Design walking control by sequential stimulation of leg muscles	77
5.2.2 Walking gait control by sequential stimulation of leg muscles.....	83
5.3 Walking speed and step length control	84
5.3.1 Experimental setup for walking speed and step length analysis	85
5.3.2 Experiment results of walking speed and step length analysis	87
5.4 Remote walking control	94
Chapter 6: Investigation and improvement of beetle leg spine functions in walking	98
6.1 Anisotropic function of beetle's natural leg spine	98
6.1.1 Beetle's natural leg spines prevent slipping in forward walking	98
6.1.2 Anisotropic leg spines resulted frequent slipping in backward walking	100
6.2 Implementation of artificial isotropic leg spine to enable both forward and backward walking.....	104

Chapter 7: Conclusion and future works.....	107
7.1 Conclusion	107
7.1.1 Graded and closed-loop control of a biological actuator	107
7.1.2 Insect-computer hybrid robot with user-adjustable walking gait and speed	108
7.1.3 New approach for biomechanical study	108
7.2 Future works	109
7.2.1 Control of all the six legs of the beetle.....	109
7.2.2 Building internal and external insect locomotion control models.....	110
7.2.3 Sensors to be integrated onto the backpack	112
7.2.4 Positioning system to be integrated onto the motion control architecture	113
List of publications.....	115
References.....	117

Figure list

- Figure 1. Six leg segments of a typical insect leg. The segments list from the most proximal to the most distal are the coxa, trochanter, femur, tibia, tarsus, and pretarsus. Figure preprinted from [33]. 6
- Figure 2. The extrinsic muscles inside the thorax control the protraction and retraction motions of the coxa of a typical insect leg. Figure preprinted from [33]. 7
- Figure 3. Intrinsic muscles connecting the coxa, trochanter, femure, tibia, tarsus, and pretarsus in a typical insect leg. Figure preprinted from [33]. 8
- Figure 4. Insect-computer hybrid robot developed by Tahmid Latif and Alper Bozkurk. (a) The stimulation backpack consists of a thin printed circuit board (PCB) mounted with a Texas Instrument's CC2530 microcontroller, a receiver for wireless communication, and miniature plugs connected with the electrodes for antenna stimulation and a 90mAh Li-Po battery. The weight of this backpack is only 500 mg. (b) the cockroach-computer hybrid robot. Figure preprinted from [25]. 9
- Figure 5. The cockroach antenna's anatomical parts, equivalent circuit diagram, and the stimulation wave forms for locomotion control. (a) The diagram of the three electrodes implantation setup for the antenna stimulation and the assessment of the voltage across the tissue-electrode interface. (b) The equivalent circuit of tissue-electrode interface. (c) The waveform of stimulation voltage V_{W-C} , the voltages between the reference and counter electrodes (V_{R1-C} and V_{R2-C}), and the voltage across the working electrode-tissue interface (V_{Rct1}). Figure preprinted from [25]. 11
- Figure 6. Implanted hawkmoth (*Manduca sexta*) in the DLMs and DVMs using fine silver wires coated with Teflon [19]. 13
- Figure 7. Electrical signals delivered to the left dorsal longitudinal muscle (LDVM) and the elicited wing motion angle. The stimulation signal (red square curves) was superimposed upon the elicited wing motion angles (blue curves). The stimulation

signal delivered into the LDVM has a magnitude of 5 V at a 1.9 s interval with 0.19 s pulse width. Figure preprinted from [19]..... 14

Figure 8. Experimental setup for spider locomotion control. Arduino based pulse generator system, the black electrode is the reference ground, and the other four are signal electrodes controlled by four push buttons respectively. Figure preprinted from [32]..... 15

Figure 9. Neuromuscular stimulation of beetles for flight initiation, cessation, and turning controls. (a) Wireless flight control devices attached on beetle *Cotinis texana* with stimulation electrodes implanted into the optic lobes and basalar muscles (placed near a quarter coin of the United States). (b) Wireless flight control devices (a custom-designed printed circuit board with microcontroller, antenna, battery, and stimulation electrodes) attached on *Mecynorrhina torquata*. Figure preprinted from [9]..... 16

Figure 10. Anatomy of the beetle brain for optic lobe stimulation. (a) Front and (b) tilted views of the *Cotinis texana* beetle's brain anatomy. The brain and optic lobe stimulation electrodes were implanted at site 1 and 2 respectively (blue crosses). The brain stimulation electrode was placed along the head's rostral–caudal midline (in the middle of the left and right compound eyes). The optic lobe stimulation electrode was implanted at the interior edge of each compound eye (site 2). Figure preprinted from [9]..... 17

Figure 11. Stimulation waveforms for the initiation and cessation control of *Mecynorrhina torquata* beetle in tethered flight. (a) Bipolar potential pulses (100 Hz, see (b) for the details of the waveform) applied between the left and right optic lobes initiated the wing oscillations and a single pulse ceased wing oscillations. Top blue curves represent the audio recording of the wing beating. (b) Pulse trains applied between the left and right optic lobes. Figure preprinted from [9]..... 18

Figure 12. Stimulation of the basalar muscle in beetle for turning control. (a) Implantation site 3 (the prothorax) was the counter electrode. The basalar muscle

stimulator was placed at site 4. (b) The basalar muscle stimulation site (site 4, blue cross) viewed in the cross-section of mesothorax. *Mecynorrhina torquata* beetle has nearly matching but scaled flight muscle structure to *Cotinis texana* beetle. Figure preprinted from [9]..... 19

Figure 13. The tripod walking gait. Thick lines indicate the duration of retraction with the foot on the ground, thin lines indicate the duration of protraction with foot in the air. L1 to L3 indicates the left front, middle, and hind leg respectively and R1 to R3 indicate the right front, middle, and hind leg respectively. The protraction time equals to the retraction time in this case. Figure preprinted from [33]. 20

Figure 14. Leg positions relative to the beetle's body in galloping gait. Solid and dotted lines indicate the positions of the front of the beetle's head and the end of the beetle's abdomen. The two front legs and the two middles legs on both sides of the beetle almost move in phase. The hind legs perform little motion relative to the beetle's body. Thick lines indicate the swing phase and thin lines indicate the stand phase. The step duration had been divided into five sections by two white strips and three gray strips. The front legs perform return and power strokes in the first three and last two sections respectively. While the middle legs perform power and return strokes in the first three and last two sections respectively. Figure preprinted from [49]..... 21

Figure 15. Duration of motoneuron activity of the middle leg motoneuron pools during free walking normalized to the period of the corresponding step. ProCx stands for protractor coxae muscles, controlling the protraction motion of the coxa (for the leg to swing forward). RetCx stands for retractor coxae muscles, controlling the retraction motion of the coxa (for the leg to swing backward). DprTr stands for depressor trochanteris, controlling the depression motion of the femur (for the leg to press down onto the ground). LevTr stands for levator trochanteris, controlling the levation motion of the femur (for the leg to lift up from the ground). Ext and flex stand for the extensor and flexor tibia muscle, controlling the extension and flexion of the tibia. DprT stands for depressor tarsi, LevT stands for levator tarsi. RetU stands for retractor unguis. TC,

CT, FT, and TT refer to the subcoxal, coxal trochanter, femur tibia, and the tibia tarsus joint respectively. Figure preprinted from [50]..... 23

Figure 16. Insect biobot with (a) three unidirectional microphones spaced 120° apart to form an omnidirectional microphone array for audio recording and streaming, (b) An unidirectional microphone to locate the audio source. Figure preprinted from [51]. 25

Figure 17. Shortening velocity (muscle lengths s^{-1} , MLs^{-1}) as a function of load during tetanic contraction. Values were obtained from release to isotonic contraction during the plateau of an isometric tetanus. The data are from five preparations, each of which is indicated by a separate symbol. Force values were expressed as a fraction of the maximum isometric force measured from each preparation. The curves are hyperbolae. The solid curve is the best-fitting hyperbola for all data points ($r=0.805$), the dotted curve is for points excluding those with force values greater than $0.78P_0$ ($r=0.812$). Figure preprinted from [60]. 27

Figure 18. The progression of muscle contraction force generated by electrically stimulating the axons of the innervating motoneurons of the extensor tibia muscle of a stick insect with frequencies ranging from 30 Hz to 200 Hz (positive current pulse train, pulse width = 0.5 ms, and amplitude = 0.00345 mA). The muscle resting force level is indicated by the dotted line. Figure preprinted from [61]. 28

Figure 19. Multiternimal innervation of insect muscle fibers. (A) Innervation of the tergo-trochanteral muscle of a blowfly (*Calliphora erythrocephala*) and (B) the metathoracic depressor tibiae muscle of a locust (*Locusta migratoria*). Photomicrographs of silver-stained preparations are illustrated (1) along with diagrammatic representations (2). Figure preprinted from [66]. 29

Figure 20. The beetle pictures before and after half of the upper prothoracic cuticle was removed. (a) Intact beetle before the anatomy was carried out. (b) Half of the cuticle on the upper side of the prothorax was removed with anatomical instruments and techniques described in section 3.2. The muscles connecting to the coxa of the left front

leg were exposed. (c) Muscles controlling the protraction and retraction motions of the coxa are easily identifiable.....36

Figure 21. Electrode implantation into a beetle's muscles. The protraction muscle and the retraction muscle (refer to Figure 20 for anatomy pictures) are implanted by two silver electrodes respectively for the purpose of both muscle stimulation and muscle signal recording. Blue circle indicates the implantation site for the protraction muscle and red circle indicates the implantation site for the retraction muscle.....38

Figure 22. Electrical circuit diagram for muscle EMG collection. The two electrodes were inserted into the target leg muscle. The other ends of the electrodes were connected to pin 2 and 3 of the LT1920 amplifier. The LT1920 amplifier was connected to a customized battery source through pins 4, 5, and 6. The amplified EMG signal was sent out through pins 5 and 6 of the amplifier to P0_2 and P0_3 of the CC2431 microcontroller for data collection and storage.40

Figure 23. Motion capturing system for biomechanical study. The 3D motion capturing system for tracking the leg and body motion of the beetle. (a1) six T40s VICON® cameras placed at the top of the arena. (a2) The VICON® server computer for data processing and storing. (a3) A personal computer for data analysis.41

Figure 24. Backpack for remote walking control and the electrical circuit diagram. (a 1-3) Top, bottom, and top with battery view of the backpack. The CC2530 microcontroller was preprogrammed to send muscle stimulation signals to sequentially elicit leg motions. (b) The electrical diagram of the custom designed PCB. J1, J2, and J3 corresponding to the three females connectors shown in (a1). Sixteen resistors were used as voltage dividers of the eight stimulation channels for leg motion control.43

Figure 25. Anatomy of the beetle's front leg revealed the muscle groups responsible for the corresponding leg motions. Red crosses indication the positions for inserting stimulation electrodes [8].....45

Figure 26. Experiment setup for leg motion capturing. (a) Markers with diameter of 3 mm were placed on the beetle's front leg (markers 1 and 2) and the body (marker 3) for motion capturing purpose. (b) The 3D motion capturing system recognized the markers as point objects and displayed on a computer screen. The two markers on the leg were recognized as a line segment. (c) 3D motion capturing system with six T40s VICON cameras (C1). The camera frame rate was set at 100 Hz. The resolution of each camera is 4 megapixels (2336×1728). (C2) A VICON server was used for camera signal collection, processing, and display. (C3) A computer is used to calculate the leg motion angular displacements with custom-written Matlab® code [8]. 47

Figure 27. Maximum protraction and retraction angular displacements elicited at stimulation voltage ranged from 0.25 V to 2.5 V (number of beetles = 5, $17 \leq$ number of data points at each stimulation voltage for each motion type ≤ 22). Blue lines and red lines indicate the protraction and retraction maximum angular displacement respectively. The dotted lines show the average angular displacement of each beetle. The solid lines indicate the average values with error bars indicate the standard deviation calculated from the total number of data points (five beetles). A pulse-width-modulation wave of frequency equals to 30 Hz and pulse width equal to 1 ms was used as the stimulation signal. The maximum angular displacement for both the protraction and retraction motions increased with the stimulation voltage until approximately 1.5 V [8]. 49

Figure 28. Change of maximum angular displacement with stimulation frequency for all the six motions of the front leg. The colored dotted lines indicate that five beetles were used for obtaining the motion data for one motion type. The black lines indicate the average maximum angular displacement of five beetles with standard deviations indicated by the black error bars (number of beetles = 5, number of data points at each stimulation frequency for each motion type = 25). The stimulation frequency was increased from 20 Hz to 100 Hz at the increment of 20 Hz each time [8]. The black dotted in lines (a-1) and (a-2) indicate the voltage sweep data at 1.5 V, 30 Hz, and 1ms

PWM stimulation input (stimulation signal used to confirm the threshold voltage in Section 4.1.2).51

Figure 29. Average angular velocity (degree/s) monotonically increased with the stimulation frequency. The stimulation frequency ranged from 20 Hz to 300 Hz with step increment of 20 Hz when the stimulation is less than 100 Hz and with step increment of 50 Hz when the stimulation frequency is greater than 100 Hz. The colored dotted lines indicate different beetles used in the experiment (number of beetles = 5, number of data point at each stimulation frequency = 25 for each motion type). The thick black line indicated the mean of the average angular velocity from 25 data point at each stimulation frequency. The black error bars indicate the standard deviations of the average angular velocity [8].53

Figure 30. Retraction muscle group EMG signal as a function of time synchronized with the captured leg motion. (a) Retraction muscle group EMG signal collected. (b) The protraction and retraction motions of the beetle's front leg. The retraction motion was indicated as a decrease in the leg's angular displacement [8].55

Figure 31. Average retraction angular velocity as functions of the average muscle EMG frequency and the electrical stimulation signal frequency. (a) A linear relationship existed between the average angular velocity and the average EMG frequency of the muscle ($R^2 = 0.76$, number of beetles =4, total number of data points = 43). (b) The average angular velocity was directly proportional to the input stimulation frequency ($R^2 = 0.75$, number of beetles = 5, number of data point at each stimulation frequency = 25). The least-squares linear regression lines were drawn in black in both graphs. Error bars in (b) indicates the standard deviation [8].57

Figure 32. Schematic representation of the closed-loop control system. The error is the difference between the predefined leg angular position and the actual leg angular position. A proportional control algorithm is embedded into the controller [8].59

Figure 33. Typical closed-loop control of protraction/retraction motion of the beetle's front leg at K_p values equal to 0.1, 0.5, 1.0, and 1.5 and update time intervals equal to 100 ms, 200 ms, and 300 ms. The predetermined angular displacements are ± 10 degrees (positive value for retraction motion and negative value for protraction motion) [8]..... 61

Figure 34. Closed-loop leg motion control system performance evaluation in terms of overshoot angle and reaching time. (a) the overshoot angle and (b) the reaching time of the front leg's protraction and retraction motion at various K_p and system update time settings. Each color of the bar indicates a particular experiment setting. Error bars indicate the standard deviations [8]. 63

Figure 35. Simulation signal and current flow through the muscle. (a) PWM wave of 1.5 V amplitude, 100 Hz frequency, and 1 ms pulse-width was used as the stimulation signal. (b) A typical current profile that passed through the depression leg muscle group of a beetle's front leg [8]..... 64

Figure 36. Repeatability test: cycles of leg motion induced by the electrical stimulation of the retraction and protraction muscles. A leg's motion (over a duration of 200 s) tracked in the repeatability test. Custom-developed Matlab® code was used to automatically detect the angular positions of extreme retraction (green circle) and extreme protraction (red circle) with respect to the maximum protraction position reached during the 30 min stimulation (angular position = 0 degree). The beetle's leg keeps moving between the extreme retraction and protraction positions every second due to the applied electrical stimulation. The difference between adjacent retraction and protraction positions is the leg's angular displacement. 68

Figure 37. Repeatability test: The average and standard deviation of four beetle's front leg angular displacement ($N = 4$ beetles, $n = 1800$ data points). Every beetle was stimulated for more than thirty minutes per day for seven consecutive days. The four different colors of the columns indicate the four tested beetles. The standard deviations are indicated by the black bars. The mean and standard deviation of the leg's angular

displacement of each beetle in each day were calculated from more than 1800 data points (refer to Figure 36 for the data points allocation method) resulted from the more than 30 minutes stimulation (as the beetles' protraction and retraction muscles were stimulated alternatively for 1 second).....69

Figure 38. Motion capturing system for walking gait study. (a) The 3D motion capturing system for tracking the leg and body motion of the beetle. (a1) six T40s VICON® cameras placed at the top of the arena. (a2) The VICON® server computer for data processing and storing. (a3) A personal computer for data analysis. (b) Markers L1, L2, R1, R2, B1, and B2 attached to walking beetle (c) The 3D marker positions captured on screen [75].....72

Figure 39. Stick diagram for walking gait analysis. The stick diagram illustrates the lateral view of the motion trajectory of the tibia segment of the beetle's front leg. The time span between two consecutive sticks equals to 10 ms. The horizontal axis indicates the distance travelled by the tibia segment in the beetle's walking direction. The vertical axis indicates the vertical distance travelled by the tibia segment of the beetle's front leg [75].....74

Figure 40. Time intervals of the four motions normalized to the corresponding step duration (N = 5 beetles, n = 25 steps). The four motion types (protraction, retraction, levation and depression) were performed at different timings to generate the cyclic power and return strokes during voluntary walking. During protraction and retraction, the whole front leg (coxa, trochanter, femur, tibia, and tarsus) move simultaneously due to the rotation of the coxa. During levation and depression, the rotation of the coxa-trochanter joint moves the femur, tibia, and tarsus together. First, retraction and depression (red and green bars, respectively) were executed concurrently during the power stroke (comprising the first 61±22% of a complete walking step). The percentage standard deviation is indicated by the black error bars. During the following return stroke, protraction (blue bar) was executed throughout, whereas levation (orange

bar) was switched to depression (purple bar) at 78+15% of the normalized step duration [75]. 76

Figure 41. Insect platform for legged robot with its front leg anatomy. (a) Beetle's front leg anatomy. The protraction and retraction muscles (inside the prothorax) enabling the leg to swing forward and backward about the thorax–coxa joint. (b) The levation and depression muscles (inside the coxa) enable levation and depression motions of the femur about the coxa-trochanter joint. Red crosses mark the sites of stimulation electrodes implanted into each muscle. 78

Figure 42. The circuit drawing for the insect-computer hybrid robot. (a) The CC2530 microcontroller with the external circuit for one muscle group stimulation. Eight identical external circuits were controlled separately by Channel 1 to 8 for stimulation of the eight muscle groups inside the beetle's two front legs. (b) The beetle with sixteen stimulation electrodes (two electrodes in one muscle group controlled by one stimulation channel) implanted into eight muscles groups (protraction, retraction, levation, and depression muscle groups in both front legs). Stimulation Channels 1 to 8 generate stimulation signals in predefined sequence for the walking control of the beetle [75]. 80

Figure 43. Sequential leg motion control demonstration for both the tripod walking gait and galloping walking gait. Videos were shot from the beetle's ventral view for clear view of resultant leg motions when predefined stimulation sequence shown in Table 2 was applied. LED lights near the beetle's head indicate the on and off status of the corresponding stimulation channel [75]. 84

Figure 44. Experiment setup for walking speed versus step frequency analysis. (a) Instantaneous image of an insect-computer hybrid robot walking at galloping walking gaits with both front legs at their first AEPs (b) The insect-computer hybrid robot walked one step with both front legs at their second AEPs. The pixel coordinates of the articulation connecting the beetle's left leg tibia and tarsus were indicated by the red crosses for step length calculation. 86

Figure 45. Average normalized walking speed as a function of step frequency (N = 6 beetles, n = 90 data points). In total, ninety data points were obtained for the walking speed study (nine data points for one step frequency in one walking gait); the black bars indicate the standard deviation of the normalized walking speed. The walking speeds obtained from six beetles were normalized to their respective body lengths. The blue and red numbers indicate the percentage change of the average normalized walking speed when the step frequency was doubled from the previous value. 88

Figure 46. Insect-computer hybrid robot's average normalized step length vs step frequency (N = 6 beetles, n = 150 data points). The blue and red numbers indicate the percentage change of the average normalized step length when the step frequency was doubled from the previous value..... 89

Figure 47. Normalized step length as a function of the stance duration. The average and standard deviation of the normalized step lengths were calculated from 150 step length values of 3 beetles. The red number between two adjacent bars shows the percentage change in the average value. The value in brackets below each stance duration is the corresponding step frequency. The average normalized step length remained almost constant (+1%) when the stance duration was increased from 549 ms to 1098 ms..... 93

Figure 48. Normalized walking speed as a function of the stance duration. The average and standard deviation of the normalized walking speeds were calculated from 15 walking speed values from 3 beetles. Each walking speed value was calculated from the distance travelled by the insect-computer hybrid robot in five continuous steps. The red number between two adjacent bars shows the percentage change in the average value. The value in brackets below each stance duration is the corresponding step frequency. The change in the average normalized walking speed (-36%) was almost the same as the change in the step frequency (-35% from 0.95 Hz to 0.62 Hz) when the stance duration was increased from 549 ms to 1098 ms..... 94

Figure 49. Backpack and insect-computer hybrid robot. (a 1-3) Top, bottom, and top with battery view of the backpack. The CC2530 microcontroller was preprogrammed to

send muscle stimulation signals to sequentially elicit leg motions. (b) Fully assembled insect-computer hybrid robot, sixteen electrodes enabled control signals to be sent to eight leg muscles. *Mecynorrhina torquata* is used as the insect platform. 97

Figure 50. Natural leg spines before and after cutting. (a) Overview of the beetle. (b) The beetle's front leg spines (inside the red circle) are curved towards the posterior direction. The spines' curvature created significant traction for the power stroke in forward walking (blue arrow) but reduced the traction for the power stroke in backward walking (orange arrow). (c) The leg spines are removed by cutting (inside the red circle). 99

Figure 51. Tibia-tarsus trajectories before and after the removal of leg spines. Blue lines are the tibia-tarsus trajectories traced under a 3D motion capturing system. (a) No slip was observed in forward walking power stroke before the leg spines were cut. (b) Obvious slipping (indicated by the red dotted arrows) occurred after the leg spines were cut. This proved that the natural leg spines provide significant traction in the forward walking power stroke. 100

Figure 52. Body trajectory before and after adding the artificial leg spines. (a) A typical body trajectory of backward walking before the addition of artificial isotropic leg spines. (b) A typical body trajectory of backward walking after the addition of artificial isotropic leg spines. The body trajectory became more linear after the addition of artificial leg spines (red dotted lines represent the linear regression lines of the body trajectories). This proved that the artificial isotropic leg spines significantly improved the leg traction in backward walking. 103

Figure 53. Artificial leg spine added to the leg. (a) A segment of insect pin (#3, Indigo Instruments) was used as the artificial leg spine. By being straight (not curved in any direction and hence isotropic), the artificial leg spine provides a passive mechanism that provides traction for power strokes in the both forward and backward directions. (b) Side view of the artificial spine on leg. The artificial leg spine was about 45° with respect to the tip of the natural leg spine. 105

Table list

Table 1. Mean and standard deviation of power consumption (μW) for all the six muscle groups of the beetle's front leg across five beetles.....	65
Table 2. Stimulation sequences of the beetles' walking control in tripod and galloping gaits. Filled and empty dots indicate that the stimulation channel is switched on and off during the motion, respectively [75]......	82
Table 3. Stimulation sequences for forward and backward walking control in galloping walking gait.....	102

Abbreviation list

Abbreviation	Description
3D	Three-dimensional
ANOVA	Analysis of variance
AEP	Anterior extreme position
CO₂	Carbon dioxide
CSV	Comma-separated values
CT	Coxal trochanter
DC	Direct current
DLM	Dorsal longitudinal muscle
DVM	Dorsal ventral muscles
DprT	Depressor tarsi
DprTr	Depressor trochanteris
EMG	Electromyography
Ext	Extensor tibia muscle
Flex	Flexor tibia muscle
FT	Femur tibia
GND	Ground
GPS	Global positioning system
IMU	Inertial measurement unit
I/O	Input/output
K_p	Proportional gain
LED	Light emitting diode
LevT	Levator tarsi
LevTr	Levator trochanteris
MEMS	Micro electro mechanical systems
PCB	Printed circuit board

PEP	Posterior extreme position
Ph.D.	Doctor of Philosophy
PWM	Pulse-width modulation
RetU	Retractor unguis
TC	Subcoxal
TVOC	Total volatile organic compounds
TT	Tibia tarsus

Chapter 1: Introduction

1.1 Background of insect-computer hybrid robots

Engineers attempted to design better hexapod robots by learning from the natural movements of insects [1-5]. The passion of research in hexapod robot is largely driven by the fact that legged robots can move across uneven surfaces and climb over obstacles more effectively than wheeled robots [6, 7]. However, despite promising progress in the area of hexapod robot research, even the state-of-art manmade hexapod robots have undeniable limitations in terms of manufacturability, cost, agility, power efficiency, and control algorithms compared to natural insects.

As an alternative way to overcome the current technical difficulties in the hexapod robot research, scientists and engineers came out with the idea of insect-computer hybrid robot. There are major engineering and scientific merits to develop insect-computer hybrid robots.

First, insect-computer hybrid robots can be manufactured at low cost and short lead time. Unlike manmade robots where many small sensors and actuators need to be manufactured, the platform of the insect-computer hybrid robot (mechanism, kinematic structure and actuators) is nature's ready-made live insect itself. Therefore, the supply of the robot platform is essentially unlimited (through the medium of insect reproduction) and incurs only the cost of food supply (fresh sugar jelly) and rearing places for the insects.

The second advantage of insect–computer hybrid robots over man-made robots is their low power consumption (a few milliwatts [8, 9]). By comparison, man-made miniature robots of similar size consume a few hundred milliwatts [10]. Moreover, thanks to advanced biofuel cell technology, the insect–computer hybrid robot can be self-powered by energy harvesters embedded in the living insect platform [11-16].

Third, the insect–computer hybrid robot neither requires complex structural design nor complicated locomotion control algorithms. In man-made robotics research, the kinematic structure design to realize a robust miniature robot is highly intricate. Furthermore, the complicated control algorithms for traversing complex terrain or maintaining posture have caused various challenges. However, the insect–computer hybrid robot has developed its own intricate kinematic structure (the motor neuron network and locomotive appendages) through millions of years of natural evolution. The insect is capable of maintaining its posture in the absence of any control input, negating the need for complicated control algorithms.

Fourth, study on electrical stimulation to elicit appendage motions contributes not only to the development of insect-computer hybrid robots but also to biomechanical research as a new method to explore animal locomotion. For example, a cockroach’s step frequency ranges from 3.7 to 13.5 Hz in its voluntary walking [3]. Implementation of electrical stimulation for motion control would be used to test the hypothesis if the cockroach’s step frequency limits are the physical limit of animals’ neuromuscular systems or it is possible to achieve higher step frequency by electrically eliciting the leg motions. Furthermore, insect neuromuscular stimulation research can reveal functions and roles of muscles which are not certainly determined by traditional anatomical study.

For instance, recent study of a small flight muscle, known as the third axillary muscle or wing folding muscle which had been traditionally understood to solely have the wing folding function in Coleopteran, was revealed, by using muscle stimulation, to have the left-right turning functions in flight [17].

Research in insect-computer hybrid robot had progressed significantly in the past few decades. The insect platforms used for building an insect-computer hybrid robot include moth (*Manduca sexta* and *Agrius convolvuli*) [18-24], cockroach (*Periplaneta Americana* and *Gromphadorhina portentosa*) [25-31], spider (*Heteropoda venatoria*) [32], and beetle (*Mecynorrhina torquata*) [9, 17]. By electrically stimulating the antenna of the moth, researchers were able to control the flight direction. Researchers stimulated the antenna of the cockroach and the leg ganglion of the spider to steer the walking direction. Flight initiation and cessation of the beetle was accomplished by stimulating the optic lobe. Moreover, beetle flight turning control was achieved by stimulating the flight muscles.

1.2 Objectives and scopes

The main objective of my research is to construct an autonomous insect-computer hybrid robot. When under user control, the elicitation of the desired appendage motion and overall locomotion should be reliable (aim to achieve 100% success rate in motion control) and precise. Moreover, the control signals send to the target neuromuscular sites should not cause any potential damage to the biological tissue. As such, when the control signal is shut off, the insect should be able to use its intrinsic locomotion control system to navigate itself. Finally sensors should be integrated onto the control

board of the insect-computer hybrid robot and the environment information can be sent back to users through wireless communication. According to the main objective of my research and based on the limitations in the current insect-computer hybrid robot, three main scopes for my Doctor of Philosophy (Ph.D.) research topic were confirmed:

- The first scope would be to achieve precise appendage motion magnitude control to build a biological micro actuator. Precise appendage motion magnitude control would enable us to accurately monitor the magnitude of the walking step of the insect-computer hybrid robot. Moreover, the power consumption and the fatigue characteristics of the biological actuator will also be analyzed.
- Secondly, the development of walking control hardware and software to control both the walking gait and speed of the insect will be done. The initial walking control is expected to be implemented on a tethered beetle.
- Thirdly, after succeeding in reliable and accurate walking control of the tethered insect; wireless control hardware and software would be developed for remote motion control. In wireless control experiments, innovative walking gaits (e.g. backward walking, turning control etc.) control protocols would be developed to further improve the applicability of the robot.

Among the commonly used insect platforms for building hybrid robots (cockroaches, spiders, moths, and beetles), beetles displayed advantages in terms of payload capacity when flying (compared to moths) and robustness (relatively stronger cuticle compared to cockroaches and spiders). For example, when moth (payload capacity is

about 1 g or 67% body weight) was used as the platform for building hybrid robot, a balloon was needed to provide additional lift as the moth itself cannot carry the heavy payload (remote communication and neuron stimulation devices) [20]. Attachment of the balloon reduced the applicability of the hybrid robot as it will not be able to pass through spaces that are narrower than the diameter of the attached balloon. On the other hand, the beetles used in this study weight 7 to 8 g on average so that a payload of 3 to 4 g could be carried by the beetle in real application. As such, the beetle (*Mecynorrhina torquata*) was chosen as the insect platform in this research.

Muscular stimulation was used instead of neural stimulation as the locomotion elicitation method. This was because, compared to neurons, muscles are relatively easily identified by a conventional laboratory microscope. The relatively large size of the muscles eased the electrode implantation. Small drifts in the position of the implanted electrodes would not affect the elicitation of the target appendage motion. On the other hand, stimulation of neurons can generally steer the locomotion of an insect (e.g. motion initiation, cessation, and left and right turnings). However, due to the higher hierarchical position of neuron clusters (i.e. brain, ganglia etc.) compared to muscles in locomotion control, stimulation of muscle groups provides a more direct method that makes it possible to control the appendage motion magnitude and frequency and hence to control the locomotion speed of an insect.

Chapter 2: Literature review

2.1 Anatomy of insect legs

Knowing a typical insect's leg structure and the leg muscle arrangement is crucial for leg motion control by electrical stimulation of leg muscles. A typical insect leg consists of six segments namely the coxa, trochanter, femur, tibia, tarsus, and pretarsus. The segments are connected to each other by either mono- or dicondylic articulations. Figure 1 shows the six basic leg segments of a typical insect leg.

Figure 1. Six leg segments of a typical insect leg. The segments list from the most proximal to the most distal are the coxa, trochanter, femur, tibia, tarsus, and pretarsus. Figure preprinted from [33].

The leg muscle structure and arrangement need to be well studied so that precise electrical stimulation locations can be defined during the leg motion control. Basically, the insect's leg muscle can be classified into two main categories, namely the extrinsic

and intrinsic muscles. The extrinsic muscles arise from the external region of the leg, while the intrinsic muscles are completely located inside the leg and connecting the leg segments from one to the other [33]. The contraction and relaxation of the extrinsic muscles inside the thorax control the protraction and retraction motion (forward and backward swing) of the coxa (Figure 2).

Figure 2. The extrinsic muscles inside the thorax control the protraction and retraction motions of the coxa of a typical insect leg. Figure preprinted from [33].

Apart from the coxa, the other five leg segments (trochanter, femur, tibia, tarsus and pretarsus) are connected by intrinsic leg muscles, except that the trochanter depressor muscle is extrinsic (Figure 3).

Figure 3. Intrinsic muscles connecting the coxa, trochanter, femure, tibia, tarsus, and pretarsus in a typical insect leg. Figure preprinted from [33].

2.2 Motion control methods

Neuron stimulation and muscle stimulation are the two most common methods used in the locomotion control of an animal-computer hybrid robot [9, 17-32, 34-44]. The following sections describe the locomotion control protocols used in various animals.

2.2.1 Motion control of cockroach

R. Holzer and I. Shimoyama [28], Tahmid Latif and Alper Bozkurk [25] stimulated the antenna of two different species of cockroach to control the cockroach's walking direction. R. Holzer and I. Shimoyama used the *Periplaneta Americana* as the insect platform, while Tahmid Latif and Alper Bozkurk used the *Gromphadorhina portentosa* as the insect platform. Figure 4 below shows the assembly of the insect-computer hybrid robot developed by Tahmid Latif and Alper Bozkurk.

Figure 4. Insect-computer hybrid robot developed by Tahmid Latif and Alper Bozkurk. (a) The stimulation backpack consists of a thin printed circuit board (PCB) mounted with a Texas Instrument's CC2530 microcontroller, a receiver for wireless communication, and miniature plugs connected with the electrodes for antenna stimulation and a 90mAh Li-Po battery. The weight of this backpack is only 500 mg. (b) the cockroach-computer hybrid robot. Figure preprinted from [25].

The cockroach's two antennae have multifunctional sensing capabilities that are able to sense temperature, smell (olfactory), tactile, and humidity changes [25]. When come across an obstacle or a predator, the flagellum of the antenna is able to transmit signals to the cockroach's nervous system and escaping behavior (in terms of turning and running away from the danger site) is elicited [25, 45]. Both R. Holzer and I. Shimoyama and Tahmid Latif and Alper Bozkurk utilized the well-known fact that electrical stimulation is able to control excitable tissues such as muscles and nerves as their fundamental theory for the control of the locomotion of the cockroaches. Custom printed circuit board (PCB) with preprogrammed microcontroller was used as the backpack to generate stimulation signals. Tahmid Latif and Alper Bozkurk used 3 V direct current (DC) pulses as their stimulation signals, while in R. Holzer and I. Shimoyama's experiment, rectangular pulses with 50% duty cycle (pulse width varies from 0.1 ms to 10 ms) were used as the stimulation signal. The current across the excitable tissue is about 10 to 100 μA . Figure 5 below shows the implantation, equivalent circuit, and the voltages implanted by Tahmid Latif and Alper Bozkur in the cockroach locomotion control experiment [25].

Figure 5. The cockroach antenna's anatomical parts, equivalent circuit diagram, and the stimulation wave forms for locomotion control. (a) The diagram of the three electrodes implantation setup for the antenna stimulation and the assessment of the voltage across the tissue-electrode interface. (b) The equivalent circuit of tissue-electrode interface. (c) The waveform of stimulation voltage V_{W-C} , the voltages between the reference and counter electrodes (V_{R1-C} and V_{R2-C}), and the voltage across the working electrode-tissue interface (V_{Ret1}). Figure preprinted from [25].

Turning control by antennal stimulation was achieved in both R. Holzer and I. Shimoyama and Tahmid Latif and Alper Bozkurk's experiments. However, according to R. Holzer and I. Shimoyama, prolonged stimulation time lead to declined turning reaction, the most favorable stimulation duration is only about 100 to 200 ms [28].

Moreover, the method of antenna stimulation made it impossible to control the appendage motion magnitude, the walking gait, nor the speed of the cockroaches. Better control techniques need to be developed to overcome the current technical obstacles in insect-computer hybrid robot locomotion control.

2.2.2 Motion control of moth

To reproduce the wing flapping motion of the hawkmoth, *Manduca sexta*, Tubbs et al. artificially stimulated the dorsal longitudinal muscles (DLMs) and the dorsal ventral muscles (DVMs) using electrical signals [19] (refer to Figure 7 below for details of the stimulation signals). The contraction of the DVMs causes the wings to move upward, while the downward movement of the wings is indirectly elicited when the DLMs contract. Note that the DLMs and the DVMs are indirect muscles for the wing flapping. The tergum of the moth is pulled down by the contraction of the DVMs which causes the upward stroke of the wings. When the DLMs contract, the center of the tergum bows upward that causes the wings to move downward [19]. The tethered hawkmoth with silver wires implanted in the DLMs and DVMs is shown in Figure 6 below. Both the flight muscles' natural electromyographical (EMG) signals and pulse width modulation (PWM) waves were used as the muscle stimulation signal. Obvious wing flapping motions were elicited when the DLMs and DVMs were stimulated; however, the amplitude of the stimulated wing motion is smaller compared to the amplitude of natural wing movement. As such, actual flight control of the hawkmoth had not been achieved yet. Elicitation of the insect's appendages to their maximum achievable

amplitude is thus an essential mile stone for building a reliable insect-computer hybrid robot.

Figure 6. Implanted hawkmoth (*Manduca sexta*) in the DLMs and DVMs using fine silver wires coated with Teflon [19].

Figure 7. Electrical signals delivered to the left dorsal longitudinal muscle (LDVM) and the elicited wing motion angle. The stimulation signal (red square curves) was superimposed upon the elicited wing motion angles (blue curves). The stimulation signal delivered into the LDVM has a magnitude of 5 V at a 1.9 s interval with 0.19 s pulse width. Figure preprinted from [19].

2.2.3 Motion control of spider

Yang Z.L. et al. stimulated the leg ganglia of a spider (*Heteropoda venatoria*) for the left and right steering control [32]. An Arduino Uno board was programmed to generate the electrical stimulation signals (Figure 8). Involuntary spasms in a leg of the spider were induced by electrical stimulation to steer the spider to the contralateral direction. The electrical stimulation signal parameters of 2 ms pulse duration, 100 ms stimulation duration, $80 \pm 20 \mu\text{A}$ stimulus amplitude, and 5 V input voltage were found to be the optimal in left-right steering control. The problem of habituation to electrical

stimulus caused the success rate of a V-shaped path following control to be only 50%. Furthermore, the elicited turning motion appeared to be erratic revealing the stimulation protocol is not adequately robust.

Figure 8. Experimental setup for spider locomotion control. Arduino based pulse generator system, the black electrode is the reference ground, and the other four are signal electrodes controlled by four push buttons respectively. Figure preprinted from [32].

2.2.4 Motion control of beetle

Remote motion control (flight initiation, cessation, and turning) of beetles (*Cotinis texana* and *Mecynorrhina torquata*) was demonstrated by Sato et al. (Figure 9 below) [9].

Figure 9. Neuromuscular stimulation of beetles for flight initiation, cessation, and turning controls. (a) Wireless flight control devices attached on beetle *Cotinis texana* with stimulation electrodes implanted into the optic lobes and basalar muscles (placed near a quarter coin of the United States). (b) Wireless flight control devices (a custom-designed printed circuit board with microcontroller, antenna, battery, and stimulation electrodes) attached on *Mecynorrhina torquata*. Figure preprinted from [9].

The flight initiation and cessation control of the beetles were achieved by stimulating the optic lobe (Figure 10 below); the turning control was achieved by basalar muscles

stimulation. By applying alternating positive and negative stimulation pulses to the optic lobe of the *Cotinis texana* beetle, the flight initiation motion was induced at a success rate of 56% ($N = 9$ beetles). The threshold voltage for flight initiation was found to be 3.2 V. The beetles were found to start flying either during or immediately after the negative stimulation pulse. Similar stimulation protocol was applied to *Mecynorrhina torquata* beetle for flight initiation (Figure 11). All the ten tested beetles initiated flight when the stimulation signals were applied to the optic lobe (100% success rate). The median time taken for the beetles to start flying after receiving the first stimulation signal was found to be 0.5 s. No significant difference was found in flight initiation by applying stimulation voltages between 2 V to 4 V.

Figure 10. Anatomy of the beetle brain for optic lobe stimulation. (a) Front and (b) tilted views of the *Cotinis texana* beetle's brain anatomy. The brain and optic lobe stimulation electrodes were implanted at site 1 and 2 respectively (blue crosses). The brain stimulation electrode was placed along the head's rostral–caudal midline (in the middle of the left and right compound eyes). The optic lobe stimulation electrode was implanted at the interior edge of each compound eye (site 2). Figure preprinted from [9].

Figure 11. Stimulation waveforms for the initiation and cessation control of *Mecynorrhina torquata* beetle in tethered flight. (a) Bipolar potential pulses (100 Hz, see (b) for the details of the waveform) applied between the left and right optic lobes initiated the wing oscillations and a single pulse ceased wing oscillations. Top blue curves represent the audio recording of the wing beating. (b) Pulse trains applied between the left and right optic lobes. Figure preprinted from [9].

In turning control, the success rates of left and right turn elicitation were 74% (38 *Cotinis texana* beetle) and 75% (52 *Mecynorrhina torquata* beetles). Flight turning was elicited by stimulating the contralateral basalar muscle (Figure 12 below) for 0.5 s by using 1.3 V, 100 Hz positive pulse trains for *Cotinis texana* beetle and 2 V, 100 Hz positive pulse trains for *Mecynorrhina torquata* beetle.

Figure 12. Stimulation of the basalar muscle in beetle for turning control.

(a) Implantation site 3 (the prothorax) was the counter electrode. The basalar muscle stimulator was placed at site 4. (b) The basalar muscle stimulation site (site 4, blue cross) viewed in the cross-section of mesothorax. *Mecynorrhina torquata* beetle has nearly matching but scaled flight muscle structure to *Cotinis texana* beetle. Figure preprinted from [9].

2.3 Walking gaits and leg motion control during walking

2.3.1 Insect walking gaits

The primary function of the legs of most insects is terrestrial locomotion (i.e. walking and running). A walking step is usually divided into two phases, namely the stance phase (when the leg perform power stroke) and the swing phase (when the leg performs return stroke) [33]. The stance phase is the duration in which the foot is pressed down onto the ground and the leg is swung backward with respect to the body so that it pushes the body forward. During the swing phase, the foot is lifted up from the ground and protracted forward to get the leg ready for the next power stroke. The leg position at the start of a power stroke is denoted as the anterior extreme position (AEP), while the leg position at the end of a power stroke is denoted as the posterior extreme position

(PEP). The AEP and PEP are crucial points for maintaining the coordination of the legs [46, 47]. Various alternating tripod walking gaits are adopted by around three million species of insects [48], that the insect's body is always supported by three legs: middle leg on one side and the front and hind legs on the other side of the body (Figure 13).

Figure 13. The tripod walking gait. Thick lines indicate the duration of retraction with the foot on the ground, thin lines indicate the duration of protraction with foot in the air. L1 to L3 indicates the left front, middle, and hind leg respectively and R1 to R3 indicate the right front, middle, and hind leg respectively. The protraction time equals to the retraction time in this case. Figure preprinted from [33].

A new galloping walking gait was recently found in a wingless dung beetle (Pachysoma) [49]. In the galloping gait, the beetle's two front legs and two middle legs move in phase to perform power stroke and return stroke at the same time. Figure 14

below shows the leg positions relative to the beetle's body when it moves in the galloping gait.

Figure 14. Leg positions relative to the beetle's body in galloping gait. Solid and dotted lines indicate the positions of the front of the beetle's head and the end of the beetle's abdomen. The two front legs and the two middle legs on both sides of the beetle almost move in phase. The hind legs perform little motion relative to the beetle's body. Thick lines indicate the swing phase and thin lines indicate the stand phase. The step duration had been divided into five sections by two white strips and three gray strips. The front legs perform return and power strokes in the first three and last two sections respectively. While the middle legs perform power and return strokes in the first three and last two sections respectively. Figure preprinted from [49].

2.3.2 Neuromuscular firing in insect walking

From the leg motion coordination point of view, the six legs of an insect are coordinated in different temporal sequence or walking gaits. The various walking gaits are controlled by coordinating mechanisms that coordinate segmental neural networks for walking pattern generation [50]. H. Fischer et al. investigated the activity patterns of the leg muscles and motoneurons controlling the coxa-trochanteral joint, the femur-tibia joint, and the tarsal leg joints of the middle leg of a stick insect (*Cuniculina impigra* Redthenbacher). Figure 15 below shows the muscle activities of the middle leg of free walking stick insects [50].

Figure 15. Duration of motoneuron activity of the middle leg motoneuron pools during free walking normalized to the period of the corresponding step. ProCx stands for protractor coxae muscles, controlling the protraction motion of the coxa (for the leg to swing forward). RetCx stands for retractor coxae muscles, controlling the retraction motion of the coxa (for the leg to swing backward). DprTr stands for depressor trochanteris, controlling the depression motion of the femur (for the leg to press down onto the ground). LevTr stands for levator trochanteris, controlling the levation motion of the femur (for the leg to lift up from the ground). Ext and flex stand for the extensor and flexor tibia muscle, controlling the extension and flexion of the tibia. DprT stands for depressor tarsi, LevT stands for levator tarsi. RetU stands for retractor unguis. TC, CT, FT, and TT refer to the subcoxal, coxal trochanter, femur tibia, and the tibia tarsus joint respectively. Figure preprinted from [50].

The retractor coxae were activated through the entire stance phase. The depressor trochanteris was activated at the first half of the stance phase, and the levator trochanteris was activated at the second half of the stance phase. In the swing phase, the protractor coxae were activated through the entire phase. The levator trochanteris was activated at the first half of the swing phase, and the depressor trochanteris was activated at the second half of the swing phase. The stick insect's walking gait was controlled by the muscle activation sequence. Knowing the muscle activation sequence would help in the design of the artificial controller of the insect-computer hybrid robot. For achieving successful walking, the control signal sequences from the artificial controller should be similar to that of the natural neural firing sequences for locomotion control.

2.4 Sensors integrated onto insect-computer hybrid robot

Advances in neuromuscular engineering had enabled researchers to build a variety of insect-computer hybrid robots. The applicability of insect-computer hybrid robots would be further improved if we can integrate sensors onto them. With micro cameras integrated, we could get instantaneous images of the robots' surrounding environment. With infrared sensors or acoustic sensors built on the robots, it would be easier to find survivors after disasters.

E. Whitmire et al. had recently developed low-power insect-mounted acoustic sensors for biobot (insect-computer hybrid robot using cockroach platform) [51]. The directional and omnidirectional acoustic sensors could help in detecting voices from victims buried under rubble (Figure 16). E. Whitmire et al. integrated three

unidirectional microphones placed 120° apart (Figure 16 (a)) or one unidirectional microphone (Figure 16 (b)) on the locomotion control PCB for the biobot. The three unidirectional microphones placed 120° apart formed an omnidirectional microphone array for detecting the audio source location. The direction of the voice source was calculated by analyzing the relative amplitude of each signal received by each of the microphones. The insect biobot was able to automatically locate and approach a sound source with the help from the microphone array. The microphones (PUI Audio, PUM-3046L-R) used on the cockroach biobot have the dimension of 6 mm (length) $\times$ 6 mm (width) $\times$ 3mm (height). The sensitivity of the microphone is -46 ± 3 dB.

Figure 16. Insect biobot with (a) three unidirectional microphones spaced 120° apart to form an omnidirectional microphone array for audio recording and streaming, (b) An unidirectional microphone to locate the audio source. Figure preprinted from [51].

2.5 Biomechanical properties of insect muscles

Understanding of the biomechanical properties of insect muscles will help in the design of muscle stimulation protocol for insect locomotion control. For example, knowing the two extensor muscles of the cockroach act as a motor and a brake respectively will help in the control of the action and cessation of the leg motion [52]. Moreover, the muscle contraction dynamics depend on the temporal components of the motor neural inputs [53-59]. Understanding how motor neuron system generates muscle contractions will help in the design of muscle stimulation signals for appendage motion elicitation. The muscle contraction dynamics is generally governed by the Hill equation:

$$(F + a) \times (V + b) = (F_0 + a) \times b$$

Where V represents the muscle shortening velocity that varies inversely with the muscle force F . F_0 is the maximum isometric tension of the muscle, and the intrinsic muscle characteristics determine the constants a and b . Therefore, although the Hill equation governs the general muscle contraction dynamics, the constants a and b vary from muscle to muscle. The curvature of the force-velocity relationship is determined by the ratio a/F_0 . For example, Figure 17 below illustrates the relationship between the muscle shortening velocity (muscle length/s, MLs^{-1}) and the loading during tetanic contraction of the metathoracic second tergocoxal muscle of the locust *Schistocerca Americana* [60].

Figure 17. Shortening velocity (muscle lengths s_1 , MLs $_1$) as a function of load during tetanic contraction. Values were obtained from release to isotonic contraction during the plateau of an isometric tetanus. The data are from five preparations, each of which is indicated by a separate symbol. Force values were expressed as a fraction of the maximum isometric force measured from each preparation. The curves are hyperbolae. The solid curve is the best-fitting hyperbola for all data points ($r=0.805$), the dotted curve is for points excluding those with force values greater than $0.78P_0$ ($r=0.812$). Figure preprinted from [60].

Furthermore, in electrically elicited muscle contraction, Guschlbauer et al. found that the contraction force of extensor tibia muscle of the stick insect (*Carausius morosus*) increased with the stimulation frequency (Figure 18) [61]. As such, the stimulation

frequency could be used as a manipulated input variable to control the induced force, hence the leg motion acceleration or velocity.

Figure 18. The progression of muscle contraction force generated by electrically stimulating the axons of the innervating motoneurons of the extensor tibia muscle of a stick insect with frequencies ranging from 30 Hz to 200 Hz (positive current pulse train, pulse width = 0.5 ms, and amplitude = 0.00345 mA). The muscle resting force level is indicated by the dotted line. Figure preprinted from [61].

Lastly, the insect skeletal muscles are connected to the motor neuron branches through multiterminal innervation [62-66]. Multiterminal innervation means that more than one axons are connected to a muscle fiber. Figure 19 below shows the innervation characteristics of the muscle fibers of a blowfly and a locust [66].

Figure 19. Multiterminal innervation of insect muscle fibers. (A) Innervation of the tergo-trochanteral muscle of a blowfly (*Calliphora erythrocephala*) and (B) the metathoracic depressor tibiae muscle of a locust (*Locusta migratoria*). Photomicrographs of silver-stained preparations are illustrated (1) along with diagrammatic representations (2). Figure preprinted from [66].

2.6 Evaluation of current research achievements

From the above mentioned literature research, the insect locomotion control methods could be categorized into two general groups: locomotion control by neuron stimulation [20, 21, 23, 25-30, 32, 35-40] and locomotion control by muscle stimulation [17, 19, 22, 24]. Both approaches (neuron stimulation and muscle stimulation) had advantages and disadvantages.

Firstly, neuron stimulation may not precisely alter the insect's locomotion path but only generally steering the insect toward the desired direction. This is because unlike muscle stimulation where stimulation of one muscle directly induces the desired appendage motion, neuron stimulation indirectly induces the desired locomotion. For example, in the flight path control of moth, W. M. Tsan et al. stimulated the nerve cord to alter the moth's abdominal angle so that to change the flight path [21]. The success rate of turning control was 70%. Tsan et al. stated that the induced abdominal deflection of the moth could rudder the flight to cause the turning behavior. As such, the turning would also depend on the insect's instantaneous motion parameters (e.g. flight speed, body orientation change etc.) that affect the aerodynamic forces on the deflected abdomen. As such the precision of locomotion control (i.e. turning radius, turning angular velocity etc.) could not be monitored in this case. Furthermore, in the line following control of the Madagascar hissing cockroach, Tahmid Latif and Alper Bozkurt stimulated the cockroach's antennae for the walking direction steering [25]. Sensors on the cockroach's antenna are able to detect olfactory, tactile, thermal, and humidity of the environment. When encountering obstacle or predators, sensory input from the

antenna will result the escaping behavior [67]. By electrically stimulating the antenna, the escape behavior of the cockroach is induced so that the cockroach turns and runs toward the opposite direction of the stimulated antenna. However, similar to the above mentioned turning control of moth, although general direction steering could be achieved at relatively high success rate, the success rate of controlling the cockroach to follow a predefined S-shaped path was on about 10% [25]. The success rate was similar to the work done by Moore et al., where 10-20% of the Madagascan hissing cockroach could be steered by stimulation of the antenna or cercus [30]. Moreover, Sanchez et al. stimulated the prothoracic ganglia of American (*Periplaneta americana*) and discoid (*Blaberus discoidalis*) cockroaches to control the turning behavior with 60% repeatability [26]. As such, neuron stimulation (e.g sensory neurons, motor ganglia etc.) was able to generally steering the insects' locomotion. However, the success rate for the insect to follow a predefined path and inducing turning is relatively low (below 20% for path following and 60% for inducing turning). As such, I set one of the goals of my thesis to be precisely control the locomotion of an insect by either neuron or muscle stimulation.

Secondly, prolonged stimulation time or the implantation of electrodes into neurons leads to declined or even ineffective locomotion control of the insects. The problem of ineffective locomotion control by neural stimulation occurred in the directional steering control of the spider by stimulation of the leg ganglia [32], the success rate of path following experiment was only 50% due to habituation. Zhaolin Ynag et al. suggested that the spider's leg might be damaged not only by electrical stimulation but also by the

surgical implantation of the electrodes into the leg ganglia [32]. Ineffective locomotion control also occurred in the control of Madagascan hissing cockroach where the control was ineffective in about 50% of the tested subjects [36]. To my best knowledge, the highest success rate of inducing desired locomotion in insects by neuron stimulation was achieved by Giampalmo et al. [37]. By stimulation the metathoracic T3 ganglion, jumping behavior of the American grasshopper was induced at a success rate of more than 95%. Note that six grasshoppers were tested in total; only three grasshoppers' jumping could be controlled at the more than 95% success rate. However the total trials of jumping elicitation for the three grasshoppers are only 20, 25, and 38 times respectively. The problem of ineffectiveness in insects' locomotion control may indicate that the neuron clusters (e.g. ganglia) is fragile and easy to be damaged during electrode insertion. Hence, better implantation techniques should be developed or alternative stimulation sites (e.g. muscle) should be located to eliminate the problem of habituation in insect locomotion control.

Thirdly, it is only possible to control the turning behavior of the cockroach or spider by stimulating the antenna or leg ganglia, but the control of start, stop, walking speed, and walking gait is also crucial in the development of a biobot. Current locomotion control technique needs to be improved so that the initiation, cessation, speed and gait of a legged insect-computer hybrid robot can be artificially controlled. Muscle stimulation should be an alternative locomotion control method for solving the problem of habituation that exists in neuron stimulation. This is because muscle stimulation had already been implemented and tested to be functioning in humans for periods of years

[68]. Moreover, the individual motion of each leg segment can be controlled by stimulating the corresponding muscle; predefined stimulation sequence will elicit corresponding leg motion sequences so that different walking gaits can then be achieved by altering the stimulation sequence. It had already been demonstrated that flight muscle stimulation was implemented to control the insects' flight path (A. Bozkurt et al. [23] and H. Sato et al. [9]). A. Bozkurt et al. stimulated a moth's dorsal longitudinal and dorsal ventral flight muscles to directly steer the up and down wing strokes. The moth's flight direction could be controlled by evoking the actuation of the wings. H. Sato et al. stimulated the beetle's basalar muscles (one of the major indirect flight muscles of the beetle) for steering the left and right turnings. Therefore, one of my approaches in walking control in this thesis will be stimulating an insect's appendage muscles in predefined sequences for walking control. By alternating the stimulation sequences and stimulation durations, I aim to achieve the control of the insect's walking gait and step frequency.

Lastly, as discussed above, muscle stimulation was potentially better than neuron stimulation in terms of reliability in motion elicitation and ability to control individual appendage motions (e.g. up and down stroke control of the moth's wings [23]). However neuron stimulation for insects' locomotion control can have the following advantages. First, the locomotion control by neuron stimulation may need far less stimulation electrodes. This is because neuron stimulation usually indirectly elicit the desired motions (e.g. to activate the cockroach's escaping motion by stimulating the antenna or cercus [36]). As such, in the turning control of cockroach, only three

electrodes are needed (two inserted in to the two antennae, and one inserted into the body as the ground electrode). However, in muscle stimulation, two electrodes are needed for one muscle. An insect's leg joint has at least two degrees of freedom (e.g. protraction and retraction for the coxa joint of a beetle or a cockroach). In order for effective walking control, at least four motions of each leg should be controlled (protraction/retraction for forward and backward swings, levation/depression for lifting from and pressing down onto the walking substrate). As such, minimally, eight electrodes are needed for the motion control of one leg by muscle stimulation. Secondly, the control algorithm is easier in locomotion control by neuron stimulation than by muscle stimulation. For example, when stimulating the antenna of a cockroach, the cockroach will escape by turning to the opposite direction. The cockroach's intrinsic escaping behavior eliminates the need for researchers to design any walking or turning algorithm to control the cockroach. The cockroach just turns itself as it senses dangers or obstacles. However, when controlling the leg motions by sequentially stimulating individual leg muscles, researchers need to design the control algorithms (the stimulation sequence, the stimulation duration, and the cooperation of the legs under control etc.). As such, more complicated control algorithm will be needed when stimulating the leg muscles, however the good outcome is the more accurate control in the step frequency, step length, walking gait, and walking speed. Therefore, my aim is to control the individual appendage motions of an insect for better locomotion performance compared to existing literatures that adopting neuron stimulation for general locomotion steering, even though I need to develop more complicated control algorithms.

Chapter 3: Materials and methods

3.1 Study animal

Adult beetle (*Mecynorrhina torquata*) was used as the insect platform for building the insect-computer hybrid robot. The beetles are reared in plastic vivarium of 15 cm (length) $\times$ 15 cm (width) $\times$ 20 cm (height). Fresh sugar jelly is fed to the beetles every 2 to 3 days. One beetle is reared in a single plastic vivarium at constant temperature of around 25 °C and at constant relative humidity of around 60 %. Only male beetles with length between 6 to 8 cm and weight of about 7 g were used in the experiments.

3.2 Anatomy study

As discussed in the introduction and literature review, this project mainly focuses electrical stimulation of the beetle's muscles to elicit desired leg motion for the realization of an insect-computer hybrid walking robot. Therefore, the first task is to find the muscles that control individual leg motions through anatomy.

Anatomy of the beetle's front leg was conducted for locating the muscle groups that controlling the leg motions. The beetle was immersed in 95 % ethanol solution for about 12 hours before anatomy. The beetle's cuticle was cut open with micro dissecting spring scissors (Vannas® straight scissors with 3 mm cutting edge, 0.15 mm tip width). The cuticle was then removed by using a tweezer (Dumont® tweezer, pattern #5, 0.05 $\times$ 0.01 mm tip size) to expose the muscles (refer to Figures 20 below for the details of anatomy to remove the beetle cuticles for muscle exposure and muscle identification).

Figure 20. The beetle pictures before and after half of the upper prothoracic cuticle was removed. (a) Intact beetle before the anatomy was carried out. (b) Half of the cuticle on the upper side of the prothorax was removed with anatomical instruments and techniques described in section 3.2. The muscles connecting to the coxa of the left front leg were exposed. (c) Muscles controlling the protraction and retraction motions of the coxa are easily identifiable.

3.3 Electrode implantation into muscles

The beetle was fixed onto a wooden block by wrapping softened dental wax (Cavex, Set Up Modeling Wax immersed in 80 °C water for 10 seconds) around the body.

Holes of 0.5 mm diameter were made on the beetle's cuticle using an insect pin (Indigo Instruments, #3 black enamel insect pin). Thin silver wires (A-M Systems, 127 μm in diameter without insulation coating, 178 μm in diameter with Teflon insulation coating) were used as the stimulation electrodes. The insulation at the end of the silver wire was removed by heating in flame. The other end of the stimulation electrode was connected to the output of a function generator (Agilent, 33220A) or the custom designed wireless control "backpack". Two electrical stimulation electrodes were implanted into one muscle. One electrode serves as the stimulation output channel; the other electrode serves as the ground channel (refer to Figure 21 below for the implantation of electrodes into the protraction and retraction muscles of the beetle's front leg).

Figure 21. Electrode implantation into a beetle's muscles. The protraction muscle and the retraction muscle (refer to Figure 20 for anatomy pictures) are implanted by two silver electrodes respectively for the purpose of both muscle stimulation and muscle signal recording. Blue circle indicates the implantation site for the protraction muscle and red circle indicates the implantation site for the retraction muscle.

3.4 Natural muscle EMG recording

The muscle EMG recording involves the collection of retraction muscle group's EMG signal and synchronizes the EMG signal with the actual leg motion. Two electrodes (silver wires, A-M Systems, 127 μm in uncoated diameter, 178 μm diameter with insulation) were inserted into the retraction muscle group of the beetle's front leg using the same implantation method described in section 3.3 (Figure 21). The electrodes were

securely fixed to the outer surface of the cuticle by using melted dental wax (Cavex, Set Up Modeling Wax) to prevent movement of the electrodes and hence to prevent potential artifacts in the EMG signal collected. An amplifier (LT1920, Burr-Brown Products) was used to amplify the EMG signal 495 times (Figure 22 below, by connecting a 100 Ohm resistor to pins 1 and 8 of the LT1920 amplifier). The amplified EMG signal was collected by the input/output (I/O) ports (Figure 22, P0_2 and P0_3) of a custom-programmed microcontroller (Texas Instruments, CC2431, $6 \times 6 \text{ mm}^2$, 32 MHz clock). The sampling rate of signal collection was set at 2000 Hz. At the above mentioned settings, the signal to noise ratio is about 300. The high signal to noise ratio made the peaks of the EMG signal distinguishable in calculating the EMG firing frequencies. The same motion capturing methods described in section 3.5 below was used. A software tool named BeetleCommanderEMG was developed with the help of a programmer from the author's research group to synchronize the EMG signal collected by the microcontroller with the motion data captured by the motion capturing system. In order to identify the maximum number of EMG spikes captured, the threshold voltage of the collected EMG signal was individually determined for each experiment result [69, 70]. Therefore, the EMG burst onset time was defined as the time at which the amplitude of the collected EMG signal exceeded the individually defined threshold value. The last detectable EMG spike was used to indicate the EMG burst termination time. The average EMG frequency was calculated as the number of EMG spikes within a single burst divided by the burst duration. The leg motion onset and offset times were defined as the first detectable retraction motion and the first detectable protraction motion [69, 70].

Figure 22. Electrical circuit diagram for muscle EMG collection. The two electrodes were inserted into the target leg muscle. The other ends of the electrodes were connected to pin 2 and 3 of the LT1920 amplifier. The LT1920 amplifier was connected to a customized battery source through pins 4, 5, and 6. The amplified EMG signal was sent out through pins 5 and 6 of the amplifier to P0_2 and P0_3 of the CC2431 microcontroller for data collection and storage.

3.5 Motion capturing techniques

A three-dimensional (3D) motion capturing system (Vicon, Figure 23 below) was used to record the Cartesian coordinates of reflective markers placed on the beetle's body or legs with time stamps when the beetle performs voluntary walking. The 3D motion capturing system consists of six T40s VICON® cameras each with resolution of 4

megapixels (2336×1728) operating at 100 frames per second. The experiment arena was a horizontal surface of about 1 m^2 . The global reference frame of the 3D motion capturing system was set in a way that the x and y-axis formed the horizontal plane parallel to the beetle's walking surface. The z-axis was thus perpendicular to the walking surface. The motion data were exported in comma-separated values (CSV) files and analyzed with custom-programmed Matlab® codes. At the experiment settings, the accuracy of the motion capturing system is $34 \pm 6.67 \text{ }\mu\text{m}$.

Figure 23. Motion capturing system for biomechanical study. The 3D motion capturing system for tracking the leg and body motion of the beetle. (a1) six T40s VICON® cameras placed at the top of the arena. (a2) The VICON® server computer for data processing and storing. (a3) A personal computer for data analysis.

3.6 Printed circuit board for remote walking control

A custom-designed printed circuit board (PCB) was used as the backpack for remotely controlling the beetle's walk (Figure 24). The length and the width of the backpack are 1.8 cm and 1.5 cm respectively. The backpack weights 0.7 g. A custom-programmed microcontroller on the backpack (Chipcon Texas Instruments, CC2530, $6 \times 6 \text{ mm}^2$, 32 MHz clock) was used for remote communication (Zigbee, 2.4 GHz, IEEE 802.15.4 wireless standard) and motion control signal generation. The Fullriver battery (3.7 V, 20mAh, 0.7 g, and with dimension of $11 \text{ mm} \times 3.8 \text{ mm} \times 14 \text{ mm}$) was used to power the backpack. One end of the stimulation electrode (Teflon-insulated silver wire with diameter of 178 μm , A-M Systems) was connected to backpack at the female connectors. The other end of the stimulation electrode was implanted into the target muscles for motion control. The input/output (I/O) ports of the CC2530 microcontroller were used as the leg motion control signal generation terminals. Timer 3 interrupt function of the CC2530 microcontroller was used to generate pulse-width-modulation (PWM) waves of 100 Hz and 1 ms pulse width from the I/O ports as the stimulation signal. The magnitude of the stimulation signal was regulated at 1.5 V.

Figure 24. Backpack for remote walking control and the electrical circuit diagram. (a 1-3) Top, bottom, and top with battery view of the backpack. The CC2530 microcontroller was preprogrammed to send muscle stimulation signals to sequentially elicit leg motions. (b) The electrical diagram of the custom designed PCB. J1, J2, and J3 corresponding to the three females connectors shown in (a1). Sixteen resistors were used as voltage dividers of the eight stimulation channels for leg motion control.

Chapter 4: A biological actuator

4.1 Graded single leg motion control

4.1.1 Insect anatomy

In order to control the leg motions by muscle stimulation, the first task is to locate the muscles that are responsible for various leg motions. This section explains the results obtained after doing the anatomy of the beetle's front leg. The beetle's front leg muscles responsible for walking control are shown in Figure 25. The protraction and retraction muscle groups inside the beetle's prothorax control the protraction and retraction motions of the front leg (for the leg to swing forward and backward about the thorax-coxa joint shown in Figure 25 (a)). The levation and depression muscle groups inside the coxa enable the femur to perform levation and depression motions (the femur rotates about the coxa-trochanter joint shown in Figure 25 (b)). The retraction muscle group is larger than the protraction muscle group, and the depression muscle group is larger than the levation muscle group. This is because the retraction and depression are performed when the leg touches the ground to execute the power stroke to push the body forward. However, the protraction and levation muscle groups are used when the leg is in the air performing the return stroke to bring the leg back to the anterior extreme point (AEP). More forces are needed during the power stroke to push the body forward. The extension and flexion muscle groups are inside the femur, controlling the extension and flexion motions of the tibia. Red crosses in Figure 25 indicate the locations for insertion of stimulation electrodes for leg motion control.

Figure 25. Anatomy of the beetle's front leg revealed the muscle groups responsible for the corresponding leg motions. Red crosses indication the positions for inserting stimulation electrodes [8].

4.1.2 Threshold stimulation voltage to elicit significant leg motion

To elicit significant leg motion by stimulating the leg muscles, the minimum stimulation voltage required is to be confirmed. The leg's angular displacement is hypothesized to be initially directly proportional to the stimulation voltage. Experiment procedures for testing the threshold voltage for leg motion elicitation are shown below.

To capture the elicited leg motion, three markers for motion tracking purpose were placed onto the beetle's body (Figure 26 (a) below). Markers 1 and 2 placed on the beetle's front leg were recognized by a 3D motion capture system (Figure 26 (c)) as a solid line segment (Figure 26 (b)). Marker 3 indicated the beetle's body position. The

3D motion capturing system was set to capture and store the 3D marker coordinates at frame rate of 100 Hz. The leg's angular displacement was calculated by using the formula below:

$$\Delta\theta = \cos^{-1} \left(\frac{(X_1 - X_2)(X'_1 - X'_2) + (Y_1 - Y_2)(Y'_1 - Y'_2) + (Z_1 - Z_2)(Z'_1 - Z'_2)}{\sqrt{(X_1 - X_2)^2 + (Y_1 - Y_2)^2 + (Z_1 - Z_2)^2} \times \sqrt{(X'_1 - X'_2)^2 + (Y'_1 - Y'_2)^2 + (Z'_1 - Z'_2)^2}} \right),$$

where X_1, Y_1, Z_1 and X_2, Y_2, Z_2 indicate the leg's initial (resting) position. X'_1, Y'_1, Z'_1 and X'_2, Y'_2, Z'_2 indicate the leg's new position as a consequence of elicited motion. For the same beetle, each time after stimulation, the leg was manually located back to its initial position by checking the 3D coordinates of the markers for it to be ready for next stimulation. However, the leg's initial position differs from beetle to beetle on the order of a few degrees.

To test for the threshold voltage for effective leg motion elicitation, the stimulation pulse width was fixed at 1 ms and the frequency was fixed at 30 Hz. The stimulation voltage ranged from 0.25 V to 2.5 V with increment of 0.25 V. The elicitation of the protraction and retraction motions was recorded by using the 3D motion capturing system (Vicon) shown in Figure 26 (c).

Figure 26. Experiment setup for leg motion capturing. (a) Markers with diameter of 3 mm were placed on the beetle's front leg (markers 1 and 2) and the body (marker 3) for motion capturing purpose. (b) The 3D motion capturing system recognized the markers as point objects and displayed on a computer screen. The two markers on the leg were recognized as a line segment. (c) 3D motion capturing system with six T40s VICON cameras (C1). The camera frame rate was set at 100 Hz. The resolution of each camera is 4 megapixels (2336×1728). (C2) A VICON server was used for camera signal collection, processing, and display. (C3) A computer is used to calculate the leg motion angular displacements with custom-written Matlab® code [8].

This paragraph explains the experiment data obtained after performing the threshold voltage testing of eliciting leg motion described above. Figure 27 below shows the maximum elicited protraction and retraction angular displacements at various stimulation voltages (number of beetles = 5, $17 \leq$ number of data points at each stimulation voltage ≤ 22). The maximum angular displacement for both the protraction motion and the retraction motion was achieved at about 1.5 V stimulation voltage (the maximum protraction angular displacement at 1.5 V = $18.09^\circ \pm 4.78^\circ$, the maximum retraction angular displacement at 1.5 V = $12.73^\circ \pm 7.04^\circ$). As we can see from Figure 27, when the stimulation voltage was further increased beyond 1.5 V, the maximum

angular displacement for both the protraction and retraction motions remained relatively constant. The elicited angular displacement with respect to input stimulation voltage profile is hypothesized due to that the beetle's leg muscles are innervated by different types of excitatory axons. The axons innervated on the muscle fibers have different activation potentials which each evoke significant muscle contractions [71]. When the input stimulation voltage increased from 0.25 to 1.5 V, the axons were gradually activated to control the muscle contraction. At stimulation voltage equals to 1.5 V, activation potentials of all the innervated axons were exceeded, hence further increment in stimulation voltage did not cause further increment in the induced muscle forces. The optimum stimulation voltage for leg motion control should be as low as possible to avoid any potential damage to the muscles, and at the same time, the stimulation voltage should be high enough to elicit the leg motion reliably. Therefore, by considering the experiment results from Figure 27, the stimulation voltage for all subsequent leg motion control experiments would be fixed at 1.5 V. In addition, by fixing the stimulation voltage at 1.5 V, I applied muscle stimulation more than 200 times within one day on one beetle and repeated the experiment on over 40 beetles; there was no obvious muscle damage observed considering the fact that the leg motion could always be elicited at the success rate of 100%.

Figure 27. Maximum protraction and retraction angular displacements elicited at stimulation voltage ranged from 0.25 V to 2.5 V (number of beetles = 5, $17 \leq$ number of data points at each stimulation voltage for each motion type ≤ 22). Blue lines and red lines indicate the protraction and retraction maximum angular displacement respectively. The dotted lines show the average angular displacement of each beetle. The solid lines indicate the average values with error bars indicate the standard deviation calculated from the total number of data points (five beetles). A pulse-width-modulation wave of frequency equals to 30 Hz and pulse width equal to 1 ms was used as the stimulation signal. The maximum angular displacement for both the protraction and retraction motions increased with the stimulation voltage until approximately 1.5 V [8].

4.1.3 The leg motion amplitude can be graded by adjusting stimulation frequency

Physiologically, the nervous system controls the motor neurons to generate command signals to induced muscle motions. The motor neuron-muscle link consists of a sequence of steps for the activation of muscle contraction [72]. Electrical stimulation

was implemented to mimic the motor neuron firing for elicitation of muscle contraction. Higher stimulation frequency could induce greater forces in the muscles [61]. This is because at low stimulation frequencies, muscle twitches result in the periodic rise and fall in the induced force. At higher stimulation frequencies, incomplete tetanus is induced due to the merge of twitch contractions. When the stimulation frequency is further increased, summation occurs where the second stimulus is applied before the first relaxation is complete. Summation results a greater tension to be developed in the muscle fibers (also known as tetanic contraction). Thus I hypothesize the leg's angular displacement and angular velocity can be graded by varying the input stimulation frequency.

For all the experiments on graded leg motion control, the stimulation voltage was fixed at 1.5 V and the pulse width of the PWM wave was fixed at 1 ms. The elicited leg motion at stimulation frequency ranged from 20 Hz to 100 Hz with increment of 20 Hz each time was studied. The motion capturing techniques described in section 3.5 were implemented to track the elicited leg motion.

This paragraph explains the experimental results obtained for graded leg motion control. Figure 28 below shows that all the six motion types (protraction/retraction, levation/depression, and extension/flexion) of the beetle's front leg monotonically increased with the stimulation frequency (number of beetles = 10). For protraction, retraction, depression, extension, and flexion, the maximum angular displacement was induced when the stimulation frequency was set at 80 Hz. However, the maximum angular displacement was reached when the stimulation frequency was set at 40 Hz for

the levation motion. The maximum angular displacement was limited by the intrinsic leg structure of the beetle.

Figure 28. Change of maximum angular displacement with stimulation frequency for all the six motions of the front leg. The colored dotted lines indicate that five beetles were used for obtaining the motion data for one motion type. The black lines indicate the average maximum angular displacement of five beetles with standard deviations indicated by the black error bars (number of beetles = 5, number of data points at each stimulation frequency for each motion type = 25). The stimulation frequency was increased from 20 Hz to 100 Hz at the increment of 20 Hz each time [8]. The black dotted in lines (a-1) and (a-2) indicate the voltage sweep data at 1.5 V, 30 Hz, and

1ms PWM stimulation input (stimulation signal used to confirm the threshold voltage in Section 4.1.2).

To further analysis the graded leg motion by changing the stimulation frequency, I calculated the average angular velocity of the elicited leg motion at different stimulation frequencies. The average angular velocity also increased monotonically with the stimulation frequency. As shown in Figure 29 below, for all the five motions (except the levation motion), the maximum angular velocity was reached when the stimulation frequency was increased to approximately 250 Hz. The maximum angular velocity of levation was reached when the stimulation frequency was set at about 100 Hz.

Figure 29. Average angular velocity (degree/s) monotonically increased with the stimulation frequency. The stimulation frequency ranged from 20 Hz to 300 Hz with step increment of 20 Hz when the stimulation is less than 100 Hz and with step increment of 50 Hz when the stimulation frequency is greater than 100 Hz. The colored dotted lines indicate different beetles used in the experiment (number of beetles = 5, number of data point at each stimulation frequency = 25 for each motion type). The thick black line indicated the mean of the average angular velocity from 25 data point at each stimulation frequency. The black error bars indicate the standard deviations of the average angular velocity [8].

4.1.4 Comparison between leg motions elicited by electrical stimulation and leg motion elicited by intrinsic neural input

It was observed that the average leg joint angular velocity of a cockroach (*Blaberus discoidalis*) was directly proportional to the natural motor neural firing frequency [69]. In Figure 29, a linear relationship also existed between the mean angular velocity and the stimulation frequency before the maximum mean angular velocity was reached. Therefore, to understand how effective our electrical stimulation is in eliciting the leg motion compared to the beetle's natural neuromuscular system in leg motion elicitation, I recorded down the front leg's retraction muscle electromyogram (EMG) and synchronized the EMG signal with the corresponding leg motion. The effectiveness of both the EMG signal and the electrical stimulation in leg motion elicitation could be judged by the slope of the linear regression line in the average angular velocity vs. EMG frequency or the stimulation frequency graphs. The steeper the slope is, the more effective for the input signal to elicit leg motion. Figure 30 below shows a typical EMG signal collected (Figure 30 (a)) and the synchronized leg motion data (Figure 30 (b)).

Figure 30. Retraction muscle group EMG signal as a function of time synchronized with the captured leg motion. (a) Retraction muscle group EMG signal collected. (b) The protraction and retraction motions of the beetle's front leg. The retraction motion was indicated as a decrease in the leg's angular displacement [8].

Figure 31 below compares the effectiveness of leg motion elicitation by the intrinsic neuromuscular input signal and the electrical stimulation signal. Figure 31 (a) shows the average angular velocity of the retraction motion as a function of the mean muscle EMG frequency (number of beetles = 4, number of total data points = 43). A linear relationship was observed between the average angular velocity and the mean EMG frequency ($R^2 = 0.76$). The average angular velocity also varied linearly with the electrical stimulation frequency (Figure 31 (b), $R^2 = 0.75$, number of beetles = 5, number of data points at each stimulation frequency = 25). Despite the expected results that the average angular velocity was directly proportional to both the muscle EMG frequency and the stimulation frequency, the slope of the linear regression in Figure 31

(a) was almost five times steeper than that in Figure 31 (b). In other words, when the average muscle EMG frequency equals to the stimulation frequency, the leg motion was five times faster if the beetle moved voluntarily compared to if the leg motion was elicited by electrical stimulation. The first possible reason could be the difference in the input signal forms (muscle's EMG spikes vs. PWM waves). The second possible reason is that the threshold charge for directly inducing muscle fiber action potentials (electrical stimulation on muscles) is much larger than the threshold for producing action potentials in neurons (natural motor neuron firing in this case) [73]. The third possible reason is that the beetle might resist the electrically elicited motion by activating the antagonistic muscles to producing a counter force so that to reduce the motion angular velocity in the elicited direction.

Figure 31. Average retraction angular velocity as functions of the average muscle EMG frequency and the electrical stimulation signal frequency. (a) A linear relationship existed between the average angular velocity and the average EMG frequency of the muscle ($R^2 = 0.76$, number of beetles = 4, total number of data points = 43). (b) The average angular velocity was directly proportional to the input stimulation frequency ($R^2 = 0.75$, number of beetles = 5, number of data point at each stimulation frequency = 25). The least-squares

linear regression lines were drawn in black in both graphs. Error bars in (b) indicates the standard deviation [8].

4.2 Closed-loop control of the leg's angular displacement

Closed-loop leg motion control could enable precise leg motion amplitude monitoring and hence help in precise locomotion control of the future insect-computer hybrid robot (i.e. to accurately manipulate the step length of the legged robot). The closed-loop control of the leg's angular displacement is hypothesized to be feasible due to the following reasons. Firstly, each of the muscle groups is controlled by separated stimulation channels that work independently. In other words, the stimulation of one muscle group does not interfere with the rest of the stimulation channels. Secondly, stimulation frequency was proved to be a reliable manipulated variable to grade the leg's angular displacement and angular velocity (i.e. the leg's angular displacement and angular velocity monotonically increase with the input stimulation frequency). The result of the closed-loop leg motion control is expected to be that the leg's angular displacement will be moved to predefined values by stimulating a pair of antagonistic muscle groups. The performance of the closed-loop control algorithm could be judged in terms of the overshoot angle and reaching time.

4.2.1 Techniques designed for closed-loop leg motion control

Figure 32 below shows the schematic drawing of the closed-loop leg motion control system.

Figure 32. Schematic representation of the closed-loop control system.

The error is the difference between the predefined leg angular position and the actual leg angular position. A proportional control algorithm is embedded into the controller [8].

The controller (BeetleCommander) is custom-developed software that is able to receive the instantaneous leg motion data and generate stimulation signals. The algorithm of proportional control was embedded into the software to adjust the step increment or decrement of the stimulation frequency (proportional control formula is shown below).

$$f_{out}^* = f_{out} + K_p e(t)$$

where f_{out} is the last output stimulation frequency from the final control element, f_{out}^* is the updated output stimulation frequency from the final control element (its value is limited to the 10–200 Hz range), K_p is the proportional gain (user-adjustable), and $e(t)$ is the instantaneous angular displacement error at time t .

The final control element consists of a microcontroller (Texas Instruments, CC2431, $6 \times 6 \text{ mm}^2$, 32 MHz clock) with its external circuit. Electrical stimulation signals were generated from two independent stimulation channels to control a pair of antagonistic

muscle groups (the protraction and retraction muscle groups of the front leg in this case). The stimulation channels generate stimulation signals according to instantaneous error (the difference between the predefined target angular position and the actual instantaneous leg angular position). If the actual angular position is larger than the predetermined position, the output stimulation frequency on the corresponding muscle group will decrease and the stimulation frequency on the antagonistic muscle group will increase. Similarly, if the actual angular position is smaller than the predefined value, the stimulation frequency on the antagonistic muscle groups will decrease and the stimulation frequency from the other stimulation channel will increase to bring the leg closer to the desired position. Moreover, both the proportional gain (K_p) and the system's update time (the duration of which the system update the instantaneous leg angular position and output stimulation signals) are user-adjustable. In summary, two independent channels stimulating a pair of antagonistic muscle groups according to the proportional control algorithm to set the leg at the predefined angular position.

4.2.2 Experimental results of closed-loop leg motion control

This paragraph explains the experimental data obtained from closed-loop leg motion control. Figure 33 below shows a typical closed-loop control of the leg's protraction and retraction motion. The red lines indicate the predetermined leg angular positions (positive for retraction motion and negative for protraction motion). The proportional gain (K_p) and the update time interval were set at various values shown in the Figure 33 to illustrate the system performance at different settings. The aim of the closed-loop control was to alternatively set the leg at 10 degrees protraction angle for 10 seconds

and set the leg at 10 degrees retraction angle for 10 seconds. As we can see that when K_p equals to 0.1, for all the system update time (100 ms, 200 ms, and 300 ms), the leg was not always brought to the predefined angular positions. This is because for the same angular error, the relatively small proportional gain resulted in small increment or decrement of the output stimulation frequency (the $K_p e$ term in formula) that is not always sufficient to move the leg to the predefined positions. In total of the 243 experiment data points for $K_p = 0.1$, the leg reached the predetermined angular positions for 104 times (42.8 % success rate).

Figure 33. Typical closed-loop control of protraction/retraction motion of the beetle's front leg at K_p values equal to 0.1, 0.5, 1.0, and 1.5 and update time intervals equal to 100 ms, 200 ms, and 300 ms. The predetermined angular displacements are ± 10 degrees (positive value for retraction motion and negative value for protraction motion) [8].

Figure 34 below shows the quantitative evaluation of the performance of the closed-loop control system in terms of overshoot angle and the reaching time at different settings of the K_p value and the update time interval (number of beetles = 5, $35 \leq$ number of data points at each experiment setting ≤ 49). The general trend is that when the K_p value was increased, the leg's overshoot angle increased while the reaching time decreased. This is because larger K_p value would result in greater change in the updated stimulation frequency for the same instantaneous angular error. Larger stimulation frequency would elicit higher angular velocity. Hence the reaching time was reduced and the angular overshoot increased. For instance, at update time equals to 100 ms, when K_p value is increased from 0.5 to 1.5, the protraction overshoot angle increased from $10.47^\circ \pm 3.66^\circ$ to $22.12^\circ \pm 7.75^\circ$ and the retraction overshoot angle increased from $11.03^\circ \pm 5.06^\circ$ to $17.48^\circ \pm 4.94^\circ$; in contrast, the protraction reaching time decreased from 0.518 ± 0.133 s to 0.299 ± 0.188 s and the retraction reaching time decreased from 1.249 ± 0.917 s to 1.111 ± 0.488 s. If we fix the K_p value and increase the system update time interval, the overshoot angle reduced and the reaching time increased. This is because longer system update time interval reduces the likelihood that the system overly increases or decreases the output stimulation frequency, and hence results in less overshoot angle but longer reaching time. For example, for $K_p = 1.0$, as the update time was increased from 100 to 300 ms, the protraction overshoot angle decreased from $17.51^\circ \pm 5.79^\circ$ to $16.01^\circ \pm 8.39^\circ$ and the retraction overshoot angle decreased from $15.97^\circ \pm 5.38^\circ$ to $6.52^\circ \pm 4.34^\circ$, whereas the protraction reaching time increased from 0.350 ± 0.184 s to 0.564 ± 0.150 s and the retraction reaching time increased from 0.492 ± 0.217 s to 0.875 ± 0.308 s.

Figure 34. Closed-loop leg motion control system performance evaluation in terms of overshoot angle and reaching time. (a) the overshoot angle and (b) the reaching time of the front leg's protraction and retraction motion at various K_p and system update time settings. Each color of the bar indicates a particular experiment setting. Error bars indicate the standard deviations [8].

4.3 Power consumption of the muscle stimulator

To build the insect-computer hybrid robot by electrical stimulation of leg muscles, power consumption is an important aspect for consideration. This is because due to the small size of the beetle (6 – 8 cm body length, and approximately 7 g body mass); great limitations will be exposed on the size and weight of the battery. However, as the electrical stimulation signal was only used to elicit the beetle's leg motion, the actual energy for muscle contraction and hence moving the leg was driven from the beetle itself. As such, the power consumption for leg motion elicitation by stimulating the corresponding muscle groups is expected to be low and in the order of μW .

The power consumption for the stimulation of all the six muscle groups in the beetle's front leg was measured. A function generator was used to generate the PWM wave

with 1 ms pulse width, 100 Hz frequency, and 1.5 V amplitude. The current was measured by using an oscilloscope (Yokogawa DL 1640). Figure 35 (a) below shows the PWM wave used for muscle stimulation. Figure 35 (b) shows a typical current profile through the depression muscle group when the PWM wave shown in (a) was applied.

Figure 35. Simulation signal and current flow through the muscle. (a) PWM wave of 1.5 V amplitude, 100 Hz frequency, and 1 ms pulse-width was

used as the stimulation signal. (b) A typical current profile that passed through the depression leg muscle group of a beetle's front leg [8].

The mean and standard deviations of the power consumption for the stimulation of all the six muscle groups of the front leg is shown in Table 1 below (number of beetles = 5, number of data points collected from each muscle group = 40). The average power consumption of electrical stimulation of a muscle group is about 148 μ W.

Table 1. Mean and standard deviation of power consumption (μ W) for all the six muscle groups of the beetle's front leg across five beetles

	Power consumption (μ W) of the six front leg muscle groups					
Beetle mass (g)/ length(cm)	Protraction	Retraction	Depression	Levation	Extension	Flexion
8.47 g/ 6.5 cm	182.34 $\pm$ 2.25	136.70 $\pm$ 6.56	137.67 $\pm$ 1.05	151.85 $\pm$ 1.61	125.75 $\pm$ 1.40	163.29 $\pm$ 4.72
6.06 g/ 5.3 cm	123.16 $\pm$ 10.15	101.55 $\pm$ 3.89	72.42 $\pm$ 1.92	176.00 $\pm$ 3.20	114.84 $\pm$ 3.73	120.07 $\pm$ 1.50
7.76 g/ 5.5 cm	126.54 $\pm$ 4.44	197.48 $\pm$ 16.15	202.81 $\pm$ 9.56	200.34 $\pm$ 1.92	164.40 $\pm$ 1.71	149.77 $\pm$ 6.73
6.57 g/ 5.9 cm	133.68 $\pm$ 0.72	128.70 $\pm$ 0.72	173.96 $\pm$ 3.78	136.05 $\pm$ 2.87	227.05 $\pm$ 9.55	210.96 $\pm$ 3.38
10.35 g/ 6.7 cm	135.22 $\pm$ 19.15	94.62 $\pm$ 1.59	119.88 $\pm$ 0.42	139.62 $\pm$ 4.36	144.18 $\pm$ 3.33	154.18 $\pm$ 6.37

4.4 Repeatability of leg motion elicitation

For reliable implementation of the biological actuator in constructing an insect-computer hybrid robot, the repeatability of leg motion elicitation by muscle stimulation is an important consideration. The reliability of the leg motion elicitation (e.g. when electrical stimulation is applied on the same animal after one hour, one day, or one week etc.) significantly affects the robot's lifespan. Therefore experiments were done to confirm the repeatability of leg muscle stimulation over a timespan of one week.

4.4.1 Techniques used for repeatability test

To test the repeatability of electrically induced leg motion, the protraction/retraction muscles of the front leg were alternatively stimulated for 1 second to elicit cycles of protraction and retraction motions of the beetle's front leg: the channel stimulating the protraction muscle was turned on for 1 second, then that channel was turned off and another channel stimulating retraction muscle was turned on for 1 second (thus, the frequency of the cycle is 0.5 Hz). The cycle was repeated for at least 30 minutes per day for 7 consecutive days. The leg's motions were tracked and recorded using the same technique as described section 3.5. The beetle and test day effects on the mean and standard deviation of the leg's angular displacement were quantitatively analysed by using two-factor analysis of variance (ANOVA) at 95% confidence level.

4.4.2 Repeatability test results

Unlike man-made robots and actuators, there are always relatively large variances or errors in motor actions of electrically stimulated muscles (e.g. Figures 27 to 29) due to biological experimental factors such as individual animal differences and implanted electrode drift. However, the levels of variances and errors in muscle motor actions themselves are independent of the trials and tested animals. The variances were mostly less than 20% as shown in the standard deviations, which indicate the standard of the repeatability in the insect-computer hybrid robot. As seen in Figure 36, the angular displacement (the length between green and red plots) did not largely change throughout the trial. Furthermore, ANOVA test results showed that no clear day-by-day or animal-to-animal difference in the mean angular displacement ($F_{6,18} = 0.67$, $P =$

0.67 for test day effect, $F_{3,18} = 1.82$, $P = 0.18$ for beetle effect) and the standard deviation ($F_{6,18} = 0.82$, $P = 0.57$ for test day effect, $F_{3,18} = 2.83$, $P = 0.07$ for beetle effect). The variation of the mean and standard deviation of the leg's angular displacement with respect to individual beetle and test day is also shown in Figure 37. We thus conclude the insect-computer hybrid robot can repeatedly demonstrate desired and expected performances, i.e. the walking speed and step length are controllable by the step frequency. The relatively large variance (standard deviation in the order of 20%) does not strongly affect the walking performances. Such a large variance should be problematic if more sophisticated and precise locomotion control, for example, regulating individual legs in pre-determined motion paths. For that case, closed-loop (feedback) control coupled with leg motion detection should be adopted for the insect-computer hybrid robot (closed-loop leg angular displacement controlled was demonstrated in section 4.2).

Figure 36. Repeatability test: cycles of leg motion induced by the electrical stimulation of the retraction and protraction muscles. A leg's motion (over a duration of 200 s) tracked in the repeatability test. Custom-developed Matlab® code was used to automatically detect the angular positions of extreme retraction (green circle) and extreme protraction (red circle) with respect to the maximum protraction position reached during the 30 min stimulation (angular position = 0 degree). The beetle's leg keeps moving between the extreme retraction and protraction positions every second due to the applied electrical stimulation. The difference between adjacent retraction and protraction positions is the leg's angular displacement.

Figure 37. Repeatability test: The average and standard deviation of four beetle's front leg angular displacement (N = 4 beetles, n = 1800 data points). Every beetle was stimulated for more than thirty minutes per day for seven consecutive days. The four different colors of the columns indicate the four tested beetles. The standard deviations are indicated by the black bars. The mean and standard deviation of the leg's angular displacement of each beetle in each day were calculated from more than 1800 data points (refer to Figure 36 for the data points allocation method) resulted from the more than 30 minutes stimulation (as the beetles' protraction and retraction muscles were stimulated alternatively for 1 second).

The little fluctuation of the variance level also implies that the animal's voluntary motor action did not significantly affect the motor action induced by the electrical stimulations. If the animal voluntarily disturbed the electrically induced motor action and it strongly influenced the resultant displacement, the angular displacement as seen

in Figure 36 would be abruptly changed, but such a big change is not seen in the data. Furthermore, the repeatability tests proved that the stimulation materials and techniques implemented in this thesis are suitable for the beetle's leg motion control over a relatively long period (seven days). Although silver electrodes are commonly used in insect-computer hybrid systems, however, for electrodes that make ohmic contact with tissue, gold, platinum, platinum-iridium, tungsten, and tantalum are better alternatives. The criteria for judging the suitability of material for implanted electrodes are tissue response, allergic response, electrode-tissue impedance, and radiographic visibility [74]. In this study, silver wires were proved to be suitable muscle stimulation electrode in terms of good tissue response (able to elicit muscle contractions in seven consecutive days) and radiographic visibility [36]. The other two properties of silver as electrodes (allergic response and electrode-tissue impedance) will be tested in my future works for the improvement of the electrode-tissue interface performances.

Chapter 5: Insect-computer hybrid walking machine

5.1 Beetle's natural walking gait

After successfully demonstrating control of the front leg's individual motion in graded and closed-loop approaches, the next goal is to control the leg motions in predefined sequences to mimic the leg motion when the beetle walked voluntarily. Therefore, the natural walking gait of the beetle needs to be studied first by extracting the leg motion sequences (which motion is executed at what time during a complete step) from the walking gait study. This data would help in the design of stimulation sequence for the walking control of our insect-computer hybrid robot.

5.1.1 Experimental setup for walking gait study

We used the same three-dimensional (3D) motion capturing system (Figure 38 (a)) to record the Cartesian coordinates of the beetle's front leg tibia sections with time stamps when the beetle performs voluntary straight line walking. The 3D motion capturing system consists of six T40s VICON® cameras each with resolution of 4 megapixels (2336×1728) operating at 100 frames per second (Figure 38 (a1)). As shown in Figure 38 (b), each of the beetle's front leg tibia segments was attached with two reflective markers (L1, L2, R1, and R2) for motion capturing purpose. Two reflective markers (B1 and B2) were attached to the beetle's body so that we could track the beetle's walking direction and body orientation. The experiment arena was a horizontal polystyrene foam surface of about 1 m^2 . The global reference frame of the 3D motion capturing system was set in a way that the x and y-axis formed the horizontal plane

parallel to the beetle's walking surface. The z-axis was thus perpendicular to the walking surface. The 3D motion data collected were displayed as three independent segments to represent the two front legs' tibia segments and the body segment (Figure 38 (c)). The motion data were then exported in comma-separated values (CSV) files and analyzed with custom-programmed Matlab® codes. The walking gaits of five beetles were recorded and analyzed.

Figure 38. Motion capturing system for walking gait study. (a) The 3D motion capturing system for tracking the leg and body motion of the beetle. (a1) six T40s VICON® cameras placed at the top of the arena. (a2) The VICON® server computer for data processing and storing. (a3) A personal computer for data analysis. (b) Markers L1, L2, R1, R2, B1, and B2 attached to walking beetle (c) The 3D marker positions captured on screen [75].

5.1.2 Beetle's walking gait study results

We studied the beetle's front leg motion by drawing out the stick diagram of the leg's tibia segment (Figure 39) [76]. Leg motion data when the beetles walked freely in a straight line were used. Figure 39 illustrates the lateral view of the motion trajectory of the tibia segment of the beetle's front leg. As the camera frame rate for motion capturing was set at 100 Hz, thus the time span between two consecutive sticks equals

to 10 ms. The horizontal axis indicates the distance travelled by the tibia segment in the beetle's walking direction. For example, if the beetle travels along the x-axis of the global reference frame (e.g. the y-coordinates of the two markers on the beetle's body remained constant), the horizontal axis of Figure 39 would be the same as the x-axis of the global reference frame. The vertical axis of Figure 39 indicates the vertical distance travelled by the tibia segment of the beetle's front leg (same as the z-axis of the global reference frame). The blue sticks in Figure 39 represent the anterior extreme positions (AEPs) of the beetle's front leg while the red sticks represent the posterior extreme positions (PEPs) of the beetle's front leg. As such, when the sticks progress from a blue stick (AEP) to a consecutive red stick (PEP), this means the leg is performing a power stroke (retraction to swing the leg backward relative to the body). Similarly, when the sticks progress from a red stick (PEP) towards a consecutive blue stick (AEP), this means the leg is performing a return stroke (protraction to swing the leg forward relative to the body). Moreover, an increase of the marker's vertical position indicates that the leg is performing the levation, and a decrease in the marker's vertical position indicates the leg is performing the depression.

Figure 39. Stick diagram for walking gait analysis. The stick diagram illustrates the lateral view of the motion trajectory of the tibia segment of the beetle's front leg. The time span between two consecutive sticks equals to 10 ms. The horizontal axis indicates the distance travelled by the tibia segment in the beetle's walking direction. The vertical axis indicates the vertical distance travelled by the tibia segment of the beetle's front leg [75].

During free walking, the power stroke and return stroke were executed recurrently. The four motion types (protraction, retraction, levation, and depression) were performed at different timings to generate the cyclic leg motions. The duration of each motion type could be extracted from Figure 39 by counting the number of sticks involved in that motion (the time interval between two consecutive sticks equals to 10 ms). A complete walking step is defined as the duration between two consecutive AEPs (indicated by the blue sticks in Figure 39) [76]. Figure 40 shows the duration of the front leg's four

motion types normalized to the corresponding step period (number of beetles = 5, number of steps = 25). The average duration of a complete walking step is 900 ± 600 ms (mean $\pm$ standard deviation). Retraction and depression were executed concurrently in the power stroke and occupied the first 61 ± 22 % of a complete walking step (red bar and green bar in Figure 40). The return stroke consists of the protraction, levation, and depression. The protraction was executed throughout the entire return stroke, while the levation was switched to the depression at 78 ± 15 % of the normalized step duration. The abovementioned leg motion sequence was similar to the firing sequence of the motor neurons seen in the middle leg motion of a free walking stick insect, where the motor neuron pools controlling the retraction and depression were activated in power stroke. Moreover, throughout the return stroke, motor neuron pools controlling the protraction were activated. Next, in the early part of the return stroke, motor neuron pools controlling the levation were activated, and then in the later part of the return stroke, motor neuron pools controlling the depression were activated [50].

Figure 40. Time intervals of the four motions normalized to the corresponding step duration (N = 5 beetles, n = 25 steps). The four motion types (protraction, retraction, levation and depression) were performed at different timings to generate the cyclic power and return strokes during voluntary walking. During protraction and retraction, the whole front leg (coxa, trochanter, femur, tibia, and tarsus) move simultaneously due to the rotation of the coxa. During levation and depression, the rotation of the coxa-trochanter joint moves the femur, tibia, and tarsus together. First, retraction and depression (red and green bars, respectively) were executed concurrently during the power stroke (comprising the first 61+22% of a complete walking step). The percentage standard deviation is indicated by the black error bars. During the following return stroke, protraction (blue bar) was executed throughout, whereas levation (orange bar) was switched to depression (purple bar) at 78+15% of the normalized step duration [75].

5.2 Design of sequential muscle stimulation protocol for walking control

After obtaining the data of the natural leg motion sequences of free walking beetles, sequential stimulation of the leg muscles can be designed in a way to elicit similar leg motion sequences for walking control of the beetle. The elicited leg motions should be able to perform cyclic power strokes and return strokes so that the insect-computer hybrid robot would be able to walk step by step.

5.2.1 Design walking control by sequential stimulation of leg muscles

To build an insect-computer hybrid walking robot, minimally we need to control the front leg's protraction/retraction (for the leg to swing forward and backward in the frontal plane) and the levation/depression (for the leg to lift up from and press down onto the walking surface). Therefore, eight stimulation channels were used to separately control the protraction/retraction and levation/depression of the two front legs. Figure 41 shows the anatomy of one front leg of the beetle with implantations sites (red crosses) to control the protraction/retraction and levation/depression motions.

Figure 41. Insect platform for legged robot with its front leg anatomy. (a) Beetle's front leg anatomy. The protraction and retraction muscles (inside the prothorax) enabling the leg to swing forward and backward about the thorax-coxa joint. (b) The levation and depression muscles (inside the coxa) enable levation and depression motions of the femur about the coxa-trochanter joint. Red crosses mark the sites of stimulation electrodes implanted into each muscle.

Pulse-width modulation (PWM) waves were applied to different muscle groups at predefined timings to elicit desired leg motions for walking control. The PWM stimulation signal was produced from a custom-programmed microcontroller (Texas Instruments, CC2530, $6 \times 6 \text{ mm}^2$, 32 MHz clock) and custom-made external circuit. Using the function of timer interrupt embedded in the CC2530 microcontroller, the pulse width and frequency of the stimulation signal were programmed to be user-adjustable. Figure 42 (a) shows the CC2530 microcontroller with the external circuit for one muscle group stimulation. The control input for the PWM waves' generation was from the input/output (I/O) pins and the ground (GND) pin of the microcontroller.

For the stimulation of each muscle group, the two inputs of an optocoupler (ISOCOM Components, ISP817X) were connect to one I/O pin (set at output mode) and the GND pin of the microcontroller. The two outputs of the optocoupler were connected in series with a 1.5 V battery (Sony Corporation, dry battery, AA R6 SUM3) and the target muscle group. For fast release of extra charge remained in the optocoupler, a 470 Ω resistor (Multicomp, wirewound, 3 W) was connected parallel to the muscle group to be stimulated. As such, the optocoupler functioned as a switch in the muscle stimulation circuit shown in Figure 42 (a). The I/O pins from the CC2530 microcontroller were used to control the on and off of the optocoupler and also used to power the light emitting diode (LED) in the external circuit for the indication of muscle stimulation status. PWM wave with 1 ms pulse width and 1.5 V amplitude could elicit muscle contraction (and hence the corresponding leg motion) at 100% success rate [8]. As such, 1.5 V batteries were used in the muscle stimulation circuit and PWM waves with pulse width of 1 ms were used as the stimulation signals in all the experiments in this study. Figure 42 (b) shows the beetle implanted with sixteen stimulation electrodes (two stimulation electrodes for one muscle group). The distance between the two electrodes implanted in the same muscle group is about 2 mm. As we were only controlling the protraction/retraction and levation/depression of the two front legs, all other leg motions were restricted by inserting insect pins at the corresponding leg joints.

Figure 42. The circuit drawing for the insect-computer hybrid robot. (a) The CC2530 microcontroller with the external circuit for one muscle group stimulation. Eight identical external circuits were controlled separately by Channel 1 to 8 for stimulation of the eight muscle groups inside the beetle's two front legs. (b) The beetle with sixteen stimulation electrodes (two electrodes in one muscle group controlled by one stimulation channel) implanted into eight muscles groups (protraction, retraction, levation, and depression muscle groups in both front legs). Stimulation Channels 1 to 8 generate stimulation signals in predefined sequence for the walking control of the beetle [75].

Various alternating tripod walking gait was adopted by around three million species of insects [48], and a new galloping gait was recently discovered in the species of flightless desert dung beetles (*Pachysoma*) [49]. In the tripod walking gait of a six-legged insect, one leg always moves out of phase with its contralateral pair (i.e. the middle leg on one side moves synchronously with the contralateral front leg and hind leg). On the other hand, in a galloping walking gait, legs of a pair always move synchronously (in-phase). Strictly speaking, the insect-computer hybrid robot in our walking control experiment neither walks in tripod gait nor galloping gait as we only controlled the motion of the two front legs. The middle and hind legs' motion was restricted and the four legs not under control were merely dragged when the insect-

computer hybrid robot walked forward. For convenience, we named the two walking gaits of our insect-computer hybrid robot as tripod and galloping. In real life, a six-legged insect was also observed to move exclusive with two legs. The cockroach (*Periplaneta americana*) was recorded propelling itself only with their two hind legs by increasing its body's angle of attack during fast running (running speed above 1 m/s) [77]. For our insect-computer hybrid robot, as we can control the individual motions of each leg, the locomotion gait can be more flexible compared to the natural walking gaits adopted by insects.

A full walking step of a legged robot involves a power stroke and a return stroke [46]. From the walking gait study, we represented the durations of the four leg motion types as percentages of the corresponding durations of the walking steps (Figure 40). In the actual walking control of the beetle, we simplified the leg motions by making the return stroke consist of protraction and levation only and making the duration of the return stroke and the duration of the power stroke same for easy walking analysis (step frequency and step length analysis). As such, in the actual walking control, the power stroke starts from the leg's AEP when the leg performs both the retraction (for the leg to swing backward) and depression (for the leg to press onto the ground) to generate propulsion for the body to move forward. At the end of the power stroke, the leg is brought to the PEP and the depression is switched to levation. Then the leg performs both the protraction (for the leg to swing forward) and the levation (to lift the leg up from the ground) during the return stroke. At the end of the return stroke, the leg is brought back to the AEP and the levation is switched to depression. As such, the

resulting leg motion control of the beetle consists of cyclic repetition of power strokes and return strokes. The leg muscle stimulation sequence is shown in Table 2. For the beetle's right leg control in both the tripod walking gait and galloping walking gait, stimulation sequence 1 consists of the protraction and the levation for the leg to perform return stroke. The leg is then switched from return stroke to power stroke by activating the protraction and the depression in stimulation sequence 2. Stimulation sequence 3 consists of the retraction and the depression for the leg to perform power stroke. The power stroke is then switched to return stroke by using stimulation sequence 4 where the retraction and the levation are elicited together. The stimulation sequences 1 to 4 were repeated in both the tripod walking control and the galloping walking control to generate cyclic power strokes and return strokes. In tripod walking control, in the same stimulation sequence, the muscle groups being stimulated in the left leg were always the antagonistic muscle groups being stimulated in the right leg. In galloping walking control, the same muscle groups of both the left leg and the right leg were stimulated in the same stimulation sequence so that the two front legs always performed the same motions at the same time. Moreover, the turning control was achieved by continuous stimulation of only one leg for the leg to perform cyclic power stroke and return stroke.

Table 2. Stimulation sequences of the beetles' walking control in tripod and galloping gaits. Filled and empty dots indicate that the stimulation channel is switched on and off during the motion, respectively [75].

Stimulation	Right Leg	Left Leg
-------------	-----------	----------

Sequence for Tripod Walking Gait	Protraction	Retraction	Levation	Depression	Protraction	Retraction	Levation	Depression
1	•	O	•	O	O	•	O	•
2	•	O	O	•	O	•	•	O
3	O	•	O	•	•	O	•	O
4	O	•	•	O	•	O	O	•
Stimulation Sequence for Galloping Walking Gait	Right Leg				Left Leg			
	Protraction	Retraction	Levation	Depression	Protraction	Retraction	Levation	Depression
1	•	O	•	O	•	O	•	O
2	•	O	O	•	•	O	O	•
3	O	•	O	•	O	•	O	•
4	O	•	•	O	O	•	•	O

5.2.2 Walking gait control by sequential stimulation of leg muscles

Figure 43 (a) and (b) illustrated the elicited leg motions when stimulations sequences for tripod and galloping walking control shown in Table 2 were applied respectively, (a-1) to (a-4) and (b-1) to (b-4) represented the stimulation sequence 1 to 4. The beetle's ventral view was used in Figure 43 for clear demonstration of leg motion control.

Figure 43. Sequential leg motion control demonstration for both the tripod walking gait and galloping walking gait. Videos were shot from the beetle's ventral view for clear view of resultant leg motions when predefined stimulation sequence shown in Table 2 was applied. LED lights near the beetle's head indicate the on and off status of the corresponding stimulation channel [75].

5.3 Walking speed and step length control

Besides controlling the beetle's walking gait, the second aim of this study is to control the step frequency and hence to control its walking speed. The control of the robot's step frequency was accomplished by altering the stimulation duration of each stimulation sequence shown in Table 2. The step length is expected to decrease with increasing step frequency as the angular displacement of the leg motion would be smaller when the stimulation duration is reduced. As such, a nonlinear relationship between the walking speed and the step frequency is expected.

5.3.1 Experimental setup for walking speed and step length analysis

As both the walking gait and step frequency of the insect-computer hybrid robot were user-adjustable, experiments studying the walking speed and step length due to step frequency changes in both the tripod walking gait and galloping walking gait were conducted. The horizontal arena for the walking control experiment was made of exterior polystyrene foam with dimension of 120 cm (length) $\times$ 60 cm (width) $\times$ 5 cm (height). Polystyrene foam is a commonly used substrate in insect walk and biomechanical studies [78-85]. A camera (Panasonic® HC-X920M, 20.4 mega pixels, 25 frames per second) was fixed overhead of the beetle and perpendicular to the horizontal walking surface. A 30 cm long ruler was placed parallel to the beetle's walking path for calibration purpose. Six beetles (body length equals to 7.7 cm, 7.6 cm, 7.1cm, 6.9 cm, 6.9 cm, and 6.3 cm respectively) walked at step frequencies of 2 Hz, 1 Hz, 0.5 Hz, 0.25 Hz, and 0.125 Hz in both the tripod and galloping walking gaits were filmed and analyzed. In total, ninety data points were obtained for the walking speed study (nine data points for one step frequency in one walking gait). Within each data point, five continuous steps (excluding the first and the last step) were used to calculate the average walking speed. For the step length analysis, five continuous steps (excluding the first and the last step) at each step frequency and each walking gait were measured to obtain the average step length (in total, 150 data points were obtained for the step length analysis, 75 data points were obtained from the left leg and 75 data points were obtained from the right leg). The walking control films were manually extracted frame by frame by using the Avidemux 2.6® video editor. The extracted pictures were opened in Microsoft® Paint and analyzed pixel by pixel manually. As

shown in Figure 44, the step length was obtained by measuring the distance between consecutive anterior extreme positions (AEPs) of the front leg [76]. The pixel coordinates at the articulation connecting the leg's tibia and tarsus was used to indicate the leg's contacting point with the ground (red crosses on the left leg in Figure 44). The instant that the leg travelled to its AEP was determined by examining the LEDs that indicate the stimulation signal output for walking control (the leg reached its AEP when the protraction and depression muscle groups were stimulated together and just before the protraction muscle group stimulation switched to the retraction muscle group stimulation, refer to Table 2 for more details). Similarly, the walking distance was obtained by measuring the length travelled by the beetle's horn (point indicated by the red crosses on the horn in Figure 44). Dividing the walking distance by the corresponding time interval, we obtained the average walking speed.

Figure 44. Experiment setup for walking speed versus step frequency analysis. (a) Instantaneous image of an insect-computer hybrid robot walking at galloping walking gaits with both front legs at their first AEPs (b) The insect-computer hybrid robot walked one step with both front legs at their second

AEPs. The pixel coordinates of the articulation connecting the beetle's left leg tibia and tarsus were indicated by the red crosses for step length calculation.

5.3.2 Experiment results of walking speed and step length analysis

We investigated the resultant walking speeds of the insect-computer hybrid robot when the stimulation duration of each sequence was set at 0.125 s, 0.25 s, 0.5 s, 1 s, or 2 s (as one walking step was executed when stimulation sequences 1 to 4 were applied, the corresponding step frequency would be 2 Hz, 1 Hz, 0.5 Hz, 0.25 Hz, and 0.125 Hz) for both the tripod walking control and galloping walking control. Figure 45 shows the relationship between the normalized walking speed (body length/s) and the step frequency of six beetles. Within each data point, five continuous steps (excluding the first and the last step) were used to calculate the average walking speed. As we can see from Figure 45, at the same step frequency, the average normalized walking speed of galloping gait was always higher than that of tripod gait. The reason is that during tripod gait walking, the two front legs moved out of phase, the leg that performing the power stroke always tended to not only propel the body forward but also make the body to turn to the contralateral direction. In the galloping gait walking, the two front legs moved synchronously, when both of the front legs performed the power stroke, the body was pushed straight forward in the walking direction. For this reason, at the same step frequency, the average normalized step length (all step lengths are normalized with the corresponding beetle's body length in this study) was larger when the insect-computer hybrid robot walked in galloping walking gait (Figure 45). As such, the galloping gait worked more efficiently in pushing the body forward than the tripod gait,

and the insect-computer hybrid robot walked faster in galloping gait than in tripod gait at the same step frequency.

Figure 45. Average normalized walking speed as a function of step frequency (N = 6 beetles, n = 90 data points). In total, ninety data points were obtained for the walking speed study (nine data points for one step frequency in one walking gait); the black bars indicate the standard deviation of the normalized walking speed. The walking speeds obtained from six beetles were normalized to their respective body lengths. The blue and red numbers indicate the percentage change of the average normalized walking speed when the step frequency was doubled from the previous value.

Figure 46. Insect-computer hybrid robot’s average normalized step length vs step frequency (N = 6 beetles, n = 150 data points). The blue and red numbers indicate the percentage change of the average normalized step length when the step frequency was doubled from the previous value.

The average normalized walking speed increased when the step frequency was increased from 0.125 Hz to 1 Hz for both the tripod and galloping walking gaits. However, the robot’s average normalized walking speed decreased when the step frequency was further doubled from 1 Hz to 2 Hz (Figure 45). The maximum walking speed was observed when the robot walked in galloping gait at step frequency of 1 Hz. The maximum walking speed was 0.26 body length/s (2.00 cm/s). In the walking speed study of both manmade robot [86] and voluntary walking animal [3], the walking speed was observed to be directly proportional to the step frequency. In other words, provided the step length was constant, the walking speed would be doubled if the step frequency

was doubled. However in our case, as shown in Figure 45, the normalized walking speed does not change proportionally with its step frequency (despite the fact that the normalized walking speed increased when the step frequency was increased from 0.125 Hz to 1 Hz). For instance, when the step frequency was doubled from 0.125 Hz to 0.250 Hz, the average normalized walking speed was increased by 77 % for both the tripod and galloping walking gait (blue and red numbers in Figure 45 indicate the percentage change of the averaged normalized walking speed when the robot's step frequency is doubled from the previous value). This is because higher step frequency would result in shorter stimulation duration in each sequence, and shorter stimulation duration would lead to smaller leg angular displacement and hence shorter step length, which is demonstrated in [8]. Therefore, we further investigated the robot's step length at different step frequencies (Figure 46). As we can see from Figure 46, the robot's average normalized step length generally decreased when the step frequency was increased (due to shorter stimulation duration, except when the step frequency was doubled from 0.25 Hz to 0.5 Hz). For instance, when the step frequency was doubled from 1 Hz to 2 Hz, the average normalized step length was reduced by 55 % for tripod walking and reduced by 53 % for galloping walking (in other words, the average normalized step length at 2 Hz step frequency was less than half of the average normalized step length at 1 Hz step frequency for both the tripod and galloping walking gaits). Therefore, despite the step frequency was doubled from 1 Hz to 2 Hz, the average normalized walking speed was reduced by 14 % for the tripod walking and by 15 % for the galloping walking. In summary, change in the stimulation duration in each sequence shown in Table 2 affects not only the step frequency but also the step length.

If we further double the step frequency from 2 Hz to 4 Hz (at 4 Hz step frequency, the stimulation duration in each sequence equals to 62.5 ms), the beetles did not move forward for both the tripod and galloping walking gaits. This is because the stimulation duration of 62.5 ms was too short for the leg to reach significant angular displacement for effectively walking control. The angular displacement as a function of time for all the six motion types (namely protraction/retraction, levation/depression, and extension/flexion) of the front leg at stimulation frequency ranged from 10 Hz to 100 Hz (at 10 Hz increment) and stimulation amplitude equals to 1.5 V had been studied and presented in [8]. For the protraction, levation, and depression, the leg needs around 0.2 to 0.25 second to reach near its maximum angular displacement at 100 Hz stimulation frequency. At very short stimulation duration (e.g. 62.5 ms duration to for 4 Hz step frequency walking), the angular displacement of the leg was too small. When the angular displacement of the levation was not large enough to lift the leg up from the ground, the leg would be in contact with the ground all the time and this made the beetle just moved back and forth with the two front legs remained at the same ground contacting positions.

To overcome the problem of nonlinear relationship between the walking speed and step frequency shown in Figure 45, the step length must be constant when the step frequency is altered. To maintain a constant step length, the strategy adopted by insects is to only change the stance duration while maintain the swing duration while achieving desired walking speed [87-93]. As such additional walking control experiments were conducted where only the stance duration was manipulated to change the step

frequency and hence the walking speed. I only tested the galloping walking gait. This is because, during the galloping gait walking, the two front legs moved synchronously to push the body straight forward in the walking direction. However during the tripod gait walking, the two front legs moved out of phase, the leg that performing the power stroke always tended to not only propel the body forward but also make the body to turn to the contralateral direction. As such it would be more accurate to test the relationship between walking speed and step length in the galloping walking gait. The average stance duration in natural walking beetles is 549 ms, it was then set to be halved (to 274.5 ms) and doubled (to 1098 ms) to test the effects on the resultant step length and walking speed. When the stance duration was halved from 549 ms to 274.5 ms, the average normalized step length was decreased by 23% (Figure 47, $n = 150$ step length, $N = 3$ beetles). This reduction in step length was because the stance time of 274.5 ms was too short for the leg to reach the maximum retraction position. Single factor ANOVA test also indicated that the halved stance duration had significant influence on the step lengths ($F_{1,298} = 98.69$, $p = 2.83 \times 10^{-20}$). Furthermore, the change in stance duration increased the step frequency by 35% (from 0.95 Hz to 1.28 Hz). The tradeoff between the decrease in the step length and increase in the step frequency resulted that the average normalized walking speed increased by 3% (Figure 48, $n = 15$ walking speed values, $N = 3$ beetles). On the other hand, when the stance duration was doubled from 549 ms to 1098 ms, there was no significant change in the step length (single factor ANOVA test, $F_{1,298} = 0.085$, $p = 0.77$). This increment in stance duration reduced the step frequency from 0.95 Hz to 0.62 Hz (35% reduction). As the walking speed equals to the product of the step frequency and step length [94], the average

normalised walking speed reduced by 36% (almost equal to the 35% reduction in step frequency, Figure 48). In individual tested beetles, similar trend was also observed (N = 3). When the stance duration was doubled from 549 ms to 1098 ms, the average step length remained almost constant among the three beetles (changed by +5%, +2%, and - 4% respectively), while the corresponding average walking speed was reduced by 36%, 32%, and 41%, similar to the reduction in the step frequency (i.e. 35%).

Figure 47. Normalized step length as a function of the stance duration. The average and standard deviation of the normalized step lengths were calculated from 150 step length values of 3 beetles. The red number between two adjacent bars shows the percentage change in the average value. The value in brackets below each stance duration is the corresponding step frequency. The average normalized step length remained almost constant (+1%) when the stance duration was increased from 549 ms to 1098 ms.

Figure 48. Normalized walking speed as a function of the stance duration.

The average and standard deviation of the normalized walking speeds were calculated from 15 walking speed values from 3 beetles. Each walking speed value was calculated from the distance travelled by the insect-computer hybrid robot in five continuous steps. The red number between two adjacent bars shows the percentage change in the average value. The value in brackets below each stance duration is the corresponding step frequency. The change in the average normalized walking speed (-36%) was almost the same as the change in the step frequency (-35% from 0.95 Hz to 0.62 Hz) when the stance duration was increased from 549 ms to 1098 ms.

5.4 Remote walking control

As demonstrated in section 5.2 and 5.3 above, the insect-computer hybrid robot's walking gait, step length, and walking speed could be reliably controlled. However, the

robot's operation range was limited by the tether that connects the beetle to the electronic walking control board. To increase the robot's operation range and hence to improve its applicability in real life scenarios, a wirelessly controlled insect-computer hybrid robot must be designed and implemented. As such, a wireless "backpack" that comprised all the essential electronic components for sending out motion control signals and communicating with users was designed for the beetle. Remote walking control by using the wireless "backpack" at both tripod and galloping gaits with all the step frequencies discussed in Section 5.3 (the tethered walking control experiments) had been demonstrated and recorded in video format.

The wireless backpack is a custom designed printed circuit board (PCB) with length and width of 1.8 cm and 1.5 cm respectively. The backpack weights 0.7 g. A custom-programmed microcontroller on the backpack (Chipcon Texas Instruments, CC2530, $6 \times 6 \text{ mm}^2$, 32 MHz clock) was used for remote communication (Zigbee, 2.4 GHz, IEEE 802.15.4 wireless standard) and motion control signal generation. The backpack was powered by Fullriver battery (3.7 V, 20mAh, 0.7 g, and with dimension of $11 \text{ mm} \times 3.8 \text{ mm} \times 14 \text{ mm}$). One end of the stimulation electrode (Teflon-insulated silver wire with diameter of $178 \text{ }\mu\text{m}$, A-M Systems) was connected to backpack through the female connectors shown in Figure 49 (a1). The other end of the stimulation electrode was implanted into the target muscles for motion control (Figure 49 b). The input/output (I/O) ports of the CC2530 microcontroller were used as the leg motion control signal generation terminals. Timer 3 interrupt function of the CC2530 microcontroller was used to generate pulse-width-modulation (PWM) waves of 100 Hz and 1 ms pulse

width from the I/O ports as the stimulation signal. By connecting a $1.4\text{ k}\Omega$ resistor and a $1\text{ k}\Omega$ resistor in series with two I/O ports, the magnitude of the stimulation signal was regulated at about 1.5 V [8]. By using an insect pin (Indigo Instruments, #3 black enamel insect pin), two holes of 0.5 mm diameter were made on the cuticle outside each target muscle for the implantation of motion control electrodes. The exact electrode implantation locations were explained in section 3.3. The insertion depth of the electrode is about 2 mm , measured from the outer surface of the cuticle. The complete assembly of the insect-computer hybrid robot is shown in Figure 49 (b). All the leg motions not under control were restricted by inserting insect pins into the corresponding articulations to avoid undesired interferences with the leg motions under control. All the research works done after this section were based on the remotely controlled insect-computer hybrid walking system.

Figure 49. Backpack and insect-computer hybrid robot. (a 1-3) Top, bottom, and top with battery view of the backpack. The CC2530 microcontroller was preprogrammed to send muscle stimulation signals to sequentially elicit leg motions. (b) Fully assembled insect-computer hybrid robot, sixteen electrodes enabled control signals to be sent to eight leg muscles. *Mecynorrhina torquata* is used as the insect platform.

Chapter 6: Investigation and improvement of beetle leg spine functions in walking

Traction between the foot and substrate plays a vital role in the performance of walking robots and animals [95]. The foot traction is mainly affected by two factors: active leg motion control through neural feedback and passive mechanical structure of the leg that affects the probability of substrate contact [96]. In nature, leg attachment mechanisms differ from animal to animal as a result of evolution for adaptation to environment. Cockroaches (*Periplaneta americana*) use distal tarsal claws for support in inverted walking [97]. It was also shown that the cockroach leg spines can increase the traction on coarse and inclined walking substrate [98]. Geckos use foot hairs or setae for rapid climbing on vertical surfaces [99, 100]. Hairs or macrosetae in a spider (*Hololena adnexa*) legs were proven to increase the walking speed on mesh substrate [96]. As such, studying the leg spine characteristics of the beetle can help in improving the walking capability of the insect-computer hybrid robot.

6.1 Anisotropic function of beetle's natural leg spine

6.1.1 Beetle's natural leg spines prevent slipping in forward walking

Destructive tests were conducted to prove that natural leg spines prevent slipping in forward walking. One leg walking control (the muscles of one leg were stimulated sequentially for the leg to perform alternating power stroke and return stroke) was performed before and after the leg spines were cut (Figure 50, spines in red circle).

Figure 50. Natural leg spines before and after cutting. (a) Overview of the beetle. (b) The beetle's front leg spines (inside the red circle) are curved towards the posterior direction. The spines' curvature created significant traction for the power stroke in forward walking (blue arrow) but reduced the traction for the power stroke in backward walking (orange arrow). (c) The leg spines are removed by cutting (inside the red circle).

The trajectory of the tibia-tarsus articulation was traced using a 3D motion capturing system described in section 3.5. Slip often occurred during the power stroke after the spines were cut (Figure 51 (a) compared to Figure 51 (b)). In detail, 11 slips were observed in 163 steps before the spines were cut ($N = 3$ beetles, 6.7% slip occurrence); whereas 119 slips were observed in 120 steps ($N = 3$ beetles, 99% slip occurrence) after

the spines were cut. The slip distance was 3.4 ± 2.0 mm (mean $\pm$ standard deviation, $N = 3$ beetles). This concludes that the presence of spines on leg helps in preventing slip in forward walking.

Figure 51. Tibia-tarsus trajectories before and after the removal of leg spines. Blue lines are the tibia-tarsus trajectories traced under a 3D motion capturing system. (a) No slip was observed in forward walking power stroke before the leg spines were cut. (b) Obvious slipping (indicated by the red dotted arrows) occurred after the leg spines were cut. This proved that the natural leg spines provide significant traction in the forward walking power stroke.

6.1.2 Anisotropic leg spines resulted frequent slipping in backward walking

The beetle's leg spines are curved towards the posterior direction (Figure 50, inside the red circle). This curvature makes the leg spine anisotropic. As such, it was suspected

that the gripping efficiency would be reduced in backward walking, as the direction of backward walking power stroke (orange arrow in Figure 50) is in the opposite direction of the natural leg spine curvature.

We noted the beetles walk backwards rarely. Hence, we developed a new protocol of electrical stimulation of leg muscles to elicit leg motions in certain sequence making the beetle capable of performing both forward and backward walking gaits (Table 3). In both forward and backward walking control, the sequences 1 and 2 enabled the return stroke while the sequence 3 enabled the power stroke of both front legs (Table 3). The difference between the forward and backward walking control is that the stimulation of the protraction and retraction muscles were switched. The stimulation protocol was implemented to a beetle using the custom-made wireless walking control device mountable on the beetle (the wireless backpack for walking control was described in section 5.4).

Table 3. Stimulation sequences for forward and backward walking control in galloping walking gait

	both front legs			
	protraction	retraction	levation	depression
stimulation sequence for forward walking				
1	● ^a	○ ^b	●	○
2	●	○	○	●
3	○	●	○	●
stimulation sequence for backward walking				
1	○	●	●	○
2	○	●	○	●
3	●	○	○	●

a. The black dot represents the stimulation channel for a particular motion is switched on

b. The empty dot represents the stimulation channel for a particular motion is switched off

Frequent slipping of the leg was observed in backward walking control. The beetle turned to the direction in which the leg slipped longer distances, or the beetle would stay at a constant location if the slipping of both legs happened throughout the power stroke. The leg slipping in backward walking made the walking path of the robot unpredictable and reduced the applicability of the robot (Figure 52 (a)). This finding concludes that the natural leg spines assist the leg gripping efficiency only in forward walking, but it is significantly reduced in backward walking. Furthermore, in the field of man-made robots, bio-inspired artificial leg spines were designed to improve robots' walking, climbing or jumping performances [79, 101-104]. However, those

abovementioned artificial leg spines are also anisotropic: the leg spines only increase the traction in one specific direction and thus restricted the robots' performance.

Figure 52. Body trajectory before and after adding the artificial leg spines.

(a) A typical body trajectory of backward walking before the addition of artificial isotropic leg spines. (b) A typical body trajectory of backward walking after the addition of artificial isotropic leg spines. The body trajectory became more linear after the addition of artificial leg spines (red dotted lines represent the linear regression lines of the body trajectories). This proved that the artificial isotropic leg spines significantly improved the leg traction in backward walking.

6.2 Implementation of artificial isotropic leg spine to enable both forward and backward walking

To prevent leg slipping in backward walking, an artificial leg spine was added near the leg's tibia-tarsus articulation (Figure 53). We designed the artificial leg spine to be ~1 mm longer than the natural leg spine, and we set it so that the angle between the artificial and natural spines was 45° (Figure 53 (b)). As such, the artificial leg spines became the sole ground contact points and the natural leg spines were thus suspended in air. The isotropic artificial leg spine (not curved towards any direction) provides a passive mechanism to create traction not only in backward walking but also in forward walking. Without the artificial leg spine, the backward walking trajectory of the robot was unpredictable due to frequent slipping of the legs (Figure 52 (a)). With the addition of the artificial leg spines, the walking trajectory became more linear (Figure 52 (b)). Statistically, the least-squares linear regression line was fitted to both walking paths with and without artificial leg spines. The $R^2 = 0.94 \pm 0.05$ for walking path with artificial leg spines was much larger than $R^2 = 0.27 \pm 0.28$ for walking path without artificial leg spines ($N = 4$ beetles, $n = 20$ trials). Single factor ANOVA test confirmed that the artificial leg spine provided significant improvements on the linearity of backward walking trajectory ($F_{1,38} = 114.9$, $p = 4.78 \times 10^{-13}$). These results conclude that with the implementation of isotropic artificial spine resulted significant improvement in the backward walking.

Figure 53. Artificial leg spine added to the leg. (a) A segment of insect pin (#3, Indigo Instruments) was used as the artificial leg spine. By being straight (not curved in any direction and hence isotropic), the artificial leg spine provides a passive mechanism that provides traction for power strokes in the both forward and backward directions. (b) Side view of the artificial spine on leg. The artificial leg spine was about 45° with respect to the tip of the natural leg spine.

In previous studies of artificial leg spine, researchers designed anisotropic spines for legged robots to increase the foot traction in only forward walking direction [101-104].

However, as demonstrated in this paper, anisotropic spines reduce the backward

walking capability of a legged robot. The design of isotropic leg spine improved the robot's performance by ensuring efficient leg gripping in all motion directions. Furthermore, the development of the remotely controlled beetle-computer hybrid robot helped in the study of biomechanical hypothesis that is otherwise difficult to test.

Chapter 7: Conclusion and future works

7.1 Conclusion

In this dissertation, the controlling of a beetle's front leg motions in graded and closed-loop manners at low power consumption was demonstrated. We also controlled the beetle to walk in both the tripod and galloping walking gaits. By altering the step frequency, the step length and walking speed were adjustable. This is the first demonstration of living insect locomotion control with user-adjustable walking gait, step length and walking speed. A wireless control backpack was developed and enabled the remote walking control of the beetle. Furthermore, the beetle's natural leg spines were proved to be anisotropic that only enabled efficient foot traction in forward walking. An isotropic artificial leg spine was designed to enable efficient leg gripping in both forward and backward walking of the insect-computer hybrid robot. Below are the detailed conclusions of the novel contributions made to relevant research fields presented in this dissertation.

7.1.1 Graded and closed-loop control of a biological actuator

The appendages are the key components in insect locomotion. The ability of precisely controlling the insect leg to build a biological actuator enabled the creation of an insect-computer hybrid legged robot. This dissertation demonstrated the fine manipulation of the beetle's leg motion in graded and close-loop manner. The confirmed leg motion control techniques would open a new realm of insect walking control by permitting the ability of manipulating the individual leg motion magnitude and speed. More

complicated insect walking maneuvers with various gaits and speed would be then achieved. Moreover, the confirmed high repeatability of leg motion elicitation proved the robustness of the insect-computer hybrid robot created by using the techniques demonstrated in this dissertation.

7.1.2 Insect-computer hybrid robot with user-adjustable walking gait and speed

The inability of controlling the walking gait or the speed of an insect-computer hybrid robot persisted for decades since the creation of the first cyborg insect. The first demonstration of manipulating a living insect's walking gait and speed is presented in this dissertation. The ability of walking gait and speed control greatly improved the applicability of the insect-computer hybrid robot and filled the existing research gaps. Due to the similarity in the leg muscle configuration among insects, walking control protocol presented in this study would be easily implemented on other insects with little modification.

7.1.3 New approach for biomechanical study

Creation of insect-computer hybrid robot by controlling individual appendage motions provided a new approach to test biomechanical hypothesis. For example, the traditional method of confirming the leg spine function (Chapter 6 in dissertation) was testing a single leg in isolation with artificial loading conditions. However, disadvantages such as 1) the interactions between all legs are neglected. 2) The loading direction and magnitude will not be exactly the same as the real walking scenarios. 3) No direct conclusion on the leg spine functions regarding the overall walking performance.

It is obvious that testing of the robot as a whole is more direct and meaningful than just testing an isolated leg. The implementation of insect-computer hybrid robot demonstrated in this study is a novel method (first demonstration in biomechanical research field) for biomechanical hypothesis testing and opens a new way for researchers to test the biological entity as a whole.

7.2 Future works

The current control methods enabled reliable control of the motions of the beetle's two front legs to create an insect-computer hybrid robot with adjustable walking speed and gait. The future works to be done to improve the applicability are listed below.

7.2.1 Control of all the six legs of the beetle

The control protocol should be improved so that to control the motions of all the six legs is feasible. With the ability of controlling all the six legs, more efficient walking control will be realized (compared with the current situation that the middle and hind legs are merely dragged behind). Experiments of the comparison of the maximum walking speed of the insect-computer hybrid robot and the natural walking beetle will also be carried out. Furthermore, with the ability of tracking the leg motions in real time, closed-loop control techniques demonstrated in section 4.2 can be implemented to achieve walking control with higher accuracy in step length, walking speed, and leg trajectories compared to current achievements.

To achieve the control of all the six legs of the beetle. First anatomical study needs to be done on the middle and hind legs in a similar manner as the anatomy done on the

front legs. By conducting the anatomical studies, the muscle locations and function controlling the motions of the middle and hind legs can then be confirmed. Similar electrode implantation techniques and stimulation protocols to the front leg motion control will be implemented to control the leg motions of the middle and hind legs. One research challenge in the controlling of all the six legs' motions will be the increasing difficulties in the electrode implantations. In current muscle stimulation protocol, two electrodes are needed to control the contraction of one muscle. In the front legs motion control demonstrated in this thesis, sixteen electrodes were implanted into the eight muscles of two front legs. As such, at least forty eight electrodes need to be implanted into the leg muscles to control all the motions of the six legs. The large amount of implanted electrodes may be practically difficult. One possible solution to reduce the total number of implanted electrodes will be finding a location on the beetle body where a common ground electrode can be implanted. By using the common ground electrode, only one electrode is needed to be implanted into each muscle. As such, the total number of implanted electrodes can be reduced to twenty five for controlling all the six legs.

7.2.2 Building internal and external insect locomotion control models

To act efficiently in natural environment, insects sense the surroundings by perceptive sensing organs. The surrounding information was sent and processed by the insect's central processing unit (i.e. the brain). Actions commands will be generated by the brain in order for the insect to react properly to the surrounding environment [105]. The sensorimotor control models of human and non-human primates have been developed

in the past few decades [106-110]. However, the reliance on internal sensorimotor models for actions guidance in insects is still not clearly understood [111, 112]. Thus constructing the insect's intrinsic internal neural network model would further aid in the design of more advanced control algorithms (e.g. optimal closed-loop control, predictive control, Bayesian decision theory etc.) for insect-computer hybrid robots [113]. Moreover, construction of the insect's external kinematics models would facilitate in the motion control algorithm testing in the early development stage [114, 115], negating the necessity of testing on the real robots. In conclusion, coupling the insect's internal sensorimotor model with the external kinematic model would help in the development of better locomotion algorithms.

Due to the fact that the neurons are often physiologically identifiable and the neural circuits are relatively traceable, insects are suitable and even attractive models for studying the sensorimotor models that take input (i.e. visual stimulation or mechanical stimulation) and give out corresponding output (i.e. motor neurons activation and hence locomotion behavior). Thanks to the recent development in genetic engineering, genetic tools make the labelling, targeting, and manipulating of specific neurons possible [116]. Integration of neural tracing with photoactivatable protein with electrophysiology, optic mapping, and laser-mediated micro-lesioning had successfully demonstrated the neuron circuit mapping from activation of sensory neurons to the excitation of the transmitting neuron in *Drosophila* [117]. However, the insect's intrinsic neural network is a dynamic model. As such, the same sensory input may activate various motor neuron outputs depending on the instantaneous states (i.e. the

posture, the instantaneous locomotion parameters, the time of the day, and the previous experience of the insect etc.). As such the construction of the insect's dynamic neural network model will be computationally complicated and challenging. Huge amount of experimental data is expected to be analyzed. Therefore, suitable machine learning algorithms should be developed to collect and analyze the experimental data. The neural network model should be automatically built by the machine learning algorithms instead of done manually.

7.2.3 Sensors to be integrated onto the backpack

Various sensors were implemented on manmade robots for search and rescue missions and environmental monitoring [118-121]. However, the manmade rescue robots are unable to pass through small spaces due to the relatively large size. As such, sensors should be added to the wireless control board on the insect-computer hybrid robot to collect real time environment information (such as sound source, temperature, and chemical detection etc.) in search and rescue missions. The information collected by the embedded sensors could also be used as feedback signal for autonomous walking control. For instance, during a rescue mission at a disaster site, with the sound sensors locating the direction of survivors, the robot would be able to walk towards the survivors with the implementation of appropriate locomotion control algorithm.

Searches on sensors with suitable size for the wireless “backpack” had been done. For the environment carbon dioxide (CO₂) and total volatile organic compounds (TVOC) detection (to detect the presence of human), the CCS811 ultra-low power digital gas sensor is selected. With dimensions of 4.0 mm × 2.7 mm × 1.1 mm (length × width ×

height), the CCS811 gas sensor can be easily mounted onto the wireless “backpack”. The supply voltage to the CCS811 sensor is 1.8 to 3.6 V that is compatible with the power source of the “backpack”. With the operation conditions at -40 °C to 85 °C and 10% to 95% relative humidity, the CCS811 gas sensor is able to operate in the future search and rescue missions conducted by the insect-computer hybrid system. Moreover, infrared sensors can be used for sensing the ambient temperature and aid in the detection of human beings. The HTPA 8×8 thermopilearrays are considered a suitable sensor to be integrated onto the wireless backpack. The HTPA 8×8 sensor has dimensions of 5.31 mm in diameter and 4.55 mm in height and can be easily mounted onto the “backpack”. With the operation temperature ranging from -20 °C to 85 °C, the HTPA 8×8 temperature sensor is suitable for the detection of human presence.

7.2.4 Positioning system to be integrated onto the motion control architecture

Integration of global positioning system (GPS) and inertial measurement unit (IMU, a combination of electronic accelerometer and gyroscope) onto the backpack would enable the position and orientation tracking of the robot [122-125]. With the aid of position and orientation information, control software can be designed to make the insect-computer hybrid robot autonomous. For example, the user only need to input the desired destination, the robot will go there automatically (the software will tell the robot when to turn and when to go straight) without the need of real-time user control commands. To further illustrate the feasibility of implementing GPS on insects, the detailed specifications of a GPS chip (U-blox, UBX-M8230-CT) are provided below. The UBX-M8230-CT chip has a dimension of $3.21 \times 2.99 \times 0.36$ mm (length $\times$ width $\times$

height). The average power consumption of the chip is less than 10 mW in instantaneous tracking. The low power consumption makes it suitable in the application of remotely controlled insect-computer hybrid system. The required supply voltage of the GPS chip is 1.4 V to 3.6 V which can be easily integrated into the current “backpack” for the beetle motion control. The horizontal position accuracy that the chip can provide is 2.5 m circular error probable (CEP).

List of publications

- [1] **Cao, F.**, Zhang, C., Choo, H.Y. & Sato, H. 2016 Insect–computer hybrid legged robot with user-adjustable speed, step length and walking gait. *J R Soc Interface* **13**. (doi:10.1098/rsif.2016.0060). (cover article, Altmetric Score: 388, In the top 5% of all research outputs scored by Altmetric, One of the highest-scoring outputs from this journal (#8 of 1,423))
- [2] **F. Cao**, C. Zhang, H. Y. Choo, and H. Sato, "Insect-machine hybrid robot: Insect walking control by sequential electrical stimulation of leg muscles," in *Robotics and Automation (ICRA), 2015 IEEE International Conference on*, 2015, pp. 4576-4582. Oral presentation.(acceptane rate 41%)
- [3] **Cao, F.**, Zhang, C., Vo Doan, T.T., Li, Y., Sangi, D.H., Koh, J.S., Huynh, N.A., Aziz, M.F.B., Choo, H.Y., Ikeda, K., et al. 2014 A Biological Micro Actuator: Graded and Closed-Loop Control of Insect Leg Motion by Electrical Stimulation of Muscles. *PLoS ONE* **9**, e105389. (doi:10.1371/journal.pone.0105389).
- [4] **Cao, F.** & Sato, H. 2017 Remote Radio Controlled Insect-Computer Hybrid Legged Robot. In *Transducers, 2017 IEEE International Conference on*. Oral presentation. (acceptane rate 55%)
- [5] **Cao, F.** & Sato, H. Anisotropic and isotropic functions of natural and artificial leg spines in insect locomotion verified by remotely controlled tetherless walking. *J R Soc Interface*. (under review)

- [6] Zhang, C., **Cao, F.**, Li, Y. & Sato, H. 2016 Fuzzy-controlled living insect legged actuator. *Sensors and Actuators A: Physical* **242**, 182-194.
- [7] Li, Y., **Cao, F.**, T. T. V., & Sato, H. (2016). Controlled banked turns in coleopteran flight measured by a miniature wireless inertial measurement unit. *Bioinspiration & Biomimetics*, *11*(5), 056018.
- [8] Doan, T.V., Li, Y., **Cao, F.** & Sato, H. 2015 Cyborg beetle: Thrust control of free flying beetle via a miniature wireless neuromuscular stimulator. In *2015 28th IEEE International Conference on Micro Electro Mechanical Systems (MEMS)* (pp. 1048-1050), IEEE. (acceptance rate 41%)
- [9] Choo, H.Y., Li, Y., **Cao, F.** & Sato, H. 2016 Electrical Stimulation of Coleopteran Muscle for Initiating Flight. *PloS one* **11**, e0151808.

References

- [1] R. J. Full, T. Kubow, J. Schmitt, P. Holmes, and D. Koditschek, "Quantifying dynamic stability and maneuverability in legged locomotion," *Integrative and Comparative Biology*, vol. 42, pp. 149-157, Feb 2002.
- [2] F. Delcomyn, and M. E. Nelson, "Architectures for a biomimetic hexapod robot," *Robotics and Autonomous Systems*, vol. 30, pp. 5-15, Jan 31 2000.
- [3] R. J. Full, and M. S. Tu, "Mechanics of 6-legged runners," *Journal of Experimental Biology*, vol. 148, pp. 129-146, Jan 1990.
- [4] R. J. Full, R. Blickhan, and L. H. Ting, "LEG DESIGN IN HEXAPEDAL RUNNERS," *Journal of Experimental Biology*, vol. 158, pp. 369-390, Jul 1991.
- [5] R. J. Full, D. R. Stokes, A. N. Ahn, and R. K. Josephson, "Energy absorption during running by leg muscles in a cockroach," *Journal of Experimental Biology*, vol. 201, pp. 997-1012, Apr 1998.
- [6] R. D. Beer, H. J. Chiel, R. D. Quinn, and R. E. Ritzmann, "Biorobotic approaches to the study of motor systems," *Curr Opin Neurobiol*, vol. 8, pp. 777-782, Dec 1998.
- [7] R. E. Ritzmann, R. D. Quinn, J. T. Watson, and S. N. Zill, "Insect walking and biorobotics: A relationship with mutual benefits," *Bioscience*, vol. 50, pp. 23-33, Jan 2000.
- [8] F. Cao, C. Zhang, T. T. Vo Doan, Y. Li, D. H. Sangi, J. S. Koh, N. A. Huynh, M. F. B. Aziz, H. Y. Choo, K. Ikeda, P. Abbeel, M. M. Maharbiz, and H. Sato, "A Biological Micro Actuator: Graded and Closed-Loop Control of Insect Leg

- Motion by Electrical Stimulation of Muscles," *PLoS ONE*, vol. 9, p. e105389, 2014.
- [9] H. Sato, C. W. Berry, Y. Peeri, E. Baghoomian, B. E. Casey, G. Lavella, J. M. VandenBrooks, J. Harrison, and M. M. Maharbiz, "Remote radio control of insect flight," *Front Integr Neurosci*, vol. 3, p. 24, 2010.
- [10] N. J. Kohut, P. M. Birkmeyer, K. C. Peterson, and R. S. Fearing, "Maneuverability and mobility in palm-sized legged robots," in *Micro- and Nanotechnology Sensors, Systems, and Applications Iv*. vol. 8373, T. George, M. S. Islam, and A. Dutta, Eds., ed Bellingham: Spie-Int Soc Optical Engineering, 2012, pp. 83731I-837319.
- [11] K. Shoji, Y. Akiyama, M. Suzuki, T. Hoshino, N. Nakamura, H. Ohno, and K. Morishima, "Insect-mountable biofuel cell with self-circulation system," in *Proceedings of the 2012 IEEE 25th International Conference on Micro Electro Mechanical Systems (MEMS)*, Paris, 2012, pp. 1249-1252.
- [12] T. Reissman, R. B. MacCurdy, and E. Garcia, "Electrical power generation from insect flight," *P Soc Photo-opt Ins*, vol. 7977, pp. 797702-797709, 2011 2011.
- [13] K. Shoji, Y. Akiyama, M. Suzuki, T. Hoshino, N. Nakamura, H. Ohno, and K. Morishima, "Insect biofuel cells using trehalose included in insect hemolymph leading to an insect-mountable biofuel cell," *Biomedical Microdevices*, vol. 14, pp. 1063-1068, Dec 2012.

- [14] L. Halamkova, J. Halamek, V. Bocharova, A. Szczupak, L. Alfonta, and E. Katz, "Implanted biofuel cell operating in a living snail," *J Am Chem Soc*, vol. 134, pp. 5040-5043, Mar 2012.
- [15] K. Shoji, Y. Akiyama, M. Suzuki, N. Nakamura, H. Ohno, and K. Morishima, "Gold nanoparticle-based biofuel cell using insect body fluid circulation," in *Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS & EUROSENSORS XXVII), 2013 Transducers & Eurosensors XXVII: The 17th International Conference on*, Barcelona, 2013, pp. 2811-2814.
- [16] K. Shoji, Y. Akiyama, M. Suzuki, N. Nakamura, H. Ohno, and K. Morishima, "Diffusion refueling biofuel cell mountable on insect," in *Micro Electro Mechanical Systems (MEMS), 2014 IEEE 27th International Conference on*, San Francisco, CA, 2014, pp. 163-166.
- [17] H. Sato, T. T. V. Doan, S. Kolev, N. A. Huynh, C. Zhang, T. L. Massey, J. van Kleef, K. Ikeda, P. Abbeel, and M. M. Maharbiz, "Deciphering the Role of a Coleopteran Steering Muscle via Free Flight Stimulation," *Current Biology*, vol. 25, pp. 798-803, Mar 2015.
- [18] H. Z. Wang, N. Y. Ando, and R. H. Kanzaki, "Active control of free flight manoeuvres in a hawkmoth, *Agrius convolvuli*," *J Exp Biol*, vol. 211, pp. 423-432, Feb 2008.
- [19] T. B. Tubbs, A. N. Palazotto, and M. A. Willis, "Biological investigation of wing motion of the *Manduca Sexta*," *International Journal of Micro Air Vehicles*, vol. 3, pp. 101-117, Jun 2011.

- [20] A. Bozkurt, R. F. Gilmour, and A. Lal, "Balloon-assisted flight of radio-controlled insect biobots," *IEEE Trans Biomed Eng*, vol. 56, pp. 2304-2307, Sep 2009.
- [21] W. M. Tsang, A. L. Stone, Z. N. Aldworth, J. G. Hildebrand, T. L. Daniel, A. I. Akinwande, and J. Voldman, "Flexible split-ring electrode for insect flight biasing using multisite neural stimulation," *IEEE Trans Biomed Eng*, vol. 57, pp. 1757-1764, Jul 2010.
- [22] A. Bozkurt, R. F. Gilmour, A. Sinha, D. Stern, and A. Lal, "Insect-Machine Interface Based Neurocybernetics," *Ieee Transactions on Biomedical Engineering*, vol. 56, pp. 1727-1733, Jun 2009.
- [23] A. Bozkurt, R. Gilmour, D. Stern, A. Lal, and Ieee, "MEMS based bioelectronic neuromuscular interfaces for insect cyborg flight control," in *Mems 2008: 21st Ieee International Conference on Micro Electro Mechanical Systems, Technical Digest*, ed New York: Ieee, 2008, pp. 160-163.
- [24] A. J. Hinterwirth, B. Medina, J. Lockey, D. Otten, J. Voldman, J. H. Lang, J. G. Hildebrand, and T. L. Daniel, "Wireless stimulation of antennal muscles in freely flying hawkmoths leads to flight path changes," *PLoS One*, vol. 7, p. e52725, Dec 2012.
- [25] T. Latif, A. Bozkurt, and Ieee, "Line Following Terrestrial Insect Biobots," in *2012 Annual International Conference of the Ieee Engineering in Medicine and Biology Society (Embc)*, San Diego, CA, 2012, pp. 972-975.

- [26] C. J. Sanchez, C.-W. Chiu, Y. Zhou, J. M. Gonzalez, S. B. Vinson, and H. Liang, "Locomotion control of hybrid cockroach robots," *Journal of the Royal Society Interface*, vol. 12, Apr 6 2015.
- [27] E. Whitmire, T. Latif, A. Bozkurt, and Ieee, "Kinect-based System for Automated Control of Terrestrial Insect Biobots," presented at the 2013 35th Annual International Conference of the Ieee Engineering in Medicine and Biology Society (Embc), Osaka, 2013.
- [28] R. Holzer, and I. Shimoyama, "Locomotion control of a bio-robotic system via electric stimulation," in *Proceedings of the 1997 IEEE/RSJ International Conference on Intelligent Robots and Systems, 1997. IROS '97.*, Grenoble, 1997, pp. 1514-1519.
- [29] A. Bozkurt, E. Lobaton, M. Sichitiu, T. Hedrick, T. Latif, A. Dirafzoon, E. Whitmire, A. Verderber, J. Marin, and H. Xiong, "Biobotic Insect Swarm based Sensor Networks for Search and Rescue," *Signal Processing, Sensor/Information Fusion, and Target Recognition Xxiii*, vol. 9091, 2014.
- [30] T. E. Moore, S. B. Crary, D. E. Koditschek, and T. A. Conklin, "Directed locomotion in cockroaches: biobots," *Acta entomologica slovenica*, vol. 6, pp. 71-78, 1998.
- [31] D. R. Lemmerhirt, E. M. Staudacher, and K. D. Wise, "A multitransducer microsystem for insect monitoring and control," *Ieee Transactions on Biomedical Engineering*, vol. 53, pp. 2084-2091, Oct 2006.
- [32] Z. L. Yang, K. Y. Chun, J. Y. Xu, F. Lin, D. Q. Li, H. L. Ren, and Ieee, "A Preliminary Study of Motion Control Patterns for Biorobotic Spiders,"

presented at the 11th Ieee International Conference on Control and Automation (Icca), Taichung, 2014.

- [33] R. F. Chapman, S. J. Simpson, and A. E. Douglas, *The Insects: Structure and Function*: Cambridge University Press, 2012.
- [34] D. C. Daly, P. P. Mercier, M. Bhardwaj, A. L. Stone, Z. N. Aldworth, T. L. Daniel, J. Voldman, J. G. Hildebrand, and A. P. Chandrakasan, "A pulsed UWB receiver SoC for insect motion control," *IEEE J Solid-State Circuits*, vol. 45, pp. 153-166, Jan 2010.
- [35] T. Latif, E. Whitmire, T. Novak, and A. Bozkurt, "Sound Localization Sensors for Search and Rescue Biobots," *Sensors Journal, IEEE*, vol. PP, pp. 1-1, 2015.
- [36] J. C. Erickson, M. Herrera, M. Bustamante, A. Shingiro, and T. Bowen, "Effective Stimulus Parameters for Directed Locomotion in Madagascar Hissing Cockroach Biobot," *PloS one*, vol. 10, p. e0134348, 2015.
- [37] S. L. Giampalmo, B. F. Absher, W. T. Bourne, L. E. Steves, V. V. Vodenski, P. M. O'Donnell, and J. C. Erickson, "Generation of complex motor patterns in american grasshopper via current-controlled thoracic electrical interfacing," in *Engineering in Medicine and Biology Society, EMBC, 2011 Annual International Conference of the IEEE*, 2011, pp. 1275-1278.
- [38] Z. Chuan, L. Jingquan, T. Hongchang, K. Xiaoyang, R. Yuefeng, Y. Bin, Z. Hongying, and Y. Chunsheng, "Control of swimming in crucian carp: Stimulation of the brain using an implantable wire electrode," in *Nano/Micro Engineered and Molecular Systems (NEMS), 2013 8th IEEE International Conference on*, Suzhou, 2013, pp. 360-363.

- [39] C. Zhang, J.-Q. Liu, H.-C. Tian, X.-Y. Kang, J.-C. Du, Y.-F. Rui, B. Yang, and C.-S. Yang, "Implantable electrode array with platinum black coating for brain stimulation in fish," *Microsystem Technologies-Micro-and Nanosystems-Information Storage and Processing Systems*, vol. 21, pp. 139-145, Jan 2015.
- [40] A. J. Chung, B. Cordovez, N. Jasuja, D. J. Lee, X. T. Huang, and D. Erickson, "Implantable microfluidic and electronic systems for insect flight manipulation," *Microfluidics and nanofluidics*, vol. 13, pp. 345-352, 2012.
- [41] W. M. Tsang, A. Stone, Z. Aldworth, D. Otten, A. I. Akinwande, T. Daniel, J. G. Hildebrand, R. B. Levine, and J. Voldman, "Remote control of a cyborg moth using carbon nanotube-enhanced flexible neuroprosthetic probe," in *Micro Electro Mechanical Systems (MEMS), 2010 IEEE 23rd International Conference on*, Wanchai, Hong Kong, 2010, pp. 39-42.
- [42] K. Mann, T. L. Massey, S. Guha, J. P. van Kleef, and M. M. Maharbiz, "A wearable wireless platform for visually stimulating small flying insects," in *Engineering in Medicine and Biology Society (EMBC), 2014 36th Annual International Conference of the IEEE*, Chicago, IL, 2014, pp. 1654-1657.
- [43] B. Li, Z. Nenggan, Z. Huixia, H. Yaoyao, Z. Huoqing, H. Fuliang, and Z. Xiaoxiang, "Flight control of tethered honeybees using neural electrical stimulation," in *Neural Engineering (NER), 2011 5th International IEEE/EMBS Conference on*, Cancun, 2011, pp. 558-561.
- [44] H. Zhao, N. Zheng, W. A. Ribi, H. Zheng, L. Xue, F. Gong, X. Zheng, and F. Hu, "Neuromechanism Study of Insect–Machine Interface: Flight Control by Neural Electrical Stimulation," *PLoS ONE*, vol. 9, p. e113012, 2014.

- [45] J. Okada, and Y. Toh, "The role of antennal hair plates in object-guided tactile orientation of the cockroach (*Periplaneta americana*)," *Journal of Comparative Physiology a-Sensory Neural and Behavioral Physiology*, vol. 186, pp. 849-857, Sep 2000.
- [46] C. Chasserat, and F. Clarac, "Basic processes of locomotor coordination in the rock lobster .2. simulation of leg coupling," *Biological Cybernetics*, vol. 55, pp. 171-185, 1986.
- [47] U. Muller, and H. Cruse, "The contralateral coordination of walking legs in the crayfish *Astacus-Leptodactylus* .2. model-calculations," *Biological Cybernetics*, vol. 64, pp. 437-446, 1991.
- [48] D. M. Wilson, "Insect walking," *Annual Review of Entomology*, vol. 11, pp. 103-&, 1966.
- [49] J. Smolka, M. J. Byrne, C. H. Scholtz, and M. Dacke, "A new galloping gait in an insect," *Current Biology*, vol. 23, pp. R913-R915, Oct 21 2013.
- [50] H. Fischer, J. Schmidt, R. Haas, and A. Buschges, "Pattern generation for walking and searching movements of a stick insect leg. I. Coordination of motor activity," *Journal of Neurophysiology*, vol. 85, pp. 341-353, Jan 2001.
- [51] E. Whitmire, T. Latif, and A. Bozkurt, "Acoustic sensors for biobotic search and rescue," in *SENSORS, 2014 IEEE*, 2014, pp. 2195-2198.
- [52] A. N. Ahn, and R. J. Full, "A motor and a brake: two leg extensor muscles acting at the same joint manage energy differently in a running insect," *Journal of Experimental Biology*, vol. 205, pp. 379-389, 2002.

- [53] U. Bässler, and W. Stein, "Contributions of structure and innervation pattern of the stick insect extensor tibiae muscle to the filter characteristics of the muscle-joint system," *Journal of Experimental Biology*, vol. 199, pp. 2185-2198, 1996.
- [54] D. Ballantyne, and W. Rathmayer, "On the function of the common inhibitory neurone in the walking legs of the crab, *Eriphia spinifrons*," *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, vol. 143, pp. 111-122, 1981.
- [55] V. Brezina, and K. R. Weiss, "The neuromuscular transform constrains the production of functional rhythmic behaviors," *Journal of Neurophysiology*, vol. 83, pp. 232-259, 2000.
- [56] R. J. Full, "Invertebrate locomotor systems," *Comprehensive Physiology*, 1997.
- [57] R. Josephson, "Contraction dynamics and power output of skeletal muscle," *Annual review of physiology*, vol. 55, pp. 527-546, 1993.
- [58] P. Meyrand, and E. Marder, "Matching neural and muscle oscillators: control by FMRFamide-like peptides," *Journal of Neuroscience*, vol. 11, pp. 1150-1161, 1991.
- [59] L. G. Morris, and S. L. Hooper, "Muscle response to changing neuronal input in the lobster (*Panulirus interruptus*) stomatogastric system: spike number-versus spike frequency-dependent domains," *Journal of Neuroscience*, vol. 17, pp. 5956-5971, 1997.
- [60] J. G. Malamud, and R. K. Josephson, "Force velocity relationships of a locust flight-muscle at different times during a twitch contraction," *Journal of Experimental Biology*, vol. 159, pp. 65-87, Sep 1991.

- [61] C. Guschlbauer, H. Scharstein, and A. Bueschges, "The extensor tibiae muscle of the stick insect: biomechanical properties of an insect walking leg muscle," *J Exp Biol*, vol. 210, pp. 1092-1108, Mar 15 2007.
- [62] P. N. R. Usherwood, "Insect Neuromuscular Mechanisms," *American Zoologist*, vol. 7, pp. 553-582, 1967.
- [63] C. Govind, and J. Pearce, "Remodeling of multiterminal innervation by nerve terminal sprouting in an identifiable lobster motoneuron," *Science*, vol. 212, pp. 1522-1524, 1981.
- [64] G. A. Edwards, "The Fine Structure of a Multiterminal Innervation of an Insect Muscle," *The Journal of Biophysical and Biochemical Cytology*, vol. 5, pp. 241-244, 1959.
- [65] J. Hardie, "Motor innervation of the supercontracting longitudinal ventro-lateral muscles of the blowfly larva," *Journal of Insect Physiology*, vol. 22, pp. 661-668, 1976/01/01/ 1976.
- [66] J. Auber, "Observations sur l'innervation motrice des muscles des insectes," *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, vol. 51, pp. 705-724, November 01 1960.
- [67] J. Okada, and Y. Toh, "The role of antennal hair plates in object-guided tactile orientation of the cockroach (*Periplaneta americana*)," *Journal of Comparative Physiology A*, vol. 186, pp. 849-857, September 01 2000.
- [68] S. Agarwal, R. Kobetic, S. Nandurkar, and E. B. Marsolais, "Functional electrical stimulation for walking in paraplegia: 17-year follow-up of 2 cases," *Journal of Spinal Cord Medicine*, vol. 26, pp. 86-91, Spr 2003.

- [69] L. Y. Mu, and R. E. Ritzmann, "Kinematics and motor activity during tethered walking and turning in the cockroach, *Blaberus discoidalis*," *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*, vol. 191, pp. 1037-1054, Nov 2005.
- [70] J. T. Watson, and R. E. Ritzmann, "Leg kinematics and muscle activity during treadmill running in the cockroach, *Blaberus discoidalis*: I. Slow running," *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*, vol. 182, pp. 11-22, Jan 1998.
- [71] R. K. JOSEPHSON, and D. R. STOKES, "Electrical Properties of Fibres from Stridulatory and Flight Muscles of a Tettigoniid," *Journal of Experimental Biology*, vol. 99, pp. 109-125, 1982.
- [72] V. Brezina, I. V. Orekhova, and K. R. Weiss, "The Neuromuscular Transform: The Dynamic, Nonlinear Link Between Motor Neuron Firing Patterns and Muscle Contraction in Rhythmic Behaviors," *Journal of Neurophysiology*, vol. 83, pp. 207-231, 2000.
- [73] P. H. Peckham, and J. S. Knutson, "Functional electrical stimulation for neuromuscular applications," in *Annual Review of Biomedical Engineering*. vol. 7, ed Palo Alto: Annual Reviews, 2005, pp. 327-360.
- [74] L. A. Geddes, and R. Roeder, "Criteria for the Selection of Materials for Implanted Electrodes," *Annals of Biomedical Engineering*, vol. 31, pp. 879-890, July 01 2003.
- [75] C. Feng, Z. Chao, C. Hao Yu, and H. Sato, "Insect-machine hybrid robot: Insect walking control by sequential electrical stimulation of leg muscles," in *Robotics*

- and Automation (ICRA), 2015 IEEE International Conference on*, 2015, pp. 4576-4582.
- [76] M. Jamon, and F. Clarac, "Locomotor patterns in freely moving crayfish (*Procambarus-Clarkii*)," *J Exp Biol*, vol. 198, pp. 683-700, Mar 1995.
 - [77] R. J. Full, and M. S. Tu, "Mechanics of a rapid running insect - 2-legged, 4-legged and 6-legged locomotion," *Journal of Experimental Biology*, vol. 156, pp. 215-231, Mar 1991.
 - [78] V. Krauthamer, and C. R. Fournier, "Locomotory activity in the extensor and flexor tibiae of the cockroach, *Periplaneta americana*," *Journal of Insect Physiology*, vol. 24, pp. 813-819, 1978.
 - [79] J. S. LEE, R. S. FEARING, and K. CHO, "Compound Foot for Increased Millirobot Jumping Ability," in *Advances in Cooperative Robotics: Proceedings of the 19th International Conference on Clawar 2016*, 2016, p. 71.
 - [80] A. Büschges, R. Kittmann, and J. Schmitz, "Identified nonspiking interneurons in leg reflexes and during walking in the stick insect," *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, vol. 174, pp. 685-700, 1994.
 - [81] D. Graham, "Walking kinetics of the stick insect using a low-inertia counter-balanced, pair of independent treadwheels," *Biological Cybernetics*, vol. 40, pp. 49-57, 1981.
 - [82] U. BÄSSLER, "Interaction of central and peripheral mechanisms during walking in first instar stick insects, *Extatosoma tiaratum*," *Physiological Entomology*, vol. 4, pp. 193-199, 1979.

- [83] A. Buschges, B. C. Ludwar, D. Bucher, J. Schmidt, and R. A. DiCaprio, "Synaptic drive contributing to rhythmic activation of motoneurons in the deafferented stick insect walking system," *European Journal of Neuroscience*, vol. 19, pp. 1856-1862, Apr 2004.
- [84] J. Peter Gabriel, H. Scharstein, J. Schmidt, and A. Büschges, "Control of flexor motoneuron activity during single leg walking of the stick insect on an electronically controlled treadmill," *Developmental Neurobiology*, vol. 56, pp. 237-251, 2003.
- [85] T. Akay, U. Bässler, P. Gerharz, and A. Büschges, "The role of sensory signals from the insect coxa-trochanteral joint in controlling motor activity of the femur-tibia joint," *Journal of neurophysiology*, vol. 85, pp. 594-604, 2001.
- [86] D. W. Haldane, K. C. Peterson, F. L. G. Bermudez, R. S. Fearing, and Ieee, "Animal-inspired Design and Aerodynamic Stabilization of a Hexapedal Millirobot," presented at the 2013 Ieee International Conference on Robotics and Automation, Karlsruhe, 2013.
- [87] P. Rosenbaum, A. Wosnitza, A. Büschges, and M. Gruhn, "Activity patterns and timing of muscle activity in the forward walking and backward walking stick insect *Carausius morosus*," *Journal of neurophysiology*, vol. 104, pp. 1681-1695, 2010.
- [88] D. Graham, "A behavioural analysis of the temporal organisation of walking movements in the 1st instar and adult stick insect (*Carausius morosus*)," *Journal of Comparative Physiology*, vol. 81, pp. 23-52, 1972.
- [89] D. Graham, *Pattern and control of walking in insects*: Academic Press, 1985.

- [90] K. Pearson, "The control of walking," *Scientific American*, vol. 235, pp. 72-86, 1976.
- [91] C. P. Spirito, and D. L. Mushrush, "Interlimb coordination during slow walking in the cockroach: I. Effects of substrate alterations," *Journal of Experimental Biology*, vol. 78, pp. 233-243, 1979.
- [92] N. C. Heglund, and C. R. Taylor, "Speed, stride frequency and energy cost per stride: how do they change with body size and gait?," *Journal of Experimental Biology*, vol. 138, pp. 301-318, 1988.
- [93] L. Ting, R. Blickhan, and R. J. Full, "Dynamic and static stability in hexapedal runners," *Journal of Experimental Biology*, vol. 197, pp. 251-269, 1994.
- [94] Z. Wang, J. Wang, A. Ji, H. Li, and Z. Dai, "Movement behavior of a spider on a horizontal surface," *Chinese science bulletin*, vol. 56, pp. 2748-2757, 2011.
- [95] L. Ding, H. Gao, Z. Deng, J. Song, Y. Liu, G. Liu, and K. Iagnemma, "Foot-terrain interaction mechanics for legged robots: Modeling and experimental validation," *The International Journal of Robotics Research*, vol. 32, pp. 1585-1606, 2013.
- [96] J. C. Spagna, D. I. Goldman, P.-C. Lin, D. E. Koditschek, and R. J. Full, "Distributed mechanical feedback in arthropods and robots simplifies control of rapid running on challenging terrain," *Bioinspiration & biomimetics*, vol. 2, p. 9, 2007.
- [97] G. Larsen, S. Frazier, S. Fish, and S. Zill, "Effects of load inversion in cockroach walking," *Journal of Comparative Physiology A*, vol. 176, pp. 229-238, 1995.

- [98] L. M. Roth, and E. R. Willis, "Tarsal structure and climbing ability of cockroaches," *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, vol. 119, pp. 483-517, 1952.
- [99] K. Autumn, Y. A. Liang, S. T. Hsieh, W. Zesch, W. P. Chan, T. W. Kenny, R. Fearing, and R. J. Full, "Adhesive force of a single gecko foot-hair," *Nature*, vol. 405, pp. 681-685, 2000.
- [100] K. Autumn, M. Sitti, Y. A. Liang, A. M. Peattie, W. R. Hansen, S. Sponberg, T. W. Kenny, R. Fearing, J. N. Israelachvili, and R. J. Full, "Evidence for van der Waals adhesion in gecko setae," *Proceedings of the National Academy of Sciences*, vol. 99, pp. 12252-12256, 2002.
- [101] J. S. Lee, and R. S. Fearing, "Anisotropic collapsible leg spines for increased millirobot traction," in *Robotics and Automation (ICRA), 2015 IEEE International Conference on*, 2015, pp. 4547-4553.
- [102] D. Lee, S. Kim, Y.-L. Park, and R. J. Wood, "Design of centimeter-scale inchworm robots with bidirectional claws," in *Robotics and Automation (ICRA), 2011 IEEE International Conference on*, 2011, pp. 3197-3204.
- [103] S. Kim, A. T. Asbeck, M. R. Cutkosky, and W. R. Provancher, "SpinybotII: climbing hard walls with compliant microspines," in *Advanced Robotics, 2005. ICAR'05. Proceedings., 12th International Conference on*, 2005, pp. 601-606.
- [104] Y. Liu, C. Hu, X. Wu, Y. Zhang, T. Mei, and S. Sun, "Design of vertical climbing robot with compliant foot," in *Robotics and Biomimetics (ROBIO), 2012 IEEE International Conference on*, 2012, pp. 649-654.

- [105] M. Mischiati, H.-T. Lin, P. Herold, E. Imler, R. Olberg, and A. Leonardo, "Internal models direct dragonfly interception steering," *Nature*, vol. 517, pp. 333-338, 2015.
- [106] M. Zago, G. Bosco, V. Maffei, M. Iosa, Y. P. Ivanenko, and F. Lacquaniti, "Internal models of target motion: expected dynamics overrides measured kinematics in timing manual interceptions," *Journal of neurophysiology*, vol. 91, pp. 1620-1634, 2004.
- [107] J. R. Flanagan, S. King, D. M. Wolpert, and R. S. Johansson, "Sensorimotor prediction and memory in object manipulation," *Canadian Journal of Experimental Psychology/Revue canadienne de psychologie expérimentale*, vol. 55, p. 87, 2001.
- [108] M. Kawato, "Internal models for motor control and trajectory planning," *Current opinion in neurobiology*, vol. 9, pp. 718-727, 1999.
- [109] D. M. Wolpert, Z. Ghahramani, and M. I. Jordan, "An internal model for sensorimotor integration," *Science*, pp. 1880-1882, 1995.
- [110] B. Mehta, and S. Schaal, "Forward models in visuomotor control," *Journal of Neurophysiology*, vol. 88, pp. 942-953, 2002.
- [111] S. J. Huston, and V. Jayaraman, "Studying sensorimotor integration in insects," *Current opinion in neurobiology*, vol. 21, pp. 527-534, 2011.
- [112] B. Webb, "Neural mechanisms for prediction: do insects have forward models?," *Trends in neurosciences*, vol. 27, pp. 278-282, 2004.
- [113] D. W. Franklin, and D. M. Wolpert, "Computational mechanisms of sensorimotor control," *Neuron*, vol. 72, pp. 425-442, 2011.

- [114] U. Müller-Wilm, J. Dean, H.-J. Weidemann, J. Eltze, F. Pfeiffer, and H. Cruse, "Kinematic model of a stick insect as an example of a 6-legged walking system," *Adaptive Behavior*, vol. 1, 1992.
- [115] A. Cully, J. Clune, D. Tarapore, and J.-B. Mouret, "Robots that can adapt like animals," *Nature*, vol. 521, pp. 503-507, 2015.
- [116] J. H. Simpson, "Chapter 3 Mapping and Manipulating Neural Circuits in the Fly Brain," in *Advances in Genetics*. vol. 65, ed: Academic Press, 2009, pp. 79-143.
- [117] V. Ruta, S. R. Datta, M. L. Vasconcelos, J. Freeland, L. L. Looger, and R. Axel, "A dimorphic pheromone circuit in *Drosophila* from sensory input to descending output," *Nature*, vol. 468, p. 686, 12/01/online 2010.
- [118] A. H. Reddy, B. Kalyan, and C. S. Murthy, "Mine Rescue Robot System—A Review," *Procedia Earth and Planetary Science*, vol. 11, pp. 457-462, 2015.
- [119] E. Ackerman, "Gemini-scout will be the first one in to rescue trapped miners," *IEEE Spectr.*, 2011.
- [120] E. Strickland, "Meet the robots of Fukushima Daiichi," *IEEE Spectrum*), accessed May, vol. 4, 2014.
- [121] R. Bogue, "Robots for monitoring the environment," *Industrial Robot: An International Journal*, vol. 38, pp. 560-566, 2011.
- [122] A. Brown, and Y. Lu, "Performance test results of an integrated GPS/MEMS inertial navigation package," in *Proceedings of ION GNSS*, 2004.
- [123] A. Noureldin, T. B. Karamat, M. D. Eberts, and A. El-Shafie, "Performance enhancement of MEMS-based INS/GPS integration for low-cost navigation

- applications," *IEEE Transactions on Vehicular Technology*, vol. 58, pp. 1077-1096, 2009.
- [124] J. A. Rios, and E. White, "Fusion filter algorithm enhancements for a MEMS GPS/IMU," in *ION NTM*, 2002, pp. 28-30.
- [125] B. S. Davis, "Using low-cost MEMS accelerometers and gyroscopes as strapdown IMUs on rolling projectiles," in *Position Location and Navigation Symposium, IEEE 1998*, 1998, pp. 594-601.