DEVELOPMENTAL PLASTICITY IN AIMED SCRATCHING MOVEMENTS OF A LOCUST

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Developmental changes may affect aimed limb movements by altering limb mass, muscle strength and musculo-skeletal resistance or changing the position of a target on the body relative to the responding limb. Scratching movements following stimulation of wings by 5th instars and adult *Schistocerca gregaria* were compared before and after the imaginal moult, during which the wings increase in length and rotate, presenting different wing surfaces to external contact. The arrangement of mechanosensory hairs on the wings of 5th instars and adults was determined and associated with the development of scratching behaviour. The neuronal projections of mechanosensory hairs on the hind wing projected intersegmentally to anterior ventral association centre (aVAC) of the metathoracic ganglion and continued intersegmentally to the mesothoracic aVAC. Scratching movements accommodated developmental changes that occurred between the 5th instar and adulthood of *S. gregaria*. There was no change in movement characteristics between 5th instars and adults, indicating that developmental changes in the muscle strength, limb mass and musculo-skeletal resistance were compensated. Movements were appropriately aimed to accommodate the increase in wing size, and were associated with stimulation of tactile hairs on different wing surfaces in 5th instars and adults, implying different synaptic connections onto post-synaptic interneurones. Therefore, changes in limb mass, muscle strength and musculo-skeletal resistance due to growth are be compensated for, possible mechanism are proprioceptive reflexes and dynamic joint stiffness. Changes in target position caused by body growth may be facilitated by the development of new mechanosensory hairs and different synaptic connections onto postsynaptic interneurones. Wing rotation is likely to be accommodated by the intersegmental projections of wing hair afferents, and the convergence of hind wing and fore wing sensory signals.

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CONTENTS

GENERAL INTRODUCTION

Aimed limb movements are vital to invertebrates and vertebrates, and there are many hypotheses to explain the planning and control of such movements. Changes in body size during developmental growth must be accounted for in the planning of movements aimed toward stimuli on the body so that behaviours remain functional. This thesis seeks to describe how aimed limb movements adapt to developmental growth, and to identify the role of the sensory nervous system that detects stimuli on the body in any adaptation. Here, I use the model of hindleg scratching by locusts to investigate how movements adapt to stimulation of the wing before and after disproportionate wing growth. I will begin the General Introduction by discussing aimed limb movements in vertebrates and invertebrates, before discussing the use of locust hindleg scratching as a model. I will describe the developmental growth of insects and suggest why this poses a problem for aimed movements. Finally I shall state the aims of this thesis.

Aimed limb movements in vertebrates and invertebrates

Limb movements are aimed toward targets in a variety of behaviours in both vertebrates and invertebrates. Visually guided reaching movements are the most thoroughly researched type, and have been investigated in humans and primates (for review see Sabes, 2000). The visual system sends information about the spatial location of the target relative to the body, and hypothetical internal models are thought to compute the appropriate motor response to direct a limb to the target (Kawato, 1999; Jordan and Rumelhart, 1992; Wolpert *et al.*, 1995; Flanagan and Wing, 1997; Kawato and Wolpert, 1998). Invertebrates also make visually guided limb movements, for example the

praying mantis makes visually guided striking movements of its fore legs to capture prey (Maldonado *et al.*, 1974; Rodriguez and Maldonado, 1974; Balderrama and Maldonado, 1973). Aimed behaviours also occur in response to tactile stimuli in both vertebrates and invertebrates. Grooming, scratching and wiping behaviours, where a limb is directed toward a specific area of the body that has been stimulated, have been described in dogs, cats (Sherrington, 1906; 1910; Khuta and Smith, 1990), frogs (Fukson *et al.*, 1980; Giszter *et al.*, 1989), turtles (Mortin *et al.*, 1985; Robertson *et al.*, 1985), mosquitoes (Walker and Archer, 1988), honeybees (Land and Seeley, 2004) and locusts (Berkowitz and Laurent, 1996a, b; Matheson, 1997). Furthermore, there are aimed components of other behaviours such as locomotion and climbing. Obstacle avoidance during locomotion has been described in response to visual stimuli in humans (Pryde *et al.*, 1997) and cats (Drew *et al.*, 2008; Drew, 1993; Widajewicz *et al.*, 1994), where the subject must make anticipatory modifications of gait, such as step length, limb trajectory and tarsus placement. Similarly, in stick insects, aiming of hind leg stepping movements occur, whereby the hind leg tarsus is placed directly behind the tarsus of the anterior leg (Cruse, 1979). The gap crossing behaviour of stick insects also involves targeted placement of the fore leg, but in this case using spatial information from antennal contact with the far edge of the gap to aim the fore leg tarsus (Bläsing and Cruse, 2004a, b). In cockroaches, climbing over objects is achieved by altered gaits, leg position and body posture, including redirection of movements of the middle legs (Watson *et al.*, 2002a, b). Thus, the ability to aim limb movements is important in a variety of behaviours across the animal kingdom

Movements can be stimulated by and aimed at either targets within the local environment or targets on the body and rely on different sensory systems to extract

spatial information. The majority of movements toward targets within the local environment are aimed using information about target location gained from the visual system, whilst others such as stick insect gap crossing behaviour (Bläsing and Cruse, 2004) and obstacle avoidance during locomotion (Dürr and Krause, 2001) are examples of tactile detection of external targets by active movements of the antennae. In contrast, tactile stimulation of the body can elicit movements aimed toward the body, a well known example being scratching behaviour. Scratching behaviour has been investigated in cats and dogs (Sherrington, 1906, 1910; Khuta and Smith, 1990), frogs (Fukson *et al.*, 1980; Gizter *et al.*, 1989), turtles (Mortin *et al*., 1985; Robertson *et al.*, 1985), and locusts (Berkowitz and Laurent, 1996; Matheson, 1997). Tactile stimulation can also elicit reflex withdrawal of the leg away from the site of stimulation (Pflüger, 1980; Burrows, 1989; Newland, 1998). Directional running in the escape behaviour of cockroaches is elicited by directional displacement of filiform hairs on the cerci by air currents generated by the approach of a predator (Camhi and Tom, 1978; Camhi *et al.*, 1978). Thus, the spatial location of a target within the local environment or a target on the body can be extracted from different sensory systems that generate movements toward the body or toward/away from an external stimulus.

The ability to aim movements is important to animals for behaviours such as locomotion, prey capture, grooming and reaching in primates. Grooming in particular, is important as a mechanism to keep the body, appendages and sense organs clean so that they fully function, enabling the animal to survive in its environment. In the cricket *Gryllus campestris* eye-cleaning behaviour is elicited by the presence of a drop of water on the ommatidiae, but not when they are covered in fine powder (Honegger *et al.*, 1979). Crickets groom water from their eyes but not dust, as the optical properties of the

water droplet above the cornea alter the received image whilst the presence of powder does not, much like water causing distortion and dust causing blurring on a camera lens. In this case, cleaning behaviour may have evolved to respond to stimuli that reduce the function of the eye. In locusts, scratching may be a mechanism to prevent contact with predators, ectoparasites, conspecifics or debris causing damage and loss of function.

Using locusts to model an aimed limb movement

Scratching movements of the hind leg toward targets on the wing of the locust *Schistocerca gregaria* provide a useful behavioural model to researchers investigating the neuronal control of aimed movements for several reasons. First, the locust nervous system has far fewer neurones than mammalian nervous systems. The metathoracic ganglion contains approximately 10,000 sensory neurones, 70 motor neurones, 1200 local spiking or non-spiking interneurones, and 150 pairs of intersegmental interneurones with cell bodies within the metathoracic ganglion (Burrows, 1996). In contrast, the human brain has been estimated to contain 85 billion neurones (Lange, 1975). This makes the locust nervous system a less complex and therefore easier system to study contrast. Second, locusts demonstrate a range of behaviours, such as jumping, kicking, walking and flight, that have been thoroughly investigated (for review, see Burrows, 1996). The motor neurones and some interneurones underlying these behaviours have been described, and individual neurones can be repeatedly located and their roles analysed in different locusts (Siegler and Pousman, 1990a, b; Pflüger *et al.*, 1980; Robertson and Pearson, 1983; Burrows *et al.*, 1989). Furthermore, locusts are large and robust insects that are easily bred, making them suitable for electrophysiological recordings from individual neurones, for physical and morphological manipulations and for repeated trials using different subjects.

Despite the simpler nervous system of locusts in comparison to vertebrates, there are similarities in the underlying principles of the nervous systems and in limb movements. The nervous system of locusts and humans are composed of similar building blocks (neurones), and their function and interactions are governed by similar principles of operation in terms of how neurones generate action potentials, and synaptic transmission. Because of this, similarities also exist in how neuronal networks operate, such as central pattern generators, shared neuronal circuitry, command neurones and population coding (Pearson, 1993). For example, scratching movements can be generated without input from adjacent ganglia in insects and without input from the brain in vertebrates (Richardson *et al.*, 2005; Deliagina *et al.*, 1975; Sherrington, 1906; Mortin *et al.,* 1985). This demonstrates the presence of a neuronal network (central pattern generator) that can endogenously generate rhythmic outputs for cyclical scratching movements is present within the local ganglion in locusts and other insects, and within segments of the spinal cord in vertebrates (Gelfand *et al.,* 1988). Movement of the limbs is similar in locusts and vertebrates even though locusts have exoskeletons and vertebrates have endoskeletons. In both insects and invertebrates, limbs act as levers and are moved by muscles that are innervated by motor neurones (Burrows, 1996). The neuronal control of aimed movements is sufficiently complex that locusts, like many invertebrates, can take account of changes in target position during movements and modify movements accordingly. In locusts, scratches are retargeted to a stimulus moving along the wing surface during a movement (Dürr and Matheson, 2003), indicating that ongoing movements can be modified. In humans, a shift in position of a visually detected target at the time of onset of a reaching movement causes a fast on-line correction subconsciously, regardless of whether the subjects can see their

hands (Soechting and Lacquaniti, 1983; Pelisson *et al.,* 1986; van Sonderen *et al.,* 1989). Thus, such similarities between the nervous systems and limb mechanics of insects and vertebrates mean locust scratching is a useful experimental model with which to understand one of the important issues of movement control: how is spatial information encoded by the sensory nervous system?

Locust scratching

Touching a fore wing or hind wing of a locust can elicit an aimed scratching movement, whereby one or both hind legs move toward the point of stimulation (target), often in a cyclical trajectory (Berkowitz and Laurent, 1996a; Matheson, 1997, 1998). Scratching is elicited by displacement of the shafts of mechanosensory sensilla on the wings' surface (Page and Matheson, 2004. The trajectory of the effector in scratching depends both on the start position of the responding limb and the stimulus location (Matheson, 1998; Page and Matheson, 2004). Movements require integration of spatial coordinates describing stimulus location from exteroceptors, with proprioceptive information describing the position of the responding limb, as the limb can start from a variety of positions. Stimuli at different regions along the length of the wing elicit different movements that are appropriately aimed by modification of a single movement pattern (Dürr and Matheson, 2003). Hind leg movements are compensated against imposed loads, so that movement kinematics are not changed (Matheson and Dürr, 2003). Little, however, is known of how the challenges of developmental growth affect scratching behaviour.

Developmental growth of insects

Body growth and changes in mass require the nervous system to be capable of plasticity to maintain functional behaviour. Developmental growth, even when *allometric* (proportional), leads to changes in limb mass, muscle strength and joint stiffness (Queathem and Full, 1995), so that identical motor signals might produce different degrees of joint rotation, and thus different limb movements at separate times during development. In other words, the same motor commands from the nervous system would generate a reduced limb movement in the case of increased limb mass or increased joint stiffness, and over rotation of a joint in the case of increased muscle strength or decreased joint stiffness. Thus, continuous plasticity of the nervous system is required to maintain functional movements during allometric growth (Easter, 1983). If body and limb growth are allometric then movements are scaled, so that the endpoint of the effector's trajectory is in the same position relative to the body. The situation is even more complex in the case of non-allometric (disproportionate) growth, where changes in one aspect of morphology may be very different to changes in another. When body growth is disproportionate to limb growth, then an identical degree of joint rotation leads to the effector's endpoint no longer reaching the target. For example in the locust, the wings undergo a dramatic increase in size during the moult to adulthood (imaginal moult), so that the tip of the wing in 5^{th} instars is above the thorax, whilst in adults it is in a more posterior position, beyond the abdomen. Thus movements of the hind leg that move the effector to the wing tip in 5^{th} instars would need to be different to the hind leg movements that move the effector to the more posterior position of the wing tip in adults. This difference in behaviour would require a difference in the degree of rotation of the hind leg joints, the contraction of the muscles, and the motor signals generated by the hind leg motor network. In locusts, however, an additional developmental change

occurs (Uvarov, 1977; Ivanova, 1947). Immediately after the moult to 4th instar, the wings rotate about the wing hinge so that the wing tips point posteriorly and the hind wing is uppermost. The position of the wings is maintained in 5th instars, but during the moult to adulthood is reversed, so that the fore wing lies on top of the hind wing (See Chapter 1 for more detail). In 5th instar *S. gregaria*, the hind wings lie on top of the fore wing, but in adults the fore wing is uppermost. This means that the wing surface which detects scratching stimuli, changes from the hind wing in 5th instars to the fore wing in adults. Thus sensory signals from the hind wing in 5th instars, and the fore wing in adults, must converge either directly or indirectly, on the motor network controlling the hind leg. The purpose of this thesis is to determine whether scratching movements do accommodate for developmental growth.

Behavioural plasticity and insect development

Insects have three developmental pathways that have evolved to suit their requirements for behavioural plasticity: ametabolous (no metamorphosis), hemimetabolous (partial metamorphosis) and holometabolous (complete metamorphosis) (Sehnal *et al.*, 1996). The varying need for metamorphosis of holometabolous and hemimetabolous insects is discussed below.

Holometabolous insects go through pupation and metamorphosis to achieve fundamental changes in behaviour. They lose most of their larval behaviours that are suited to one habitat, and replace them with other behaviours suited to the different habitat of adult insects. For example, the caterpillar *Manduca sexta* lives and feeds on plants such as tobacco, before burrowing underground for pupation. On emergence, adult moths have developed behaviours for flight, locomotion and reproduction, which

are important to locate food and mates. This is achieved by respecification of most muscles, motor neurones and interneurones, and the degeneration of most of the larval sensory apparatus and its replacement with an adult sensory apparatus (Edwards, 1969; Sbrenna, 1971; Bate, 1976; Levine and Weeks, 1990; Truman, 1990; Weeks and Levine, 1990; Truman *et al.*, 1993, Levine *et al.*, 1995; Truman, 1996a). Whilst most larval sensory neurones degenerate, there are examples of groups of sensory neurones that persist, such as a small set of larval sensory neurones associated with leg discs (Jan *et al.*, 1985), or with the abdomen (Shepherd and Smith, 1996) that appear to act as guideposts for developing adult sensory neurones. Thus, most larval sensory organs and most associated sensory neurones degenerate during pupation and are replaced or modified to accommodate the different requirements for sensory systems associated with the development of new behaviours such as flight (Bate, 1978; Jan *et al.*, 1985).

In hemimetabolous insects, such as the locust *S. gregaria*, both nymphs and adults live in the same habitat and have similar behaviours for locomotion, jumping and kicking, although functional flight and reproductive behaviours are only present in the adult. Increases in body size, with little change in form, are achieved by periodically shedding the exoskeleton. Almost all the interneurones and motor neurones in hemimetabolous insects are generated during embryogenesis (Bate, 1976; Doe and Goodman, 1985). However, extra sensilla and sensory neurones are added at each moult, particularly during the moult to adulthood (Chiba *et al*., 1992; Lawrence and Hayward, 1971; Schafer, 1973). Therefore, whilst newly hatched larvae have almost all the motor neurones and interneurones in their CNS that they need for the rest of their lives (Shepherd and Bate, 1990), their sensory systems continue to develop postembryonically.

The motor network underlying scratching behaviour may therefore be present throughout postembryonic life, whilst the sensory system that sends signals to it may well change during postembryonic development. This thesis aims to identify changes in the neuronal pathways underlying scratching that may facilitate the accommodation of developmental growth by the movement.

Developmental growth and aimed movements in other insects

To my knowledge there is only one description of how an aimed limb movement of an insect adapts to developmental growth: the effects of developmental growth on the aimed striking component of prey capture behaviour of the praying mantis, *Stagmatoptera biocellata* has been studied by Balderrama and Maldonada (1973). They suggested that to accommodate postembryonic growth of the body, capture behaviour was retargeted, and defensive behaviour changed from crypsis (hiding) in smaller instars to diematic (intimidating) in larger instars. Prey capture is achieved by a stereotypical lunging of the forelegs and a variable displacement of the body toward the stimulus. With growth of the forelegs and body, the maximum capture distance increases, and mantids increasingly strike at stimuli that are further away (Balderrama and Maldonado, 1973). They proposed the *automatic adaptation hypothesis* based on constant proportionality during growth between the maximum capture distance and the dimensions of the visual system used to triangulate strikes (Maldonado *et al.*, 1974). They suggested that a single ommatidium or group of ommatidiae were responsible for detecting prey at the maximum capture distance, and that developmental changes in head breadth, ocular globe breadth and ocular prominence governing the position of this ommatidium changed, so that it would be receptive to stimuli that were increasingly

further away. Thus the position of ommatidiae within the compound eye automatically changes to match the new dimensions of the body, so that existing movements are rescaled (Maldonado *et al.*, 1974; Kral, 1998). This finding rejects the role of learning in adaptation to body growth, and sets a precedent for such changes to be accommodated by proportional developmental changes in the sensory apparatus detecting the stimuli. Thus the development of the sensory apparatus of the wings may be key to scratching movement adaptation to accommodate development growth.

Thesis aims

The aim of this thesis is to address two general problems for the control of movement:

1) Do movements adapt to developmental growth, and how?
2) How is the spatial location of a target encoded by tactile hairs?

The first problem will be dealt with by determining whether the locust *S. gregaria* adapts hind leg scratching movements aimed at the wing to accommodate developmental growth, comprising changes in the musculoskeletal system, the increase in size of the wings and wing rotation. I have described hind leg scratching movements following stimulation of the wing in 5^{th} instar and adult *S. gregaria* (Chapter 1). I have associated changes in behaviour with changes in the sensory apparatus of the wing that detect stimuli (Chapter 2). The second problem will be addressed by determing whether the peripherial position of tactile hairs on the wing are represented by the position of the arborisations of their sensory neurones within the central nervous system. In Chapter 3, I present the first description of the projection patterns of wing hair afferents in a hemimetabolous insect.

CHAPTER 1

Scratching movements compensate for developmental growth

Introduction

Aimed limb movements are important in a variety of behaviours, such as reaching, locomotion, prey capture. It is therefore important that movements are adapted to changes in body structure and mass during developmental growth, so that they remain functional throughout life (Easter, 1983). Aimed limb movements must compensate for developmental changes in sensory systems that detect a stimulus, and in motor systems that produce the response. Given the complexity of generating an aimed movement this is no easy feat.

The generation of an appropriate motor pattern to direct a limb to a point in space requires sensory information about both the location of the target, and proprioceptive information about the starting position of the effector limb (Kargo and Gistzer, 2008; Fukson *et al.,* 1980). Sensory signals encoding target location are integrated with limb proprioceptive signals, generating motor commands that cause controlled rotations at each limb joint by graded contractions of the innervated muscles. There are two main groups of hypotheses about how vertebrate central nervous systems compute motor outputs. First, motor patterns may be constructed by the combination of building blocks,

such as “muscle synergies” or spinal reflexes (d’Avella *et al.*, 2003; Poppele and Bosco, 2003). Such synergies can be combined in a computationally simple manner to produce an appropriate movement (Tresch *et al.*, 1999; d’Avella *et al.*, 2003). Second, that each movement is specifically calculated using complex internal models representing target position and limb posture, either encoded directly or as other variables such as elasticity or joint stiffness of the musculo-skeletal system (for review, see Kawato, 1999; Schenau, 1995). For instance, the equilibrium hypothesis suggests that limb posture arises as a result of a balance between active muscle forces and dynamic musculo-skeletal resistance, both of which are encoded internally within the nervous system (Feldman, 1966; Bizzi *et al.*, 1991). Limb trajectories result from a shift in this equilibrium point. This hypothesis has been used to explain aspects of both human (Flash, 1987) and insect targeted behaviours (Dean, 1992). Thus at present the control of movement is very much a “black box”, meaning the neuronal processes underlying movement control are unknown. There is no agreement as to which hypothesis best explains the control of movement.

Developmental growth poses a challenge to maintaining functional aimed limb movements for two reasons. First, growth or structural changes of the body alter information about the target, by either changing the location of targets on the body relative to the responding the limb, or by changes in the sensory system detecting the stimulus, such as addition of exteroceptors or changes in their properties (Dagan and Volman, 1982; Chiba *et al.*, 1988). Second, growth of the limbs may lead to changes in limb segment length, muscle strength and resistance of the musculoskeletal system, which given an unchanged motor pattern would result in a different effector trajectory. If behaviours are to be conserved during development, then plasticity of the underlying

neuronal pathways may be required. The growth of the locust *Schistocerca gregaria* provides a good model in which to investigate the affects of changes in body structure on limb movements aimed at the body. Hind leg scratching following stimulation of the wing is a well described aimed limb movement (Berkowitz and Laurent, 1996a, b; Matheson, 1997; 1998; Page and Matheson, 2004), and development of the wings from 5^{th} instar to adulthood involves dramatic growth and reorientation with respect to the body and hind leg (Uvarov, 1977; Ivanova, 1947).

In locust scratching, displacement of tactile hairs on a wing elicits movement of the ipsilateral hind leg with the distal end of the tibia and tarsus aimed toward the site of stimulation (Berkowitz and Laurent, 1996a; Matheson, 1997, 1998; Dürr and Matheson, 2003). Furthermore, stimulation of different areas along the wing surface elicits tarsal trajectories that are target-specific from onset and accurately aimed to each stimulus site (Berkowitz and Laurent, 1996a; Dürr and Matheson, 2003; Matheson, 1997, 1998). To reach progressively more distal targets requires increases in the angle of elevation of the femur and increased flexion of the femoro-tibial joint. These continuously graded changes in hind leg joint angles suggest that locusts use a single systematically variable movement pattern for scratching rather than unique combinations of joint angles specific to each location in the overall receptive field (Dürr and Matheson, 2003). In addition, locusts have demonstrated adaptive mechanisms to compensate scratching movements against perturbations caused by loading the hind leg (Matheson and Dürr, 2003). This behaviour provides a unique opportunity to investigate whether a targeted movement can adapt to changes that arise during developmental growth. The dramatic growth of the wings that occurs after moulting sets hemimetabolous insects apart from

gradual growth of vertebrates, but provides an opportunity to determine whether and how such behavioural plasticity.

The growth of insects is restricted by their exoskeletons, and is achieved by periodically shedding the exoskeleton and enlargement of a new exoskeleton before it hardens (saltatory growth). In hemimetabolous insects the same body structure with the exception of the wings and reproductive organs is maintained throughout life, enlarging with each successive moult (Uvarov, 1977). In *S. gregaria*, the wings increase in length from less than 1 cm in 5^{th} instar nymphs to over 6 cm in adults. Non-allometric growth of the wing relative to the body alters the position of the tip of the wings to a more posterior position in adults compared to 5^{th} instars. Thus scratching movements elicited by stimulation of the wings' surface must adapt to the change in size of the wing to remain functional. Furthermore, there is a second morphological change to contend with; a rotation of the wings immediately after the imaginal moult (Uvarov, 1977; Ivanova, 1947; Burnett, 1951; Khattar, 1972; Michelmore, 1934; Roonwal, 1940). The wing pad is constructed of two epidermal membranes, forming dorsal and ventral surfaces. The faster growth of one compared to the other results in the wing pads folding or rotating about their joint with the thorax. In *S. gregaria*, wing pads first develop in 1^{st} instars as ventrally directed extensions of the dorsal cuticular plates, with the fore wing pad (blue) lying anterior and adjacent to the hind wing pad (green) with the wing pad tips pointing ventrally (Fig. 1 A). In 2^{nd} and 3^{rd} instars, the wing pads remain in the same position, increasing in size (Fig. 1 B, C). Subsequent instar wing pads develop within the wing pad of the previous instar, by the epidermis forming a new layer of cuticle beneath the old cuticle of the wing. Towards the end of the 3^{rd} instar, the ventral epidermis grows faster than the dorsal epidermis, resulting in a larger

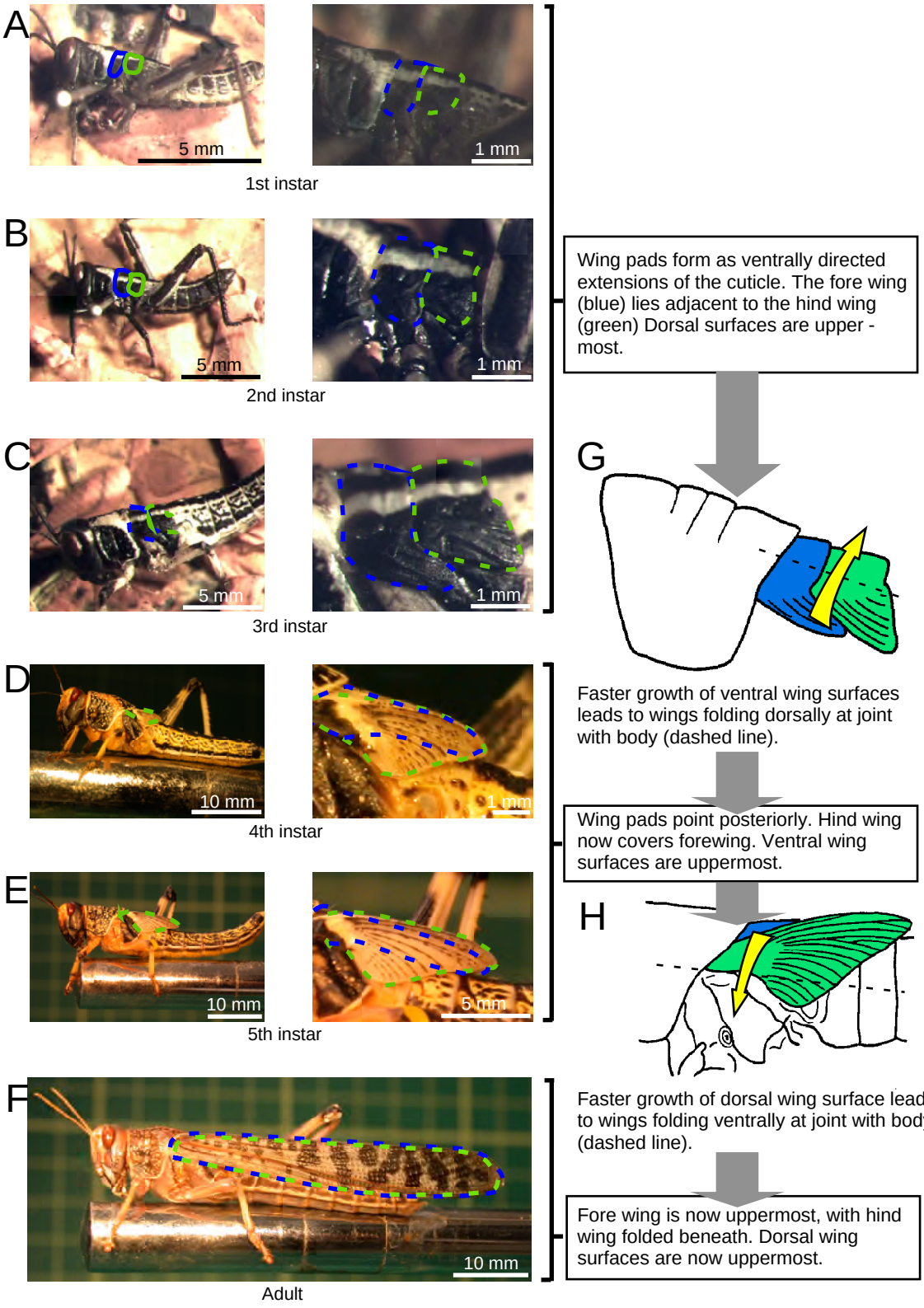


Figure 1: The rotation of the wings during development of S. gregaria with higher magnification images of the wings (note changes in scale). Each instar is shown with the position of the fore wings (blue) or hind wings (green) outlined in a dashed line. In the right hand column the mechanism of wing rotation is described.

surface area of epridermis and cuticle on one side of the wing compared to the other (Fig. 1 G). On moulting to 4th instar the wings expand, and the larger ventral surface area drives the wings to fold dorsally at the joint with the thorax (Fig. 1 D). In 4th instars the ventral surfaces are uppermost for both the fore and hind wing pads, and the hind wing pad covers the fore wing pad. In 5th instars the wing pads remain in the same position, increasing in size (Fig. 1 E). At the end of the 5th instar, again, one wing surface grows faster than the other. This time the dorsal epidermis has grown faster than the ventral epidermis (Fig. 1 H). On moulting to adulthood, the larger surface area of the dorsal epidermis and cuticle causes the wings to fold ventrally at the joint with the thorax. In adults, the dorsal wing surfaces are uppermost, and the forewing covers the hindwing (Fig. 1 F). This mechanism has been described for many orthoptera, but the instar at which it occurs varies (Uvarov, 1977). Wing rotation, therefore means that different wing surfaces are exposed to receive stimuli from the external environment at different times during development. The ventral surface of the hind wing is exposed to external stimuli in 5th instar *S. gregaria*, and in contrast, the dorsal surface of the fore wing is exposed in adults. Thus, after the imaginal moult, locusts must adapt scratching movements to the altered position of distal parts of the wing due to growth, and also to the detection of external stimuli by different wing surfaces.

Developmental changes during the imaginal moult require adaptation of locust scratching movements so that they effectively reach their targets and remain functional. Changes in musculoskeletal properties, hind leg proportions, wing length and exposed wing surface as a consequence of wing rotation mean that the motor commands underlying the movements may be modified to reach targets in relatively similar positions and those that are in different locations in 5th instar and adults. The aims of

this chapter are (1) to describe for the first time scratching movements of 5th instar locusts; (2) to determine if scratching movements have similar kinematics to those of adults; (3) to determine the effects of wing growth on scratching movements in response to wing stimulation.

Methods

Animals and experimental protocol

Experiments were carried out on six 5th instar and six adult desert locusts (*Schistocerca gregaria* Forskål) taken from a crowded laboratory culture established at the University of Leicester, UK, with stock from Blades Biological Ltd., Kent , UK, and the University of Cambridge, UK. Animals were tethered with a fine loop of wire that passed around their pronotum without obstructing movements of any of the legs. They were suspended above a Styrofoam ball on which they stood or walked. The tether allowed each animal freedom to adjust its body posture, with the exception of thorax height above the substratum, which was set to the height normally maintained by a walking locust. The hind leg was manipulated into a standardised start posture by placing the tarsus on a horizontal rod located at 67% of the distance between the anterior rim of the metathoracic coxal joint and the tip of the abdomen (Dürr and Matheson 2003). Figure 2 illustrates the mean start position with standard error bars for adults (Ai) and 5th instars (Bi). The eyes were covered with black acrylic paint to exclude visual cues. Experiments were carried out at 22-24°C, maintained by an infra-red heat lamp suspended 50cm above the animal. Figure 1 Aii, Bii show the positions of 1 mm diameter discs, cut from reflective tape (3M Scotchlite), that were attached to the body with insect glue (Thorne (Beehives) Ltd., UK). The marked points include 4 positions on the hind leg to allow measurement of all joint angles. Movement of the unguis was ignored. For illustrative purposes tarsal length was standardised to 6.75 mm (Dürr and Matheson, 2003).

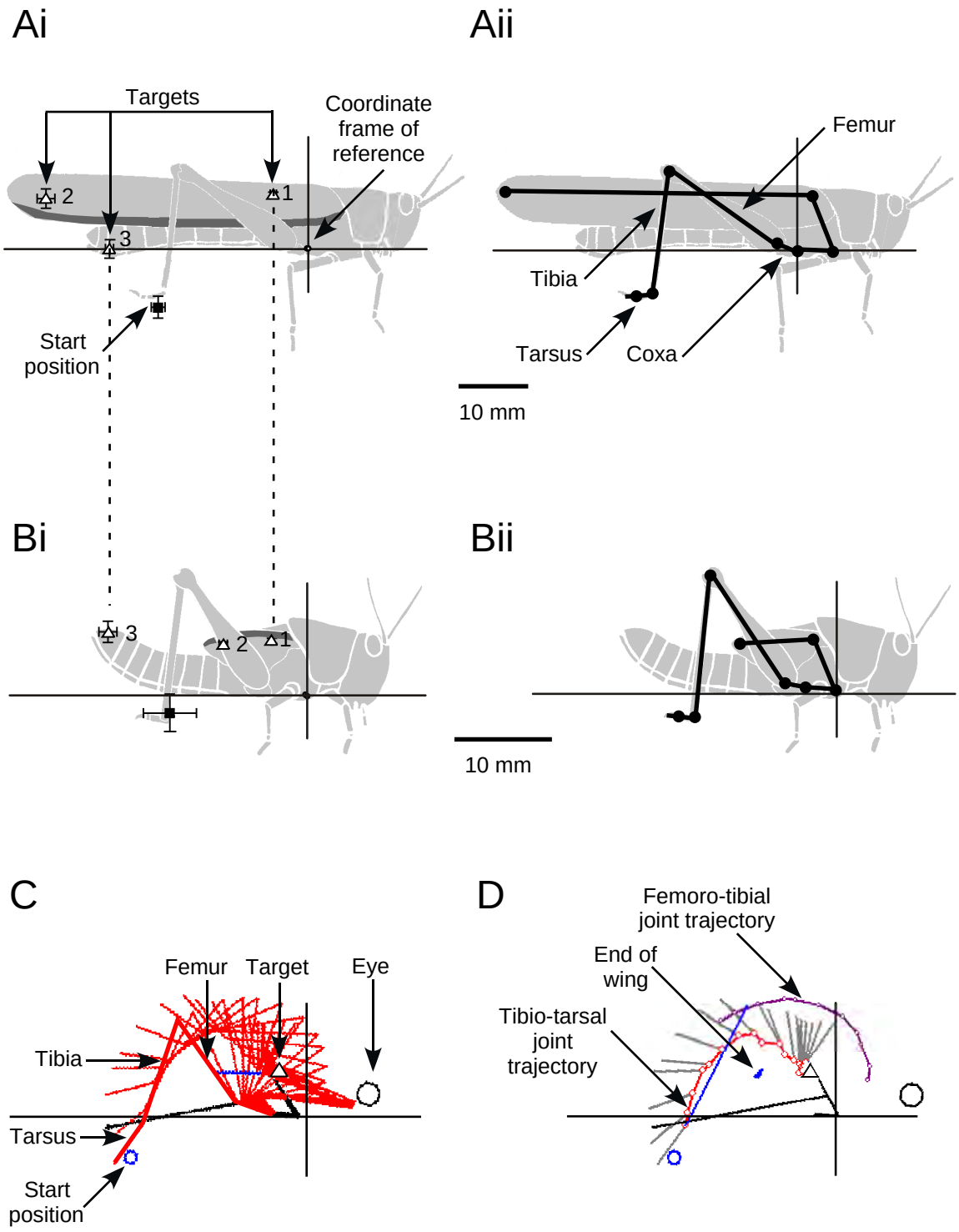


Figure 2. Stimulus positions for adult (A) and 5 th instar (B) S. gregaria. Mean stimulus location on the wing base, wing tip or abdomen tip (white triangles 1, 2 or 3 respectively, mean ± SEM). The hind leg tarsus was placed on a rod in at a single start position (black square, mean ± SEM). The body-centred coordinate frame had its origin on the anterior rim of the hind leg coxa for adults (black lines, A) and was shifted so that wing base and abdomen tip stimuli in 5 th instars were aligned with those of the adults (black lines, B). C) A body model comprising 8 points (black circles, Aii, Bii) was used to track movement of the hind leg (red lines), wing (blue line) and abdomen (black line) relative to the thorax. D) The trajectories of the tibio-tarsal (red line), and femoro-tibial (purple line) hind leg joints are shown for a wing base stimulus (white triangle) in a 5th instar. Grey straight line segments show the tarsus position in each frame (20 ms between frames).

Scratching movements of the right hind leg ipsilateral to the stimulus sites were analysed in the following way. The fore wing was notionally divided into 5 regions of equal length. The most anterior and posterior of these regions were used as wing base and wing tip stimulus sites, respectively, and in addition the posterior fifth of the abdomen was used as a posterior target. Stimulation was provided by gently touching the wing base, wing tip or abdomen tip with a fine paint brush to elicit scratching behaviour (mean ± SEM stimulus position; white triangles, 1, 2 and 3, respectively in Fig. 2 Ai, Bi). Wing base, wing tip and abdomen tip stimuli were given randomly until at least 10 scratches were recorded for each stimulus site. The results are based on analyses of 793 scratches.

Locusts rarely touch their own body surface during the first few cycles of a scratching response, meaning that responses are open-loop with respect to the stimulus. Responses in which the leg made contact with the body surface or with the stimulating brush were analysed only until the time of first contact.

Video acquisition and analysis

Animals were videotaped using a colour CCD camera (JVC-C1380) operated at a shutter speed of 1/500 s. The sVHS video signals were combined on a multiviewer (For-A MV-40PS) and time stamped using a video timer (For-A VTG-33). Images were taped on an sVHS video recorder (JVC HR-S7500), displayed on a monitor (Trinitron Sony PVM-1450MD) and played back for capture by a personal computer video interface card (MiroVIDEO DC30 plus, Pinnacle Systems) in PAL video format at a size of 720 x 540 pixels using MiroVIDEO Capture software (25 Hz frame rate for full interlaced video frames) and compressed using Microsoft “dvsd” codec. A deinterlacing

algorithm (Videotrack 2D written by J. Zakotnik in Microsoft Visual Studio) then split each frame into even and odd frames, and linearly interpolated the missing lines. Clips containing individual scratches were produced by editing recording sessions in VirtualDub (written by A. Lee in Microsoft Visual C++). A calibration display was also filmed and captured, centred in the field of view so that Videotrack could calibrate the position of the locust's body relative to the camera lens and calculate distances moved in mm.

A custom-written program, *Videotrack 2D* was used to access the AVI clips and detect the co-ordinates of the 8 markers within each frame that described the animal's body position and leg movement (See Dürr and Matheson 2003 for a discussion of the accuracy of this method; Fig. 2 Aii, Bii). Marker identifications were assigned manually, and the markers then tracked automatically through the video sequence by *Videotrack 2D*. The line between the markers adjacent to the hind leg coxa and the frontleg coxa defined the x-axis of the body-centred coordinate frame with the marker 2 mm in front of thoraco-coxal joint of the hind leg set to the origin (Fig. 2 Aii, Bii). Each frame of the sequence was filtered with a colour filter and eroded with an algorithm that removed image pixels from borders of bright areas. Any pixel with a brightness value less than the threshold was set to black. After this pre-processing, the image contained only white pixels corresponding to each marker on a black background. *Videotrack 2D* contains several algorithms to overcome marker occlusions and spurious bright spots in the video. The stimulus location and the start position of the tarsus were digitised on the first frame of each behavioural sequence (mean position ± SEM shown as white triangles and black squares, respectively, in Fig. 2 Ai, Bi). For the x-axis comparison of centre of density, the 5^{th} instar coordinates were scaled by 140% based on mean size for

body segments for six 5th instar and six adult locusts used in the behavioural experiment and wing base and abdomen tip stimuli were aligned by shifting the 5th instar frame of reference anteriorly by 2.7 mm. This standardisation permitted the direct comparison of movement trajectories between 5th instars and adults of different sizes.

Data analysis

Analysis of individual scratching responses started with the frame before the movement began and ended with the occurrence of one of the four following events; the tarsus touched the ground, the tarsus hit the brush, the leg completed three cycles of movement, or the leg stopped moving for >120 ms. The 2-dimensional co-ordinates of hind leg joints throughout a scratch were output by *Videotrack 2D* and subsequently analysed by *Scratch Analysis* (written by V. Dürr in Borland Delphi; Fig. 2 C, D). The position of the hind leg relative to the body is shown as an example, for one movement toward a wing base target in a 5th instar (Fig. 2 C). The hind leg tarsus moves from the start position (blue circle) anteriorly and dorsally toward the stimulus position (white triangle) on the wing (blue line) driven by anterior rotation of the thoraco-coxal joint and flexion of the femoro-tibial joint. Figure 2 D shows the movement of the femoro-tibial joint (purple), the tibio-tarsal joint (red), and the wing tip (blue) relative to the thorax (black) for the same movement. The output from *Scratch Analysis* contained the calculated joint angles, joint angular velocities, and joint angular accelerations for all four hind leg joints in each frame of the sequence. Scratching was described quantitatively in terms of the mean joint angles and angular velocities for each hind leg joint. Statistical tests were calculated with the Statistical Package for the Social Sciences (SPSS). For all data, apart from the density maps described below, means were

first calculated for each animal and then averaged across animals and presented together with the standard error of the mean.

To determine which part of the hind leg was being aimed at the target, the hind leg was divided into 24 equal units and the shortest distance between each unit and the target during the scratch was calculated. Having determined the point on the leg that on average most effectively reached all 3 target sites, the movement trajectory of this effector point was used to locate its closest point of approach to the target site in each scratch. The distance between the closest point and the stimulus gave a measure of the accuracy of each movement. A mean accuracy was established for each animal for movements toward wing base, wing tip and abdomen targets.

Two components of each scratching response were analysed: a short (200 ms) initial component and a second cyclic component. In earlier work, 200 ms was shown to be the mean duration of the initial straight trajectory toward the target prior to the cyclic movement (Dürr and Matheson, 2003). The first 200 ms of movement was used to calculate the initial speed and angle of movement of the effector (scaling was not applied to these values). The cyclic component was quantified by calculating a probability distribution of the location of the distal tibia across scratches. The workspace was divided into an orthogonal lattice of 1 mm grid width. The movement of the distal tibia was interpolated between each pair of consecutive video frames to calculate the area that the distal tibia passed through between those two frames. The number of frames in which the interpolated area of the distal tibia overlapped each given lattice point was used to construct a 2D frequency histogram for the whole movement (probability distributions). Probability distributions were calculated for each

trajectory, spatially smoothed with a 2D-Gaussian filter of 5 x 5 mm area and σ^2 = 2 mm^2, normalised to a standard volume, and averaged across trials point by point on the lattice. Thus the likelihood that the effector moved across each point in the workspace during a scratch was quantified as 2-dimensional probability distribution. The centre of density of the probability distribution was used as a single measure of the location of the scratch. To distinguish between pairs of movement distributions the probability distributions were treated as empirical likelihood functions, and Bayes' rule (reviewed in Joyce, 2008) was applied to decide which one of the two sites had most likely been stimulated to cause the leg to move across point *X* during a single video frame (see Dürr and Matheson, 2003 for derivation and details). The likelihood of the probability estimate being wrong for a single observation (frame) was used to determine a critical number of frames required to permit two maps to be distinguished. If the average number of frames of the two maps being compared was more than the critical number of frames needed to distinguish between them, then the two distributions were considered statistically different. In other words, the distributions were considered significantly different if observation of a single scratch of average duration would permit a correct prediction of the observed probability distribution in 95 % of cases (see Dürr and Matheson, 2003). Probability distributions were compared for pairs of target sites to determine: (1) the probability of assigning the wrong target site to a single observation (1 video frame); (2) the minimum number of observations needed to obtain a 95% chance of making a correct decision (>50% correct single assignments); and (3) the percentage volume overlap of the two distributions.

Cyclical trajectories were analysed in joint angle space for comparison with Dürr and Matheson (2003). Measurements of the thorax-coxa and the coxo-trochanteral joint

angles of the hind leg were smoothed with a sliding window having a width of five frames, weighted by binomial coefficients.

Results

Developmental growth

Measurements taken from 12 animals used in subsequent behavioural experiments revealed that growth of each segment of the hind leg was proportional to growth of the body (Fig. 3). The lengths of the exposed wing surfaces were compared; the hind wing pad in 5^{th} instars and the fore wing in adults. The mean adult body length was 156.8% larger than the mean body length of 5^{th} instars. The length of the coxa, femur, tibia, tarsus of the adult hind leg were longer by 136.4%, 148.1%, 141.6%, 130.2%, 142.3% and respectively, than in 5^{th} instars (Mean = 140.3%), whilst the adult wings were 447.0% longer than the wings of 5^{th} instars (Fig. 3 B). The increase in length of the wings (447.0%) was significantly greater than the increase in length of the hind leg (156.8%) (paired t-test, df = 5, t = -24.551, P <0.001). The ratio of growth of the coxa, femur, tibia, tarsus, and total leg compared to the body was 0.9, 0.9, 0.9, 0.8, and 0.9 respectively, whilst that for the wing was 2.9. Thus wing growth is disproportionate to growth of the body or hind leg. The tip of the adult wing is posterior to the tip to the tip of the abdomen whereas the tip of the wing pad in 5^{th} instars is relatively anterior.

Do 5^{th} instar locusts scratch?

As a consequence of wing rotation, the exposed wing surface in 5^{th} instars is the ventral hind wing surface, whilst in adults the exposed wing surface is that of the dorsal fore wing. Does stimulation of the ventral hind wing surface in 5^{th} instars elicit scratching

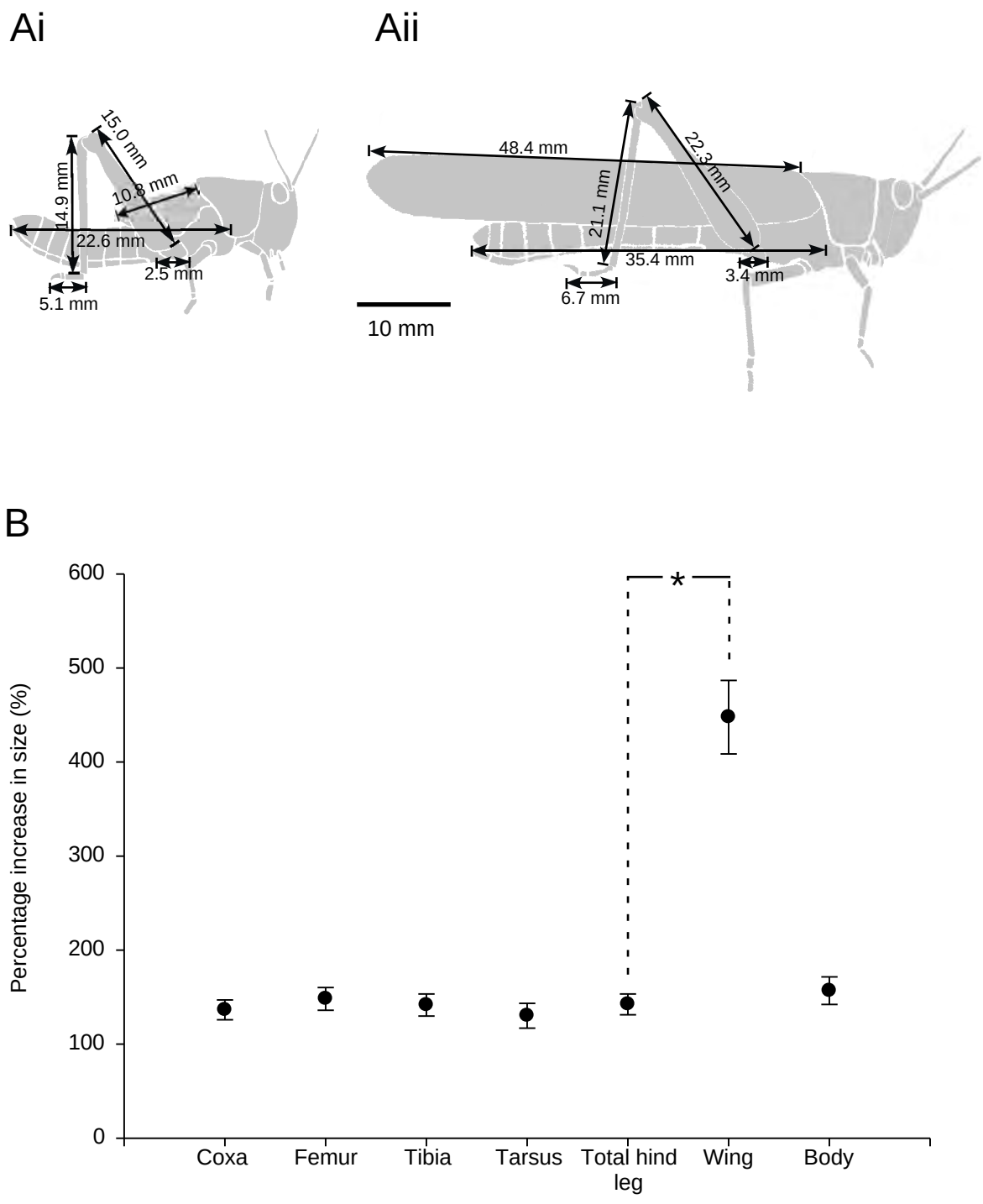


Figure 3. Comparison of length of the hind leg, body and wing of the twelve 5 th instar and adult S. gregaria used in subsequent experiments. A) Mean and SD of body segment lengths are shown for 5th instar (i) and adults (ii). B) The percentage increase in segment length between 5th instars and adults. A t-test revealed that the increase in size of the wing was significantly greater than that of the hind leg ($P < 0.001$).

behaviour, or is this behaviour associated only with stimulation of tactile hairs on the dorsal fore wing in adult *S. gregaria*?

Fifth instar locusts perform similar scratching movements to adults, toward both anterior and posterior targets (Fig. 4). Following stimulation of an anterior target at the base of the wing, 5th instars, like adults, move the ipsilateral hind leg tarsus toward the target in an anteriorly-directed arc, and perform a cyclical movement near to the target (black line, Fig. 4 Ai, Bi). Following stimulation of a posterior target on the tip of the abdomen, 5th instars again like adults, move the hind leg tarsus toward the target, in a posteriorly-directed movement and making a cyclical movement around the target (black line, Fig. 4 Aii, Bii). The wing pad tip in 5th instars is an anterior target and its stimulation elicits an anteriorly aimed movement (Fig. 4 Aiii) similar to that aimed toward the wing base (Fig. 4 Ai). In contrast, the wing tip is a posterior target in adults, and its stimulation elicits a movement that is posteriorly aimed (Fig. 4 Biii) similar to those aimed toward abdomen tip targets (Fig. 4 Bii).

Grooming effector

For movements toward anterior wing base targets in both 5th instars (ventral hindwing) and adults (dorsal forewing), two different regions of the hind leg approach the target, resulting in a "w" shaped distribution (Fig. 5 Ai, Bi). Unit 4 of the hind leg was closest for both 5th instars and adults, representing the proximal femur passing the anteriorly located target (double asterisk in Fig. 5 Ai, Bi). Additionally, unit 18 of the second minima represents the distal tibia approaching the target (single asterisk in Fig. 5 Ai, Bi). The physical constraints of hind leg movement mean that for the distal tibia or tarsus to reach anterior targets (wing base and wing tip in 5th instars, and wing base,

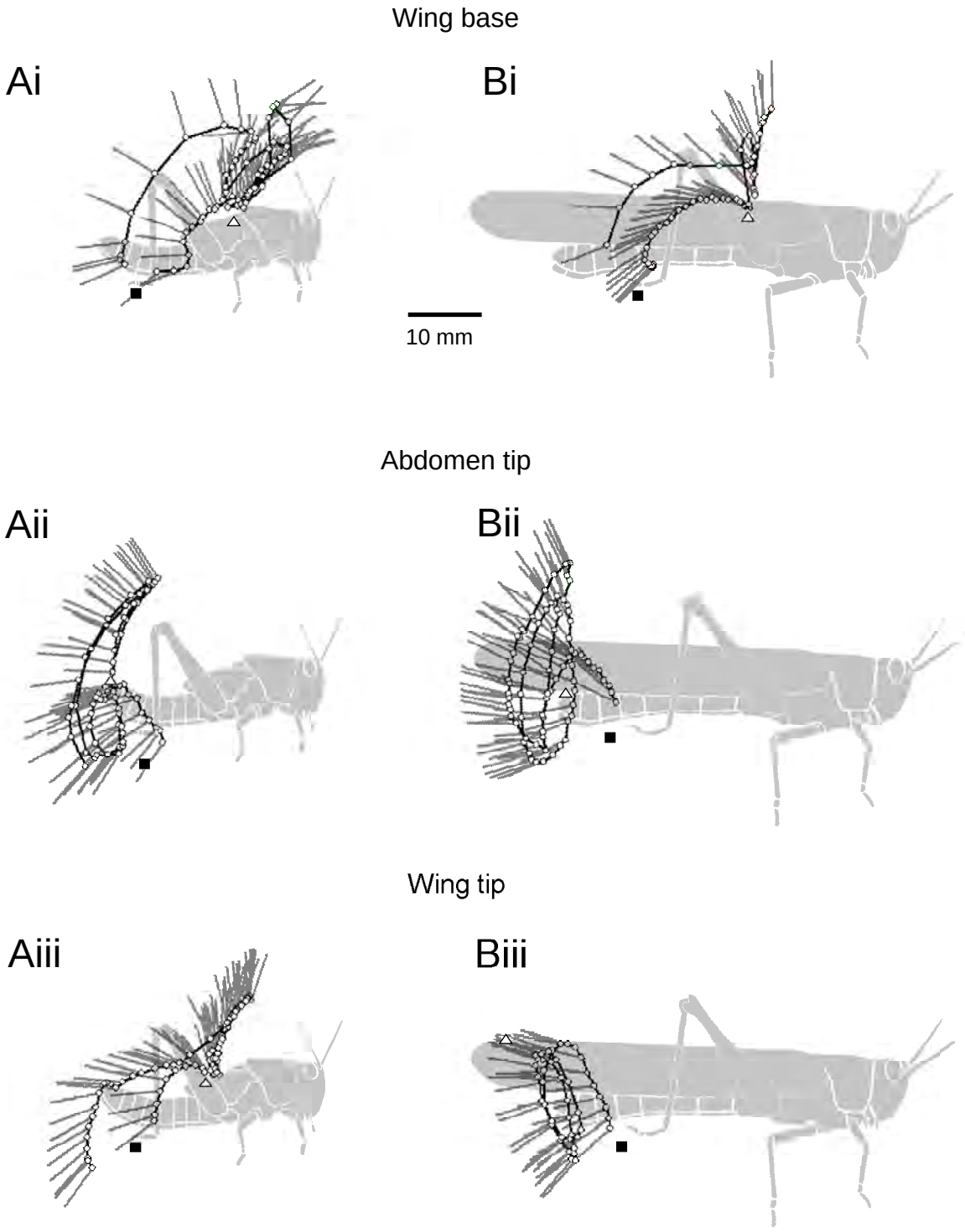


Figure 4. Trajectories of the distal tibia toward anterior and posterior targets in 5 th instar (A) and adult (B) S. gregaria. Stimulus locations on the wing base (i), abdomen tip (ii) and wing tip (iii) are represented by white triangles. Tarsal start position is shown by a black square. The position of the tibio-tarsal joint and the tarsus (grey line) are shown at intervals of 20 ms throughout a single movement.

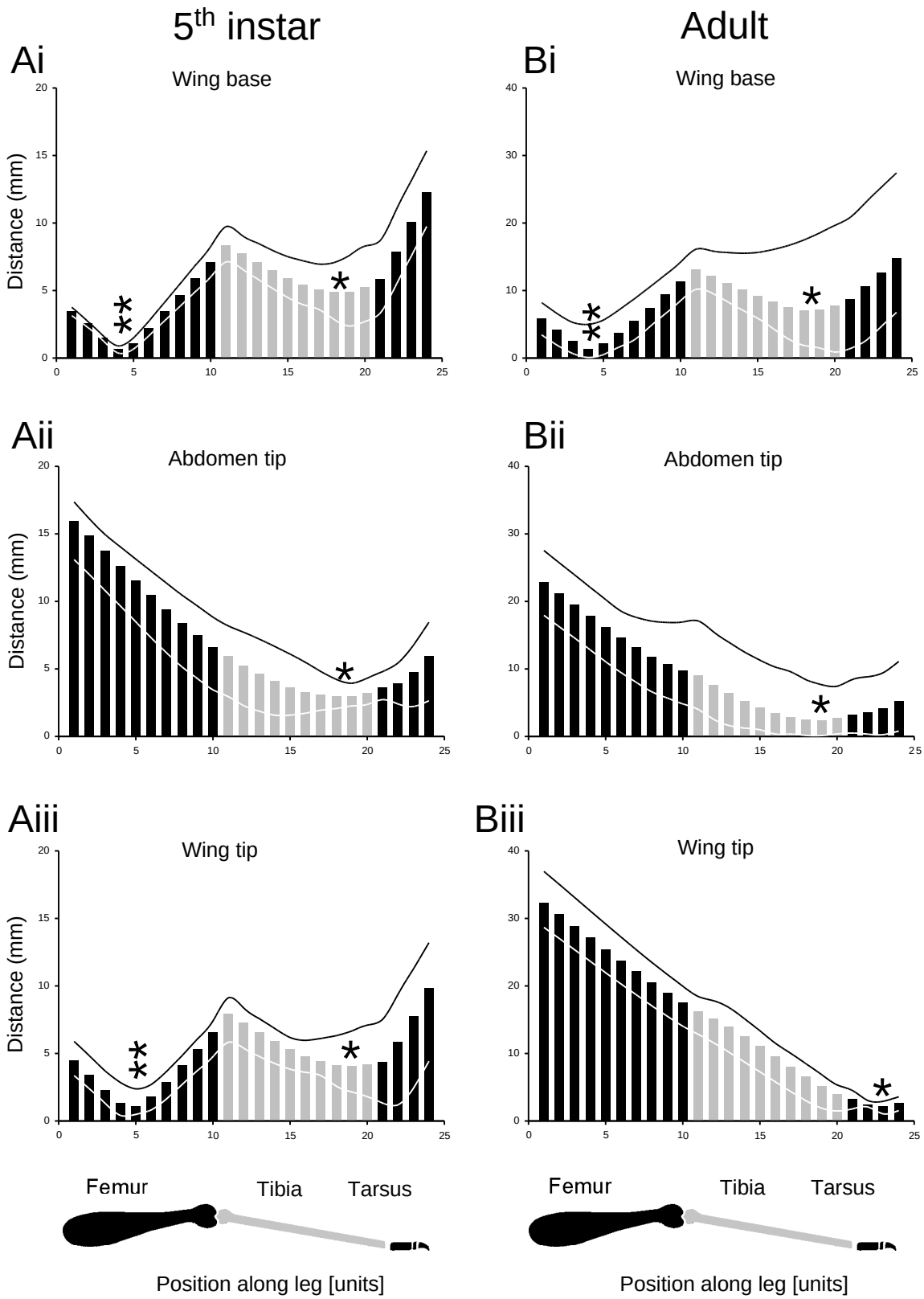


Figure 5. The mean closest approach of each leg unit to the target during movements toward fore wing base (i), abdomen tip (ii), and fore wing tip (iii) targets, for 5 th instar (A) and adult (B) S. gregaria. White and black lines display the minima (white line) and maxima (black line) closest approaches of the minimal distance per animal per trial. Grey bars correspond to units of the tibia, black bars correspond to units of the femur or tarsus (see inset diagrams below iii). The region of the leg that approaches the target most closely is labelled with a single asterisk for the "real" minima and a double asterisk for the "false" minima. Note different scales for 5 th instars and adults.

only, in adults), the femur must rotate far anteriorly, itself crossing the target site (see Fig. 2 C). The femur typically crossed the target once, and was then held anteriorly for the rest of the movement. In contrast, the distal tibia approached the target several times during the cyclic component of a scratch (Fig. 4). In other words, the minima at unit 18 reflected the aimed effector whereas that at unit 4 reflects a mechanical consequence of that aimed movement. In movements toward (posterior) abdomen tip targets in both 5th instars and adults, a single region of the hind leg closely approached the target. Unit 18 of the hind leg in 5th instars and unit 19 of the hind leg in adults were the closest to the target (see single asterisk in Fig. 5 Aii, Bii), representing the distal tibia approaching the target. The wing tip is an anterior target in 5th instars, but a posterior target in adults, and this change in target position is reflected in the parts of the hind leg that approach the target. In 5th instars both unit 5 of the femur, and unit 19 of the distal tibia approach the target, representing the femur crossing the target whilst rotating anteriorly, and the distal tibia reaching the target during the cyclic movement (double asterisk and single asterisk, respectively in Fig. 5 Aiii). In adults, only unit 23 of the tarsus reached the posterior position of the target (single asterisk in Fig. 5 Biii). Thus, the distal tibia and tarsus are used as effectors during scratching movements in both 5th instars and adults. Furthermore, the hind leg approaches the target in a similar way for adults and 5th instars in response to stimulation of anterior or posterior targets.

Targeting accuracy

The closest point of the effector to the target during each trajectory was used to determine the mean closeness of the effector as a measure of accuracy. Figure 6 shows the mean closeness of movements to the wing base, abdomen tip and wing tip in 5th instars (black) and adults (grey). For 5th instars, the mean closeness of the effector from

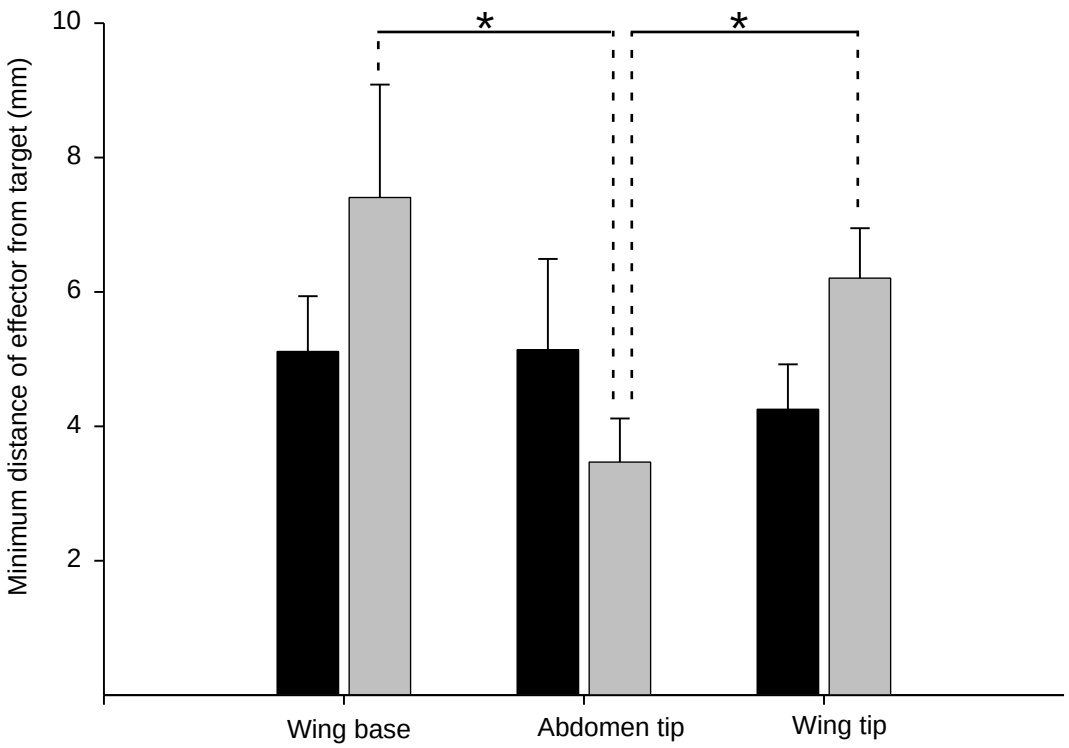


Figure 6. The minimal distance (mean ± SEM) between the effector and the target for 5 th instar (black) and adult (grey) S. gregaria for movements toward wing base, abdomen tip and wing tip targets.

the target was similar for movements toward the wing base, abdomen tip and wing tip (Repeated Measures ANOVA, Greenhouse-Geisser correction, F (1.084, 5.421) = 0.448, *P* = 0.548). The effector reached a mean distance from each target of 5.1 ± 0.8 mm, 5.1 ± 1.4 mm, and 4.3 ± 0.6 mm (Mean ± SEM) for wing base, abdomen tip and wing tip respectively (black bars in Fig. 6).

For adults, the mean closeness of the effector to the different targets was significantly different (Repeated Measures ANOVA, F (2, 10) = 4.167, *P* = 0.048). *Post hoc* t-tests revealed that although the closeness of movements toward the wing base and wing tip were not significantly different to each other (*P* = 0.537), movements toward the abdomen tip were significantly closer than movements toward the wing base (*P* = 0.028) or the wing tip (*P* = 0.036). The effector reached a mean distance from each target of 7.4 ± 1.7 mm, 3.5 ± 0.7 mm, and 6.2 ± 0.7 mm (Mean ± SEM) for wing base, abdomen tip and wing tip respectively (grey bars in Fig. 6).

When the closeness of 5th instars and adult movements to targets were compared using a One-way ANOVA, there was no significant difference for movements toward wing base targets (F (1, 10) = 1.498, *P* = 0.249), for abdomen tip targets (F (1, 10) = 1.242, *P* = 0.291), or for wing tip targets (F (1, 10) = 3.815, *P* = 0.079).

Initial direction of movement and speed

To determine whether movements were target specific from their onset, I analysed the initial 200 ms of movement that formed the out going trajectory of the effector.

For 5th instars, the initial directions of movements toward all three targets were directed dorsally and anteriorly (Fig. 7 A). The initial direction of movement was analysed with a Friedman Test, a non-parametric version of the repeated measures ANOVA, to compare the effect of target position (wing base, wing tip and abdomen tip) on movement direction in 5th instars. The initial direction of movement for the first 200 ms was similar for movements toward all three stimulus sites (Friedman χ^2 (2, 6) = 0.333, *P* = 0.846, see Fig. 7 A).

For adults, the initial direction of movement toward wing tip targets (dark grey vector in Fig. 7B) was more posterior than that for either wing base or abdomen tip targets (light grey vector and black vector respectively in Fig. 7B). Target position had a significant effect on the initial movement direction (Friedman χ^2 (2, 6) = 9.000, *P* = 0.011).

Fifth instars and adults were compared, using non-parametric Mann-Whitney tests, to compare the effect of age (5th instar, adult) on corresponding pairs of target locations. There was no significant difference between 5th instars and adults for movements toward wing base (light grey vectors and corresponding curved lines in Fig. 7 A, B; Mann-Whitney U = 18.000, Z = 0.000, *P* = 1.000) and abdomen tip targets (black vectors and corresponding curved lines in Fig. 7 A, B; Mann-Whitney U = 15.000, Z = -0.480, *P* = 0.631). There was, however, a significant difference for scratches toward wing tip targets (dark grey vectors and corresponding lines in Fig. 7 A, B; Mann-Whitney U = 0.000, Z = -2.882, *P* = 0.004).

For 5th instars, the initial speed was similar for scratches towards all three targets (vector lengths in Fig. 7B, Friedman Test, χ^2 (2, 6) = 3.000, *P* < 0.223). For adults also, the

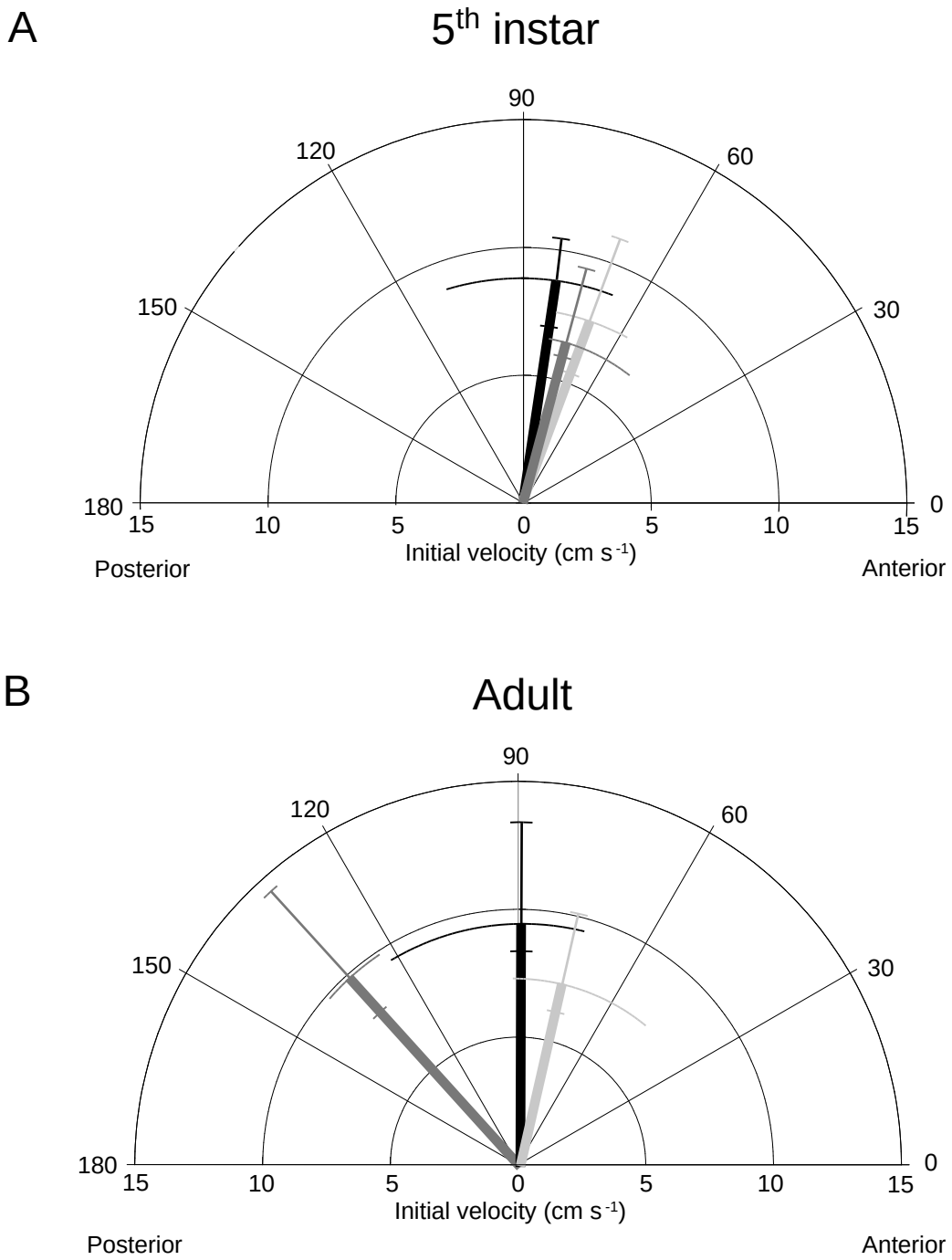


Figure 7. The initial velocity of the effector measured over the first 200 ms for movements in 5 th instar (A) or adult (B) S. gregaria toward wing base (light grey), wing tip (dark grey) and abdomen tip (black) targets. The median direction is indicated by the angle of each thick line, and the interquartile range is indicated by the length of the perpendicular thin error bars. The median veolcity of movement is indicated by the length of each thick line and the interquartile range of velocity is indicated by thin error bars. Movements toward wing tip targets were more posterior (larger angle) for adults than for the movements toward wing tip targets in 5 th instars ($P = 0.004$). Speed was similar for 5 th instars and adults for movements toward wing base ($P = 0.749$) and abdomen tip ($P = 0.631$), but different for movements toward wing tip ($P = 0.037$).

initial speed was similar for scratches towards all three targets (vector lengths in Fig. 7A; Friedman Test, χ^2 (2, 6) = 4.333, $P < 0.115$).

When 5th instars and adults were compared, initial speeds were similar for movements toward wing base (light grey vectors and corresponding lines in Fig. 7 A, B; Mann-Whitney U = 16.000, Z = -0.320, P = 0.749) and abdomen tip targets (black vectors and corresponding lines in Fig. 7 A, B; Mann-Whitney U = 15.000, Z = -0.480, P = 0.631). However the initial speed for scratches toward wing tip targets was faster in adults than in 5th instars (dark grey vectors and corresponding lines in Fig. 7 A, B; Mann-Whitney U = 5.000, Z = -2.082, P = 0.037).

Cyclic component of scratching

To identify differences in the cyclic component of scratching movements, the number of movements with 0, 1, 2, or 3 loops was determined for 5th instars and adults for each target (Fig. 8). A loop was defined by both extension and flexion of the femoro-tibial joint, and ended with the transition of the femoro-tibial joint from flexion to extension. First, relatively few scratches (9.6 ± 1.3 %, Mean ± SEM) had no loops. Second, χ^2 tests comparing the average number of scratches with 0, 1, 2, or 3 loops revealed that there was no significant difference between 5th instars and adults for movements towards wing base stimuli (χ^2 = 1.201, df = 3, P = 0.867), abdomen tip stimuli (χ^2 = 0.671, df = 3, P = 1.000) or wing tip stimuli (χ^2 = 2.237, df = 3, P = 0.588).

The overall pattern of movement during the cyclic scratching phase for a group of scratches was summarised as the effector probability distribution (Fig. 9). Regions of

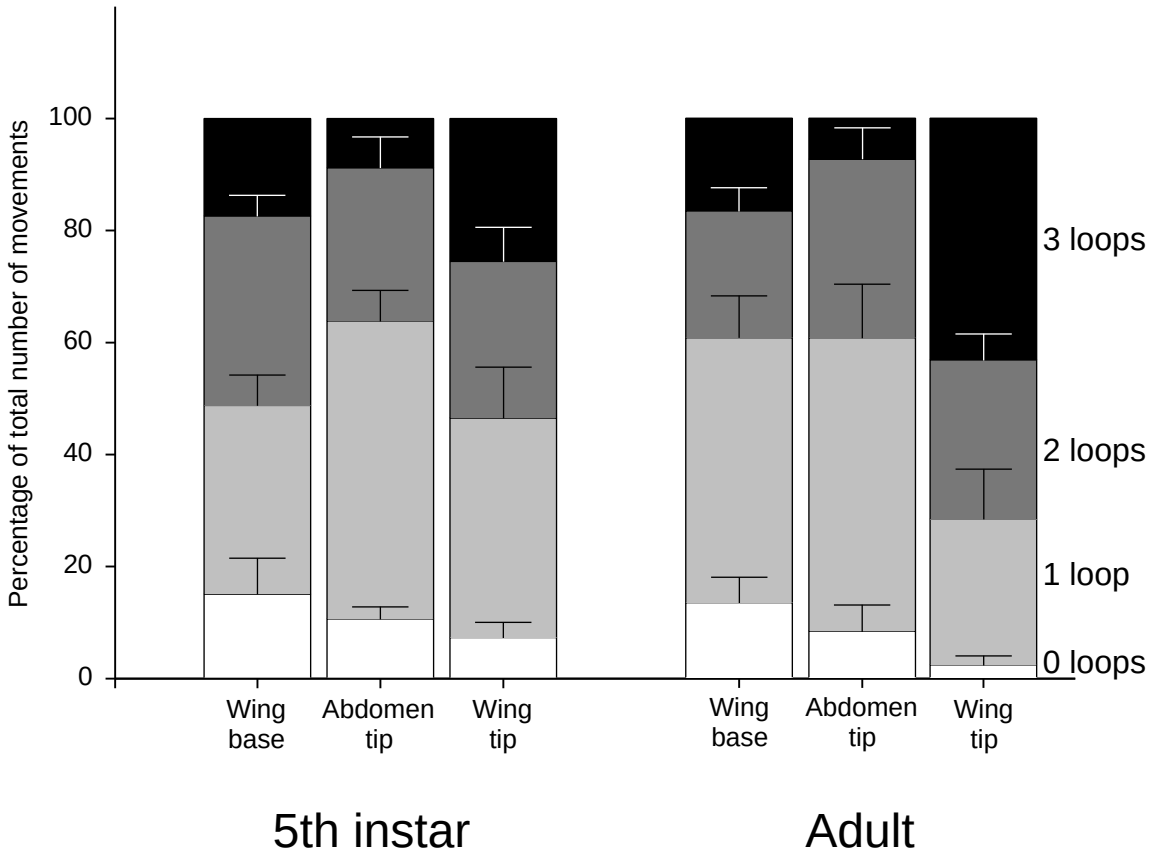


Figure 8. Percentage of total number of scratching movements with 0, 1, 2 or 3 loops. Each column shows movements with 0 loops (white), 1 loop (light grey), 2 loops (dark grey) and 3 loops (black) as a percentage of the total number of movements towards each target site for 5 th instar and adult S. gregaria. Mean percentage for 6 animals is shown with standard error of the mean.

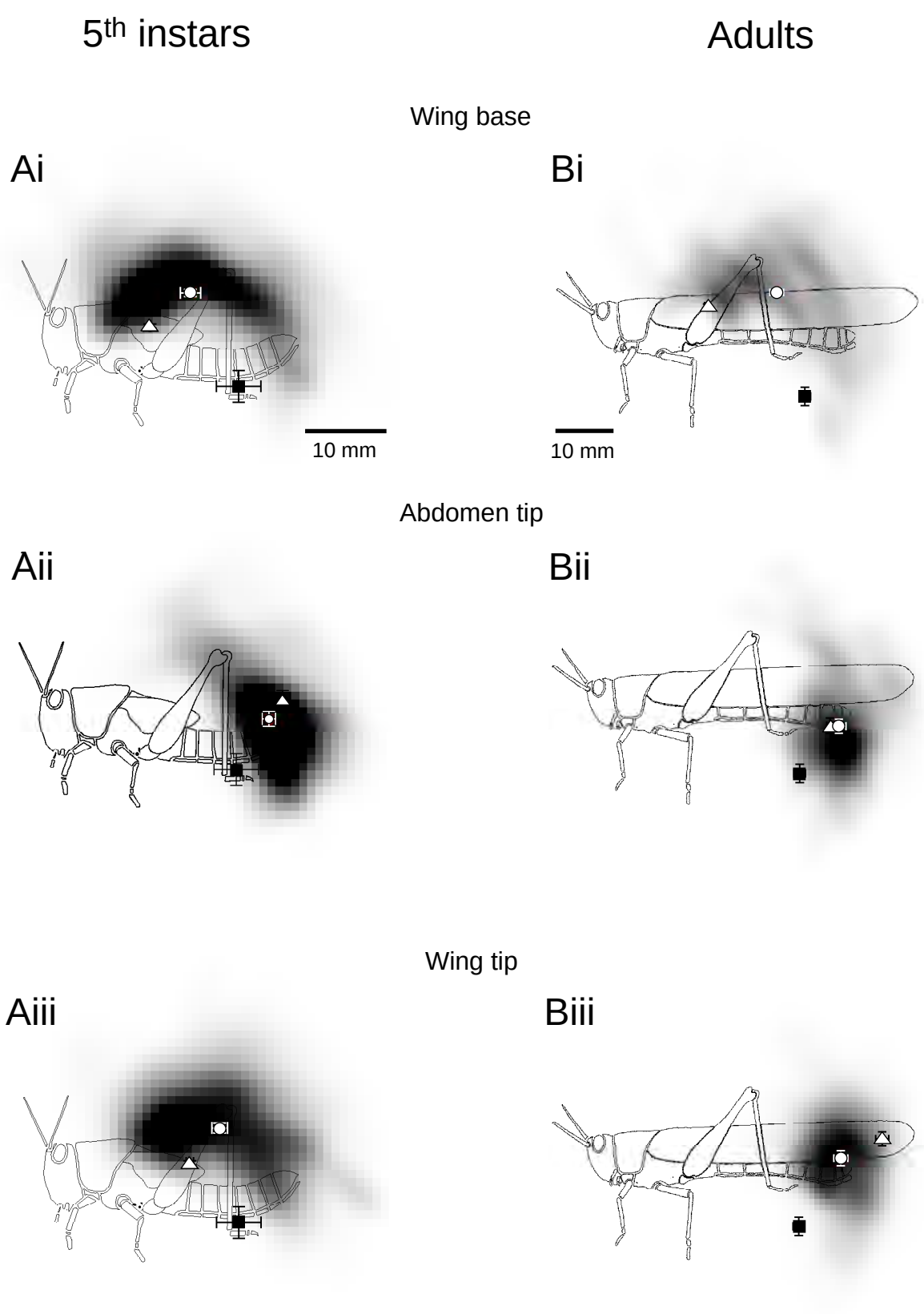


Figure 9. Effector probability distributions were for 5 th instar (A) and adult (B) S. gregaria for movements toward wing base, abdomen tip and wing tip targets (white triangles in i, ii and iii, respectively). The grey scale codes the likelihood with which a particular point in the workspace would be crossed by the effector. The centre of density for each probability distribution (white circle, mean ± SEM) represents the effector's most probable location during a group of movements. 5th instar: wing base: N = 93; abdomen tip: N = 84; wing tip: N = 86. Adult: wing base: N = 83; abdomen tip: N = 79; wing tip: N =78.

Table 1.Overlap and discriminability of probability distributions for movements toward wing base, abdomen tip and wing tip for 5 th instar and adult S. gregaria.

	Wing base	Abdomen tip	Wing tip	
Wing base Overlap, % Probability No. required		39.5 0.273 15*	75.6 0.445 223	5th instars
Abdomen tip Overlap, % Probability No. required	34.1 0.240 11*		47.4 0.324 23*	
Wing tip Overlap, % Probability No. required	36.8 0.265 11*	66.1 0.420 107		
	Adults			

Pairs of distributions within an age group were tested for an effect of target position, i.e. whether target site can be determined reliably from observations of the scratching response. * and bold numbers indicate the pairs of distributions that can be discriminated within the average number of observations per response.

highest probability are darkest in Fig. 9, and the weighted centre of the distribution is indicated by a circle (mean ± SEM) and termed the mean centre of density.

In 5th instars, the probability distributions for movements aimed at targets at the base of the wing and the tip of the wing both formed an arc (shaded region in Fig. 9 Ai, Aiii) that curved from near the start position (black square), dorsally and anteriorly, then ventrally to near the target (white triangle). The mean centres of density (white circles in Fig. 9 Ai, Aiii) were dorsal and posterior to the targets in both cases, placing the targets at one extreme of the cyclic movements. The probability distribution for movements aimed at targets at the tip of the abdomen formed a compact distribution over the target site (white triangle in Fig. 9 Aii). The centre of density (white circle in Fig. 9 Aii) was close to the target (white triangle in Fig. 6 Aii). This shape indicated that the effector moved from the start position dorsally and posteriorly toward the target, forming a cyclic movement around the target. Both distributions were denser than the probability distribution for movements toward wing base targets in adults because the movements covered a smaller area.

The probability distributions for movements aimed at the wing base and wing tip targets overlapped by 75.6% and were not significantly different for 5th instars (Table 1). Probability distributions for movements aimed at the wing base and abdomen tip in 5th instars overlapped by 39.5% and were significantly different (Table 1), as were those for movements toward wing tip and abdomen tip targets (47.4% overlap). These movement distributions were thus target-specific.

As a second measure to test whether movements were target-specific in 5th instars, I statistically compared the centres of density of movements toward wing base, wing tip or abdomen tip targets. The *x* coordinates of the centres of density were analysed using a Repeated Measures ANOVA to compare effects of target position (wing base, wing tip and abdomen tip). Target position had a highly significant effect on the x-axis location of the centre of density (Repeated Measures ANOVA $F\ (2, 10)$ =31.13 $P<0.001$). *Post-hoc* tests revealed that the centres of density of distributions for movement aimed at wing base and wing tip targets were in a similar location along the x-axis. Both were however, significantly more anterior than the centre of density for movements towards abdomen tip targets (wing base: $P = 0.004$ and wing tip: $P = 0.004$). In other words, the centre of the cyclic component of movements towards anterior targets, such as the wing base and wing tip in 5th instars, were significantly more anterior than those toward the posterior target, the abdomen tip.

In adults, the probability distribution for movements aimed at targets on the base of the wing formed an arc that curved from near the start position (black square in Fig. 9 Bi), dorsally and anteriorly, then ventrally to near the target (white triangle). The mean centre of density (white circle on Fig. 9 Bi) was dorsal and posterior to the target. The probability distributions for movements following stimulation of the wing base were less dense than those for the wing tip or abdomen tip, because the movements covered a larger area. For the majority of movements toward wing base targets, the effector moved within the arched region between the target and the ground (shaded region on Fig. 9 Bi). In adults, the probability distributions for movements aimed at targets at the abdomen tip or wing tip formed a compact cluster close to the corresponding target site (white triangle in Fig. 9 Bii, Biii). For the majority of movements toward abdomen tip

and wing tip targets, the effector therefore moved within a compact region near to the target. The centre of density was ventral and anterior to the target in movements toward wing tip targets (white circle in Fig. 9 Biii) placing the target at the posterior of the cyclic movement, whereas in movements toward targets at the abdomen tip the centre of density overlapped the target site (white circle in Fig. 9 Bii), placing the target in the centre of the cyclical movement.

To test whether trajectories were target-specific in adults, the overlap between pairs of probability distributions was compared in the same way as for 5th instars. The probability distributions for movements aimed at wing base and wing tip targets overlapped by only 36.8% and were significantly different from one another (Table 1), as were movements aimed at the wing base and abdomen tip (Table 1). Probability distributions for movements aimed at the wing tip and abdomen tip targets overlapped by 66.1% and were not significantly different for adults (Table 1).

As for 5th instars, target position had a highly significant effect on the x-axis location of the centre of density, Repeated Measures ANOVA $F(2, 10) = 61.05$, $P < 0.001$. *Post-hoc* tests demonstrated that in adults, movements toward the wing base had a significantly more anterior centre of density than movements toward the abdomen tip ($P = 0.002$) or the wing tip ($P = 0.001$). In contrast movements toward the abdomen tip and wing tip had centres of density in a similar location along the x-axis ($P = 1.000$). In other words, the centre of the cyclic component of movements towards anterior targets, such as the wing base, was significantly anterior to those toward posterior targets, such as the abdomen tip or wing tip.

Comparison of 5th instar and adult scratching movements

For movements towards an anterior target such as the base of the wing, the probability distributions for 5th instars and adults both had a similar shape (shaded area in Fig. 9 Ai, Bi), curving from near the start position (black square), dorsally and anteriorly, then ventrally to near the target (white triangle) on the base of the wing. The adult distribution was less dense as the trajectories covered a larger area (note scale bars in Fig. 9). The centres of density were both dorsal and posterior to the target for both 5th instars and adults (white circles in Fig. 9 Ai, Bi). For movements towards a posterior target, such as the tip of the abdomen, the probability distributions for 5th instars and adults also had a similar shape (shaded area in Fig. 9 Aii, Bii), forming a compact cluster close to the target (white triangle). The centres of density (white circle) were both close to the target. The location of the wing tip changes from an anterior position in 5th instar locusts to a posterior position in adults, and this was accompanied by a clear change in the behavioural response. Fifth instars made an anteriorly directed movement in response to stimulation of the wing tip (shaded area in Fig. 9Aiii) that had a similar shape to probability distributions of movements aimed at the base of the wing (Fig. 9 Ai), whereas adults made a posteriorly directed movement (Fig. 9 Biii), that had a similar shape to the probability distributions of movement aimed at the tip of the abdomen (Fig. 9 Bii).

Can 5th instar locusts distinguish between wing base and wing tip targets, because the distance between them is small? The probability distributions for the movements toward the wing pad base and wing pad tip overlapped each other by 75.6%, and were not significantly different (Table 1). There was a 5.3 ± 1.9 mm mean difference between the x-axis position of centres of density for movements aimed at wing base and wing tip

targets, however that difference was not significant (Repeated Measures ANOVA $F(2, 10) = 31.13$ $P<0.001$, *posthoc* test $P = 0.110$). The wing pad tip is only 4 mm posterior to the wing pad base, and there was a corresponding 5.3 mm posterior shift in the centre of density which nevertheless was non-significant.

Joint dynamics

To determine whether 5th instar and adult *S. gregaria* use similar patterns of inter-joint co-ordination to reach anterior or posterior targets, I plotted the combined thoraco-coxal and coxo-trochanteral joint angle against the femoro-tibial joint angle at the closest point of approach to the target of the effector in all animals (see Fig. 10). Mean joint angles for each animal were overlaid on the data for adults from Dürr and Matheson (2003) for both 5th instars and adults (Fig. 10 AB). Dürr and Matheson (2003) stimulated multiple sites along the length of the wing surface in adult *S. gregaria* and found that combinations of joint angles were gradually shifted as the target position was moved along the wing's length (grey data points, Fig. 10 A). My data for adults reaching toward anterior targets at the wing base, and posterior targets at the wing tip correspond well with the pattern described by Dürr and Matheson (2003). For anterior wing base targets the thoraco-coxal + coxo-trochanteral joint angle was small and the femoro-tibial joint angle large (Fig. 10 A), reflecting elevation of the femur and flexion of the tibia (Fig. 2 C). For posterior wing tip targets, the thoraco-coxal and coxo-trochanteral joint angle was large, and the femoro-tibial joint angle small (Fig. 10 A) reflecting depression of the femur and extension of the tibia (Fig. 2 C). Joint angle combinations used to reach the abdomen tip lie on the same curve as those used to reach the wing (Fig. 10A).

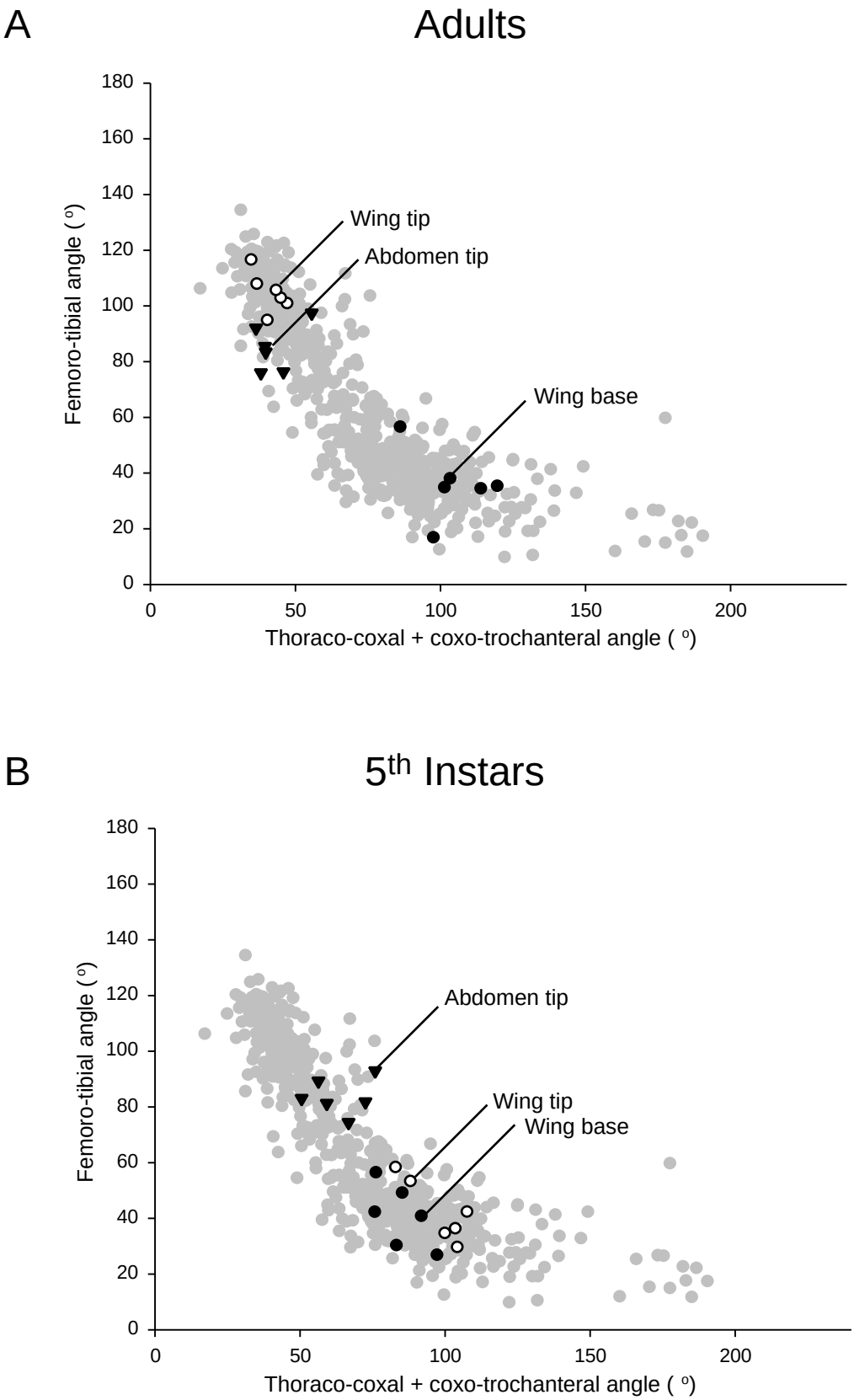


Figure 10. Hind leg joint angles at the closest point of approach. Combinations of femoro-tibial angle and the combined thoraco-coxal + coxo-trochanteral angle of the hind leg at the point of closest approach to the wing base (white circle, mean ± SEM), wing tip (black circle, mean ± SEM) and abdomen tip (black triangle, mean ± SEM) targets, are shown against data from Duĭr and Matheson (2003) (grey circles, individual scratches).

For 5th instars, movements toward the anterior wing base target also use joint angles that coincide with the joint angle combinations used to reach anterior targets in adults (Fig. 10 B). Furthermore, the combinations of joint angles used to reach the posterior abdomen tip target in 5th instars, coincide with those toward posterior targets in adults (Fig. 10 B). In contrast, the combinations of joint angles for movements toward the wing pad tip are very different to those toward wing tip targets in adults (Fig. 10 AB), but still lie on the same curve.

Adults and 5th instars therefore use the same graded combinations of limb joint angles to reach corresponding targets, demonstrating the movement pattern does not change during development. To statistically test for a difference between adult and 5th instar data, quadratic equation curves were fitted to adult and 5th instar data sets. An F-test was performed to determine if the curves were significantly different, by comparing the error for either 5th instar or adult separate samples, with the error of the 5th instar and adult samples combined. If the 5th instar population was different to the adult population, then combining the two would result in a much larger error than each group analysed separately. The continuum of joint angles used by adults and 5th instars was not significantly different, $F(3, 30) = 1.400$, $P = 0.262$.

Discussion

During the imaginal moult of *S. gregaria*, body size and structure change in two key ways. First, growth of the wings is non-allometric compared to growth of the body (447% vs. 140% increase in length). Changes in the size of the wings relative to the body mean that the tips of the wing pads lie over the thorax in 5th instar nymphs, but that the tips of the wings protrude beyond the tip of the abdomen in adults. Thus the position of the exposed wing base is unchanged relative to the body and hind leg, whilst the position of the tip of the wing is altered during the imaginal moult. Second, rotation of the wings during the imaginal moult means that the ventral surface of the hind wing is exposed in 5th instars but that the dorsal surface of the fore wing is exposed in adults. Thus, external stimuli are detected by two different wing surfaces during the 5th instar and adulthood. To successfully respond to stimulation of the wing tip, scratching behaviour must be modified to take into account the relocation of targets and the different sensory input from tactile hairs. This study illustrates that such an adaptation does indeed occur and scratches are retargeted to appropriate locations, whilst the characteristic form of movements remains the same during development.

Do 5th instar *S. gregaria* scratch?

Fifth instar locusts scratch, but in response to a stimulation of a different wing surface to adults. This is the first description of the development of scratching behaviour in locusts. In response to stimulation of the exposed wing pad (the ventral hind wing) 5th instar locusts perform rhythmic scratching movements using the ipsilateral hind leg. In adult *S. gregaria*, the dorsal fore wing is the exposed wing surface. The different wing surfaces result in different tactile hairs detecting the stimulus in 5th instars and adults. It

is therefore possible that the associated sensory afferents have different synaptic connections onto postsynaptic interneurones involved in generating the scratching response. Fifth instar sensory hair afferents (from the hind wing) send signals locally to the metathoracic motor network underlying scratching whilst adult sensory hair afferents (from the fore wing) send information intersegmentally via the mesothoracic ganglion (Altman *et al.*, 1978).

Developmental changes in scratching behaviour may be genetically "hard wired" and mediated by wing rotation

The involvement of different wing surfaces also suggests that 5th instar and adult responses to exposed wing stimulation might be "hard wired" (fixed neuronal circuitry) within the nervous system, and released when appropriate during development, and therefore genetically controlled Wing rotation is likely to be genetically predetermined by gene expression that lead to changes in the rate of cell division of the ventral wing surfaces compared to the dorsal wing surface (Ivanova 1947) as it is a characteristic shared by many different winged insects, although its function is not known. The different wing surfaces that are presented during growth can explain the why different behaviours are stimulated by touching the exposed wing surface of 5th instars to adults without any changes in the underlying neuronal pathways. The anteriorly directed behaviour of 5th instars is associated with sensory neurones from hairs on the ventral hindwing, whilst the posteriorly directed behaviour of adults is associated with sensory neurones from hairs on the dorsal forewing. Thus instead of the new behaviour arising through learning, the genetically preprogrammed change of wing rotation might allow stimulation of different wing surfaces to elicit different behaviours, without the need for modification of scratching behaviour through learning.

Do changes in other locust behaviours indicate hard wired neuronal patheways? Many other neural components underlying locust behaviour are hard wired from birth. Oviposition and flight neuronal pathways develop in the embryo but are not expressed until adulthood (Seymour, 1990; Thompson, 1986a, b; Rose, 2004; Thompson and Roosevelt, 1998). The neuronal pathways generating oviposition behaviour are hard wired from birth, exhibiting similar motor patterns in larvae and adults (Thompson and Roosevelt, 1998). Neural components involved in generating the flight motor pattern, such as the wing hinge stretch receptor, flight interneurones and motor neurones innervating flight muscles, develop their basic morphological shape embryonically (Kutsch and Hemmer, 1994; Bentley and Toroian-Raymond, 1981). The sensory apparatus that control flight, however, alters during an insect's life. For example, the number of wind-sensitive hairs on the head of the locusts increases with each instar (Svidersky, 1969). At the same time, the adoption of flight posture in response to stimuli that generate flight in adult insects becomes develops to the adult form (Cooter, 1973; Altman, 1975; Kutsch, 1985). After onset of flight behaviour, wing beat frequency is modified, increasing as body mass increases. This may be associated with changes in sensory input. Thus the interneurones and motor neurones underlying many adult behaviours develop embryonically, whilst sensory systems continually expand at each moult. This would suggest that the motor neurones and interneurones underlying scratching behaviour would also develop embryonically. This is likely for the motor neurones and several interneurones also generate walking behaviour, which is performed by 1^{st} instars. Furthermore, the sensory system of tactile hairs that elicit scratching behaviour are likely to develop with each instar. Thus the sensory system for the ventral hindwing may develop for 4^{th} and 5^{th} instars when the ventral hindwing is exposed, whilst the sensory system for the dorsal forewing may develop for adulthood

(Altman *et al.*, 1978). The development of tactile hairs on the wings of 5th instars and adults will be addressed in Chapter 2.

In the context of locust hind leg scratching behaviour directed at the wing surfaces, it is evident that further development of the wings and the sensory apparatus on them occurs between the 5th instar and adulthood. Altman and co-workers (1978) describe an increase in the number of axons innervating the locust hind wing with each successive moult. I have demonstrated that the scratching movement pattern is already present in 5th instars, but it is likely that the motor network that generates it, elements of which are also used for locomotion, may develop embryonically and is modified postembryonically. The modification of scratching behaviour may be mediated by dramatic differences in the sensory apparatus of the wing surface between the ventral hind wing and the dorsal fore wing and the connections of wing hair afferents.

Changes in spatial location of targets requires different sensory input

Plasticity of a sensory system is critical in maintaining behaviours during growth. In the prey capture behaviour of the praying mantis, catching distance is precisely estimated by a triangulation mechanism of the visual system, and the longest distance that elicits a strike is related to the length of the fore legs (Maldonado and Barros-Pita, 1970; Barros-Pita and Maldonado, 1970). A prey stimulus within this region elicits a striking response. The behaviour itself has two components: a fore leg strike and a lunge of the body. The area within which a response is elicited increases in proportion to foreleg length. The development of the visual system acts to preserve the targeting mechanism

within this space. The growth of the head and the eyes is such that throughout development the same ommatidium or group of ommatidia register a stimulus at the maximum catching distance, thus triggering the same striking movement. In other words, the movement is conserved by growth maintaining constancy in the input of the visual system to the motor network. In contrast in locust scratching, the position of the wing tip is different in 5th instar and adults, and this is associated with the involvement of sensory afferents from different wing surfaces. In other words, a change in the movement is associated with a change in sensory input. Thus the role of sensory signals in shifting the equilibrium position of the leg need to be investigated (Chapter 2 and 3).

A single movement pattern for scratching is conserved during development whilst sensory input changes

There were strong similarities between the scratches of 5th instars and adults, suggesting conservation of a single movement pattern that can be shifted toward anterior or posterior targets. First, both 5th instars and adults used the distal tibia as the effector, consistently using it to reach toward the site of stimulation. Second, both 5th instars and adults moved the effector at similar speeds for the initial 200 ms of movement. Third, the minimum distance between the effector and the target, a measure of accuracy, was similar for both 5th instars and adults. Fourth, 5th instar and adult locusts both used similar combinations of hind leg joint angles to reach targets in similar positions. The effector is the key criterion used to define forms of movement (Stein *et al.*, 1986), suggesting that there is no developmental change in the form of movement. Furthermore the consistency in accuracy and combinations of hind leg joint angles used to reach stimuli imply that a similar strategy for aiming movements exists in both ages. This

might be achieved by using the same neuronal network to generate a single movement pattern, and conserving the properties of and connections between the interneurones and motorneurones that comprise this network. This movement pattern is modifiable (Dürr and Matheson, 2003), by different sensory inputs (tactile hairs at different spatial locations on the wing). I suggest that it is the synaptic connections of these sensory neurones onto the motor network for scratching that modifies the movement pattern. In other words, sensory projections from tactile hairs in an anterior spatial location on the wing form different synapses onto the scratching motor network than those from tactile hairs in a posterior spatial position on the wing. The difference may be the region of branching within the ganglion of the central nervous system. Such somatotopic maps, where peripheral receptor position is represented by the organisation of neuronal projections within the central nervous system, have been described for tactile hairs on the locust hind leg (Newland, 1991a). The projections of tactile hairs on the wing have not been described in holometabolous insects, and Chapter 3 will pursue this.

Developmental changes in muscle strength and limb mass may also be compensated for, as movement patterns in 5^{th} instars and adult *S. gregaria* had the same characteristics. Indeed, applying loads to the leg of adults locusts are compensated, perhaps by proprioceptive reflexes and dynamic joint stiffness (Matheson and Dürr, 2003). Such mechanisms, again, would allow for the conservation of the motor network that generates scratching.

The equilibrium point hypothesis can explain the development of scratching

There is considerable debate about whether muscle forces are encoded explicitly by the nervous system, or whether limb position is encoded and the movement arises as a result of changes in a neuronally specified "equilibrium position" (Feldman, 1966). Motor commands that move the tarsus along a desired trajectory must be computed by an internal model that calculates the rotation of individual leg joints, taking into account musculo-skeletal resistance. The equilibrium point hypothesis provides an explanation in which motor commands are represented within an internal model that also encodes joint rotations, torques and muscle actions. Joint stiffness and proprioceptive reflexes might accommodate developmental changes, such as increased muscle strength or increased limb mass, in the musculo-skeletal system (Matheson and Dürr, 2003), and thus maintain an equilibrium position of the leg. The continuum of hind leg joint angles used for reaching different targets along the locust wing (Dürr and Matheson, 2003) may lend support to the equilibrium point hypothesis that tarsal trajectories are achieved by a shift in the limb equilibrium position. The observed shift of the movement pattern would require that stimulus location is encoded in such a way that it could induce a shift in the equilibrium point of the hind leg. The developmental change in desired equilibrium position from anterior (in 5^{th} instars) to posterior (in adults) is associated with sensory input from different wing surfaces. This change in sensory input could alter the equilibrium point without necessitating whilst conserving the properties and connections of the motor network.

Hind leg position is both controlled and internally represented by hind leg motor neurones and feedback from proprioceptive receptors such as chordotonal organs

(Theophilidis and Burns, 1979; Matheson and Field, 1995). The hind leg femoro-tibial chordotonal organ reaches its minimal firing frequency at a joint angle of 80º. At femoral-tibial angles greater than this, reflex excitation of tibial flexor motorneurones and inhibition of tibial excitor motorneurones occurs, and at femoro-tibial angles lower than this, reflex inhibition of tibial flexor and excitation of tibial excitor motorneurones occurs (Field and Burrows, 1982). Both act to drive the femoral-tibial joint angle toward 80º. Most receptors have such "preferred" states (e.g. joint position) whilst the passive musculo-skeletal system also has its own preferred state to which it recoils passively (Page *et al.*, 2009). Thus the actual hind leg position is a balance of these states – the equilibrium position of the leg. According to the equilibrium point hypothesis, gradually shifting the equilibrium position of the leg ultimately produces the tarsal trajectory of an aimed limb movement. Thus the known starting position of the leg, as represented by proprioceptive input, is shifted toward the target. A bias in the equilibrium position could be achieved by reducing the work capability of one of the pair of antagonistic muscle groups at a joint. This could be achieved in a number of ways; neuromodulation by dorsal unpaired median (DUM) neurones that can affect tonus and force production (Hoyle, 1978), neuromuscular inhibition by common inhibitor neurones that down regulate tonic contraction forces (Wolf, 1990), or altering the feedback of proprioceptive loops. Various mechanisms are likely to change in a co-ordinated way so as not to work against each other. In stick insects, assistive reflexes have been described during voluntary movements (Bässler, 1974, 1976; Cruse and Schmitz, 1983). Modulation of the efficacy of proprioceptive feedback pathways or changes in their preferred angle could cause a shift in the equilibrium position of the hind leg, without resistive reflexes effectively opposing. This would be a literal shift in the equilibrium point. The ensuing gain or loss of muscular contraction across one side of a joint would

cause a shift in the actual position of the leg. The conservation of movement kinematics despite probable developmental changes in the musculoskeletal system indicates a role of proprioceptive reflexes in maintaining movements through development. A logical extension of the proprioceptive reflexes is the equilibrium point hypothesis as a mechanism for the graded modification of a single movement pattern.

The idea of a “preferred state” for the femoro-chordotonal organ is perhaps simplistic, as the receptor is made up of many neurones, each of which has its own “preferred state” and a set of very specific output connections. The output of the organ is therefore much more complex than a single summed drive that is maximal at the extremes and minimal at 80º. It is, however, this complexity that might allow movements to be optimised for other parameters such as speed, force production and not simply position.

Another possible explanation for the conservation of scratching kinematics in the face of changes in the musculoskeletal system is that the scratching motor pattern matures to automatically adapt for changes in the musculoskeletal system. Flight behaviour matures over the first three weeks of adulthood, and does so endogenously without external input. Concomitant with an increase in body mass and hardening of the wings in immature adults, wing beat frequency during flight increases to almost double (Kutsch, 1971, 1973b). This maturation of the motor pattern is not affected by preventing use of the wings, preventing flight experience or removing sensory feedback from the wing. It has been suggested that changes in the sensitivity and conduction speed of the stretch receptor neurone cause more spikes to be delivered to the flight central pattern generator earlier in the wing beat cycle, advancing the timing of the

depressor phase, so that the overall frequency of wing beats increases (Gray and Robertson, 1994; Gee and Robertson, 1994).

In summary, the non-allometric growth and rotation of the wings at the imaginal moult requires adaptation of scratching movements aimed at the wings so that they remain functional. Following stimulation of the ventral hind wing, 5th instar nymphs made rhythmic scratching movements with the ipsilateral hind leg, like those of adults in response to stimulation of the dorsal fore wing. The constancy of the kinematics of scratches between 5th instar and adult locusts indicates little change in the underlying motor network and that developmental changes in the musculoskeletal system are compensated. Stimulation of the hind wing tip in 5th instars elicited an anteriorly aimed scratch, whilst stimulation of the fore wing tip in adults elicited an anteriorly aimed scratch. Thus, modification of the movement pattern and a difference in where the effector is aimed is correlated with differences in sensory pathways between 5th instar and adult locusts. This change in sensory input could alter the equilibrium point of the hind leg without necessitating any other major change to the motor network.

CHAPTER 2

Development of sensory hairs on the wings of the locust: a mini metamorphosis?

Introduction

The desert locust, *Schistocerca gregaria*, has mechanosensory and chemosensory receptors on its wings (Page and Matheson, 2004). Mechanical stimulation of both of types of receptor elicits scratching movements of the hindleg that are aimed at the wing's surface. Touching different places (targets) on the wing leads to different movements (Matheson, 1997, 1998). The tarsus follows a relatively straight trajectory toward the stimulus site, and passes the stimulus site one or more times in a cyclical movement. The aimed component relies on the sensory neurones encoding information about the position of the tactile hair receiving the stimulus, and relaying it to the metathoracic ganglion where the motor neurones of the hindleg originate (Siegler and Pousson, 1990a, b). The combinations of hindleg joint angles used to reach targets are continually graded for different positions along the wing and body in both 5th instar and adult locusts (Dürr and Matheson, 2003, Chapter 1). This demonstrates that the scratching movements are not restricted to a number of pre-programmed combinations of hindleg joint angles. Instead, a single movement pattern is modified to produce a combination of joint angles that is specific for each target. In Chapter 1, I demonstrated that 5th instar *S. gregaria* use the same movement patterns as adults to scratch the wing and abdomen with similar combinations of joint angles, effector velocities and accuracy. Changes in the sensory apparatus that detect the mechanical stimulus may

facilitate shift of the scratching movement pattern required by wing growth and rotation during development, whilst maintaining similar movement patterns and kinematics.

Allometric growth requires the nervous system to compensate for changes in muscle strength and limb mass to maintain a movement toward a target in a proportionally similar position. In contrast, non-allometric growth creates an additional challenge for the nervous system; movement must also be retargeted to account for disproportionate growth. In *S. gregaria* the non-allometric growth of the wings mean that whilst the tips of the wing pads overlie the thorax in 5th instars, the tips of the wings extend posterior to the abdomen tip in adults. Furthermore, rotation of the wings during growth means that the ventral surface of the hind wing is exposed to external stimuli in 5th instars, whilst the dorsal surface of the forewing is exposed in adults (Ivanova, 1947). Despite this, scratching movements in both 5th instars and adults remain appropriately aimed at wing tip targets and are executed with similar kinematics and hindleg joint angles, suggesting that a single movement pattern is used during development (Chapter 1). Wing rotation leads to different sensory neurones encoding the position of tactile hairs detecting a stimulus during development. In 5th instars sensory neurones from the ventral hindwing encode stimulus position, whilst in adults stimulus position is encoded by sensory neurones from the dorsal forewing. As such, the organisation of sensory apparatus of different wings may be key to how the spatial representation of a growing wing surface is achieved. As yet the developmental changes in the *organisation* of tactile hairs on the wing have not been described, and this chapter seeks to do so.

Insect sensory hair development begins with the genetic induction of the division of a single epidermal cell into 4 cells; a tormogen, a trichogen, a thecogen and a sensory cell

(Fig. 11, see Keil, 1997a for review). The thecogen cell forms a sheath around the sensory cell, and both are partially enveloped by the trichogen and tormogen cells. The trichogen cell projects an apical sprout which secretes the hair shaft, before receding to leave a cavity within the hair. The thecogen cell forms the socket. The sensory cell gives rise to a dendrite that attaches to one side of the base of the hair, and an axon that projects to the segmental ganglion. The length of the hair and its mechanical properties may influence its sensitivity to speed, acceleration, air movement or physical contact (for review see Keil, 1997b). During the growth of hairs in hemimetabolous insects, such as the hairs on the cerci of crickets (Gnatzy and Romer, 1980; Gnatzy, 1978), the epidermis separates from the cuticle around the sensillum, and the trichogen and tormogen cells begin forming a new hair beneath the existing one. The dendrite remains attached to the old hair shaft, and elongates to cross the cavity between the old cuticle and the newly forming cuticle, passing through a moulting pore in the forming hair. Thus, existing sensilla are often conserved and can be identified and tracked across many moults (Gnatzy, 1978). New hairs are generated by the division and differentiation of an epidermal cell into new thecogen, trichogen, tormogen and sensory cells, and is regulated genetically (Jan *et al.* 1985). The development of hairs on the wing of hemimetabolous insects has not been examined. The arrangement of mechanosensory sensilla on hindleg and thorax is represented by the position of hair afferents within the segmental ganglion (Newland, 1991a; Hustert, 1985), and this organisation may preserve the spatial location of a stimulus within the nervous system. If, as described by Gnatzy (1978) for the cerci, existing hairs are associated with tormogen, trichogen, thecogen and sensory cells embedded in the epidermis, then expansion of the epidermis may displace these hairs from the spatial position their sensory neurones encode. Thus, hairs on the tip of the ventral hindwing in 5^{th} instars

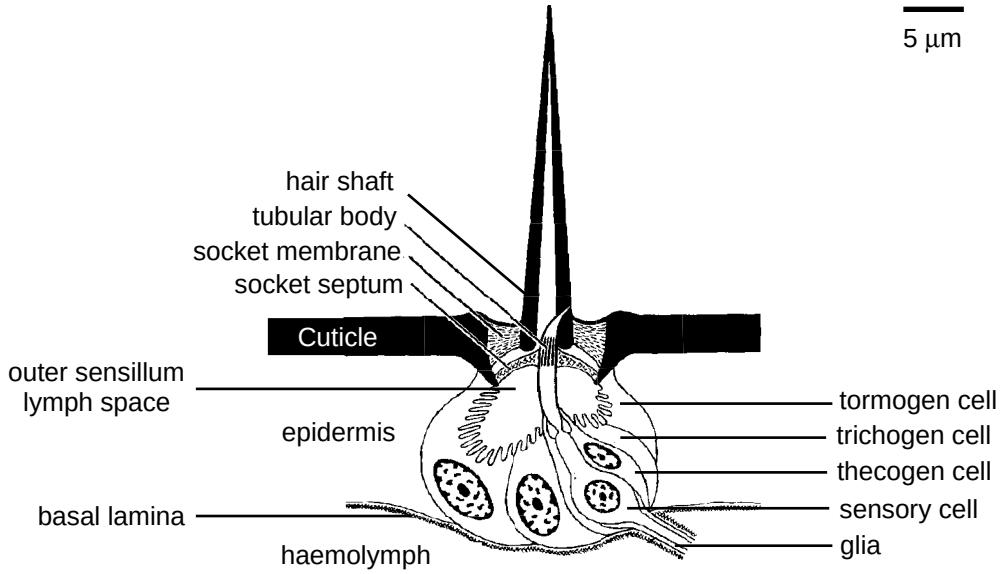


Figure 11. General organisation of an insect (hemimetabolan) mechanoreceptor (Keil and Steinbrecht 1984).

may encode an anterior spatial position, but after expansion of the wing in adults would be displaced to a more posterior position. How could sensory neurones encode the new position of their associated tactile hair?

In many hemimetabolous insects changes in the number of sensory receptors and their transduction properties occur during postembryonic development. In the cricket, *Teleogryllus commodus*, the scolopales of the tympanal organ increase in number and size from the 3rd instar to adulthood, and the subgenual organ, tympanal tracheae, and tympana mature at different rates postembryonically (Ball and Young, 1974). Whilst all receptor cells appear in the 1st instar of the locust, *Locusta migratoria*, the tympanum and associated cuticular structures continue to develop with each successive moult (Michel and Petersen, 1982). Campaniform receptors increase in number and size on the cockroach hindleg (Ridgel *et al.*, 2003). In *Locusta migratoria*, the number of contact chemosensory sensilla on the palps increases with each moult until the 5th instar, when all existing sensilla are reformed during the moult to adulthood (Blaney *et al.*, 1971). In the cercal system of crickets and cockroaches, new filiform hairs are added and existing filiform hairs increase in length (Palka and Edwards, 1974; Knyazev and Popov, 1981; Kanou *et al*., 1988; Kamper, 1992; Chiba *et al*., 1992; Dangles *et al*., 2006). The modification of sensory apparatus in crickets leads to a change in what stimulus hairs detect; from detecting the acceleration of air currents to detecting their speed (Chiba *et al*., 1992; Landolfa and Miller, 1995). Despite this, escape behaviour of orthopteroid insects remains coordinated throughout postembryonic development (Dagan and Volman, 1982). However hairs that detect air currents are different to hairs that detect direct physical contact; air currents can be used to determine the position of an approaching predator by the direction of hair displacement. In contrast, the position of a

tactile hair determines the position of the stimulus. Thus postembryonic development of filiform hairs that detect air currents may be different from the development of mechanosensory hairs that detect physical contact. The development of hairs receiving tactile stimuli may be studied on the wings of *S. gregaria*, where the position of such a stimulus is functionally relevant in the elicited scratching behaviour.

In contrast to development in hemimetabolous insects, holometabolous insects deal with dramatic changes in body structure and size; to accommodate the associated change in behavioural repertoire, almost all larval sensory apparatus is replaced with adult sensory apparatus during metamorphosis (Tissot and Stocker, 2000). The change in body structure and behaviour is associated with reorganisation of the nervous system (reviews: Levine and Weeks, 1990; Truman, 1990; Weeks and Levine, 1990; Truman *et al*., 1993; Levine *et al.*, 1995, Truman, 1996a). Might complex changes in body structure of hemimetabolous insects be accommodated by the degeneration and replacement of sensory apparatus?

This chapter presents the first description of the development of the organisation of tactile wing hairs in a hemimetabolous insect. I have described the pattern of hair distribution on the wings of 5^{th} instar 5th instar and adult locusts, *S. gregaria*, to investigate how these relate to changes in scratching behaviour.

Methods

Fifth instar and adult male and female desert locusts, *Schistocerca gregaria* Forskål, were raised in a crowded colony maintained at the University of Leicester (established with locusts from the University of Cambridge colony and Blades Biological Ltd., Kent).

Hair distribution

One to two days after moulting, fore and hind wings from six 5th instar (3 male and 3 female) and five adult (2 male and 3 female) locusts were removed, rinsed in distilled water in a sonicator to remove dust and debris, and then dried at room temperature. Intact wings were then dry mounted on aluminium stubs, sputter-coated with gold (Polaron SC7640 sputter coater, 90 s at 2.0 kV, 20 mA producing a thickness of 612 Å), and examined using a Hitachi S3000H scanning electron microscope (SEM). Three hair types were identified according to Page and Matheson (2004): short (14 – 46 µm), medium (73 – 159 µm) and long (316 – 511 µm) types were determined by measuring hairs on two adult dorsal forewings. Samples were rotated whilst using the SEM so that hairs were perpendicular to the detector to avoid foreshortened measurements due to the angle of the hair. Hairs were grouped into the non-continuous categories described by Page and Matheson (2004) for each wing surface. The distinct differences in length between categories reduce the chance of mis-classification. All veins on the wing surface were charted by systematically moving the field of view at 18,000 x magnification along each wing vein. Hairs within the area delineated by the field of view were counted. At this magnification the three hair types could be distinguished visually based on hair length and diameter. Parametric statistical tests (multivariate

analysis of variance) were carried out on hair numbers using SPSS version 12 for Windows (SPSS Inc.). Wing vein terminology is after Albrecht (1953). The wing long axis is referred to as proximal-distal, and the orthogonal axis as leading-trailing. The wing surfaces are referred to as dorsal and ventral to reflect their orientation in the adult during flight. The actual position of individual hairs on the wing surfaces varied between animals. The average distribution of hairs along each vein is shown in Figure 13. Within the literature the nomenclature of insect sensilla is inconsistent. The names of sensilla currently in use originate from Schenk's (1903) light microscope observation of shapes of sensilla on lepidoptera and hymenoptera, and include sensilla basiconica (peg-like), styloconica (peg-on-a-pillar), chaetica (spine-like), placodea (plate-like), coeloconica (peg-in-pit) and trichodea (hair-like). These morphological characteristics and the presence of pores have both been used to infer function, but the two are not always in agreement. For example on locust antennae, a class of multi-porous hairs are termed trichoid and thought to be olfactory (Ochieng *et al*., 1998), but aporous hairs on the locust wing and leg are also termed trichoid and known to be mechanosensory (Page and Matheson, 2004; Newland, 1991b). In this thesis, I shall describe the long, medium and short types of hair as long and medium mechanosensory hairs, and contact chemosensory hairs respectively, based on the physiological response properties of each hair type (Page and Matheson, 2004).

Response frequency

Ten 5th instar and ten adult locusts were tethered using a wire noose around the pronotum, and each given a polystyrene ball of 3 cm diameter on which they could walk freely (Matheson 1997). The eyes and ocelli were blacked out using water-based black

acrylic paint (Daler-Rowney, Acryla: Bracknell, UK). The forewing and hindwing on the right hand side were raised from the body and held elevated by applying beeswax at the base of the wing, to enable access to all wing surfaces. Care was taken not to damage the 5th instar wings. Five 5^{th} instar and five adult animals were set up together and allowed to rest for 30 min before the experiment began. A fine paintbrush was used to touch the middle of the dorsal forewing, ventral forewing, dorsal hindwing or ventral hindwing. Stimulus locations were tested in a pseudorandom sequence to apply 20 stimuli to each location in each animal (total of N = 1600 stimulations). No individual animal was stimulated twice within 5 min. Animals were standing still when the stimulus was given. Behaviour was scored as either a scratch or a "no scratch". Since 20 stimuli were delivered to each stimulus location the occurrence of a single scratch yielded a probability of 5%. Parametric (repeated measures or standard) ANOVAs and Student's t-tests were carried out using SPSS version 12 for Windows (SPSS Inc.).

Scratching behaviour

The method of quantitatively tracking locust scratching behaviour has been described in detail in Chapter 1. Six 5^{th} instar locusts and six adult locusts were tethered above a polystyrene ball, with their eyes and ocelli obscured by black paint. The tarsus of the left hind leg was placed on a rod to provide a constant anterior start position. In contrast to chapter 2, where movements toward the ventral hind wing in 5^{th} instars and the dorsal fore wing in adult *S. gregaria* were compared, the ultimate aim of these experiments was to determine whether movements elicited by stimulating the *same* wing surface (ventral hindwing) were retargeted after wing growth. This was achieved by raising the adult wings to allow access to the ventral hindwing.

Experiment 1 (Control): The impact of altering the position of the wing on scratching

Six adult locusts with their wings in the normal folded position whilst at rest were stimulated with a fine paintbrush which was used to touch either the base or tip of the forewing. Each site was stimulated 10-15 times in a non-sequential order. The same animals were also tested with their forewings and hindwings raised to a position perpendicular and almost vertical to the animal's body and fixed at the wing base with bee's wax, to allow access to the ventral hindwing. The vannal region of the hindwing was removed to prevent the hindleg making contact with the raised hindwing (Matheson, 1997). Three of the animals were tested with the *wings raised* condition before the *wings folded* condition, whereas the other three animals underwent the same experiments with the order reversed.

Experiment 2: The impact of the reorganisation of wing sensilla on the ventral hindwing.

Whilst adults were in the *wings raised* condition, the base or tip of the ventral hindwing was stimulated 10-15 times in a non-sequential order. This was repeated with 5^{th} instars but in this case it was not necessary to raise or fix the wings as the ventral hindwing is the uppermost surface and thus easily accessible (see Fig. 1).

Results

Hair distribution on 5th instar and adult wing surfaces

Fore wings and hind wings of six 5th instar and five adult *S. gregaria* were examined under a scanning electron microscope, and the number of long (316-511 µm), medium (73-159 µm) and short (14-46 µm) hairs along each vein were counted (Fig. 12 A, B). Figure 13 shows a diagrammatic representation of the mean number of each hair type on the dorsal forewing (i), ventral forewing (ii), dorsal hindwing (iii), and ventral hindwing (iv).

Ninety percent of all 5th instar wing hairs were found on the exposed wing surface: the ventral hindwing surface (Fig. 13 Aiv, Fig. 4). This exposed wing surface had significantly more hairs (143 ± 20) than the dorsal forewing (2 ± 2), ventral forewing (7 ± 5) or dorsal hindwing (7 ± 4; repeated measures ANOVA with Greenhaus-Geisser correction, df = 1.069, F = 242.934, $P < 0.001$; *post-hoc* tests for ventral hindwing compared to dorsal forewing: $P < 0.001$; ventral forewing: $P < 0.001$; dorsal hindwing: $P < 0.001$). The remaining hairs were located on the subcostal vein of the dorsal forewing (Fig. 13 Ai), ventral forewing (Fig. 13 Aii) and dorsal hindwing (Fig. 13 Aiii). These areas protrude above the leading edge of the ventral hindwing when folded at rest and thus are exposed to external stimuli. The majority of hairs on the 5th instar ventral hindwing were long hairs (82 ± 15, mean ± SD), compared to similar numbers of medium (27 ± 8) and short hairs (35 ± 8).

Seventy-five percent of all adult wing hairs were located on the exposed wing surface, the dorsal forewing (Fig. 13 Bi). The exposed wing surface in adults had significantly

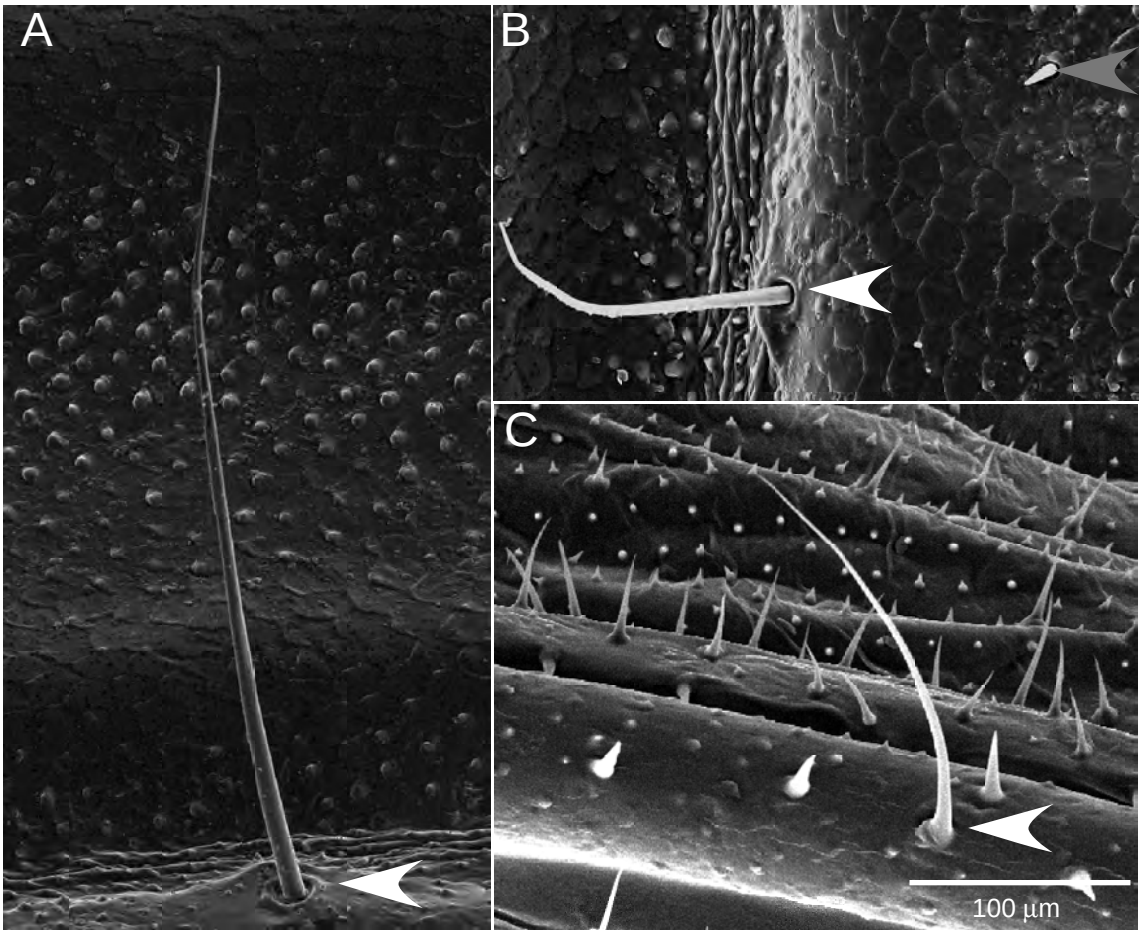


Figure 12. Hairs on the dorsal forewing (A, B) and ventral hindwing (C) of adult S. gregaria. Hairs corresponded to the categories described by Page and Matheson (2004) of long (white arrow, A), medium (white arrow, B) and short (grey arrow, B) hair lengths. All hairs were inserted into sockets with raised rims (see arrow heads). There were also many socket-less, hair-like structures (C). Page and Matheson (2004) found that socket-less hairs were not innervated so they were not included in the SEM count. All images are the same scale.

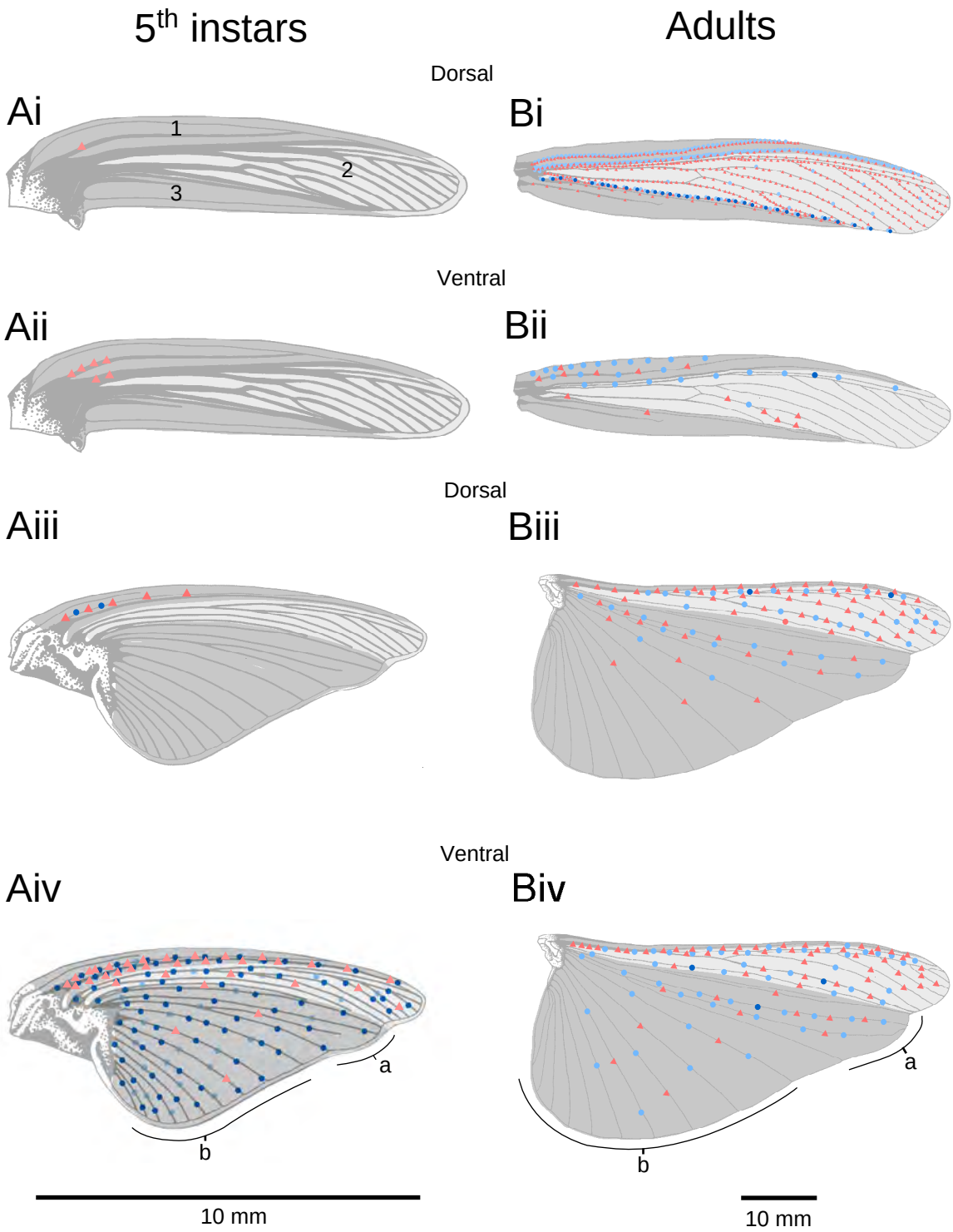


Figure 13. The average distribution of mechanosensory and contact chemosensory hairs on the (i) dorsal fore wing, (ii) ventral fore wing, (iii) dorsal hind wing, and (iv) ventral hind wing of (A) 5th instar and (B) adult S. gregaria. The mean number of contact chemosensory (red triangles), medium (light blue circles) and long (dark blue circles) hairs on each vein is represented above, actual position of individual hairs is not shown. Wings are divided into 3 regions based on vein homology shown in light and dark grey (labelled in Ai). Region 1: costa and subcosta; Region 2: radius, media, cubitus; Region 3: postcubitus and vannus. In 5 th instars (A), the ventral hind wing (iv) is uppermost and has the majority of wing hairs. For the ventral hindwing only, region 3 is further split into postcubitus and vannus 1 - 4 (a), and vannus 5 - 15 (b).

more hairs than the ventral forewing (37 ± 13), dorsal hindwing (97 ± 9) or ventral hindwing (108 ± 51; one-way ANOVA with Brown-Forsythe test for unequal variances, df1= 3, df2 = 7.330, F = 297.667, P <0.001; *post-hoc* tests for dorsal forewing compared to: ventral forewing: P < 0.001; dorsal hindwing: P < 0.001; ventral hindwing: P < 0.001). In contrast to the long hairs that cover the exposed wing surface in 5th instars, the majority of hairs on the adult dorsal forewing are short hairs (511 ± 31), with fewer medium hairs (212 ± 43), and a comparatively small number of long hairs (33 ± 4).

To determine whether new hairs develop during the imaginal moult, the mean hair distribution of each wing surface was compared in 5th instar and adult *S. gregaria* (Fig. 14). There was a significant difference in the number of hairs between 5th instars and adults (two-way ANOVA, testing the effect of age on hair number for each wing surface, DF = 3, F = 396.894, P < 0.001). *Post-hoc* Brown Forsythe tests for a difference in mean value between two samples that have unequal variance found a significant increase in hair number for the dorsal forewing (754 new hairs, P < 0.001), the ventral forewing (30 new hairs, P = 0.001), and the dorsal hindwing (90 new hairs, P < 0.001). There was no significant difference in the total number of hairs on the ventral hindwing between 5th instars and adults (P = 0.267).There was a significant difference in hair number when the hind wing was analysed by region (two-way ANOVA, testing the effect of age on hair number for each region of the ventral hindwing, df = 3, F = 3759.079, P < 0.003). The ventral hindwing was divided into region 1, 2, 3a and 3b (Fig. 13 Aiv, Biv). On average 24 and 32 hairs were lost from region 1 and region 3b respectively, and 12 and 10 hairs were added to regions 2 and 3a. The reduction in hair number in region 3b was significant (vannal region 5-15, see asterisk in Fig. 15 Aiv,

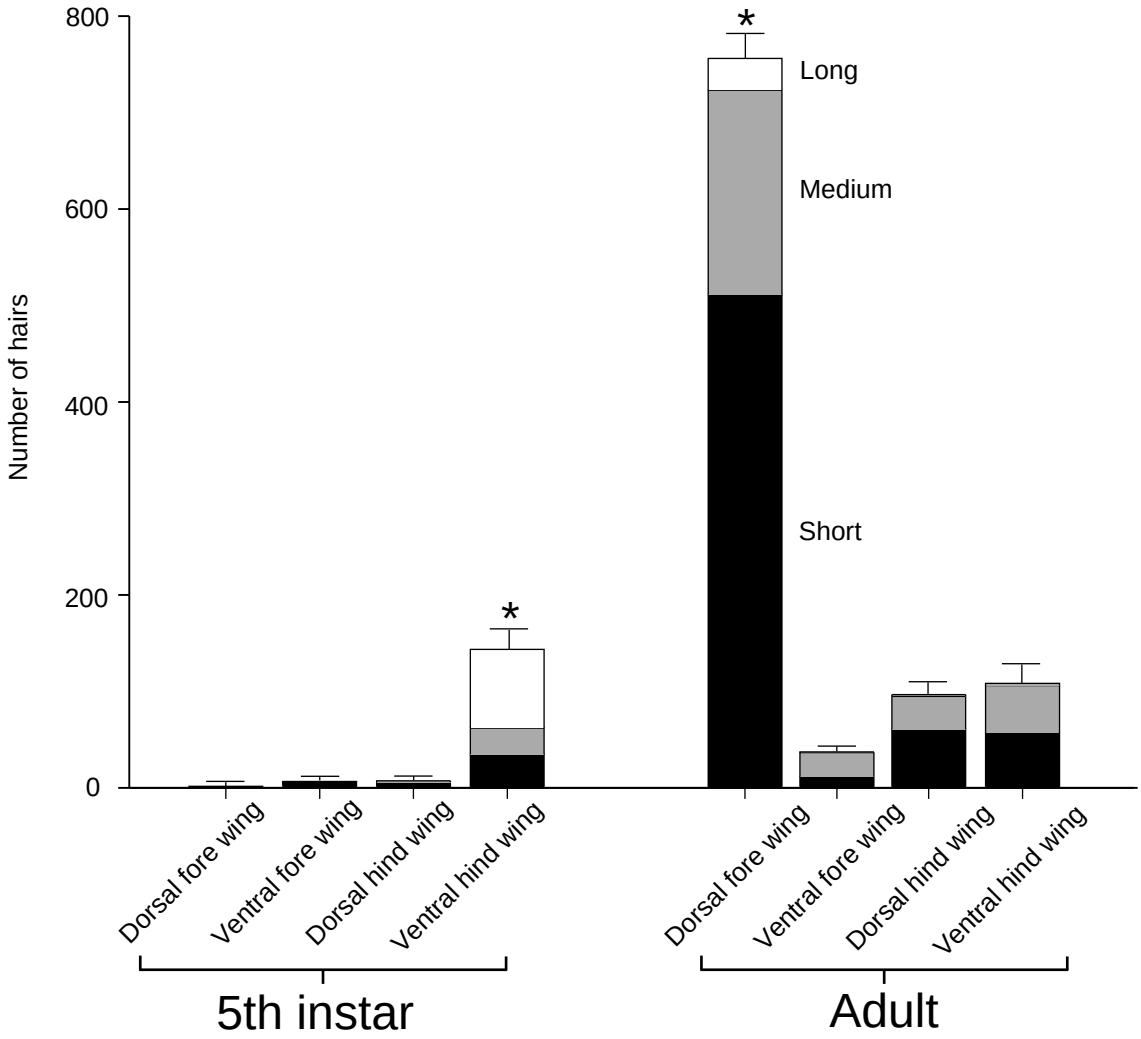


Figure 14. Mechanosensory hairs on the dorsal and ventral surfaces of the fore wing and hind wing, in 5th instar and adult S. gregaria. The mean number of short (black), medium (grey) and long (white) hairs are stacked to demonstrate the total number of hairs on each wing surface, and shown with SD of the total. The ventral hind wing in 5 th instars (asterisk, $P < 0.001$) and the dorsal fore wing in adults (asterisk, $P < 0.001$) had significantly more hairs than other wing surfaces of 5th instars and adult, respectively. Long hairs (dark grey) were most prevalent in 5 th instars, whilst short hairs (black) were most prevalent in adults.

Biv; post-hoc *t*-test, df = 8, t = 3.350, P = 0.010 where a Bonferroni adjusted alpha level = 0.0125) but the change in hair number was not significant for region 1 (df = 8, t = 2.678, P = 0.028), region 2 (df = 3.162, t = -0.930, P = 0.418), and in region 3a (df = 3.150, t = -0.888, P = 0.437).

To compare the density of hairs on the wing surfaces that would normally receive external stimuli (ventral hind wing in 5^{th} instars and the dorsal fore wing in adults), the wing surface was divided into three homologous regions based on vein morphology (see shaded regions in Fig. 15 Ai, Bi). Region 1 included the costal and subcostal veins, region 2 included the radial, medial, and cubital veins, and region 3 included the post-cubital and vannal veins. Fifth instars had a significantly higher density of hairs than adults in all three regions (one-way ANOVA, df = 1, F = 77.850, P < 0.001 for region 1, P < 0.001 for region 2, P = 0.049 for region 3). The hair density in 5^{th} instars overall was approximately double the hair density in adults (Fig. 15 Aii, Bii).

To determine if the organisation of short, medium and long hair types on the exposed wing surface was different between 5^{th} instar and adults, the density of each hair type was compared for regions 1, 2 and 3 (stacked bars in Fig. 15 Aii, Bii). In 5^{th} instars, the density of short hairs was highest in region 1 and decreased significantly toward region 3 (black bars in Fig. 15 Aii; repeated measures ANOVA, df = 2, F = 87.485, $P < 0.001$). The density of long hairs was also highest in region 1 and decreased significantly toward region 3 (white bars in Fig. 15 Aii; repeated measures ANOVA, df = 2, F = 46.053, $P < 0.001$). Medium hairs had a similar distribution across all regions of the 5^{th}

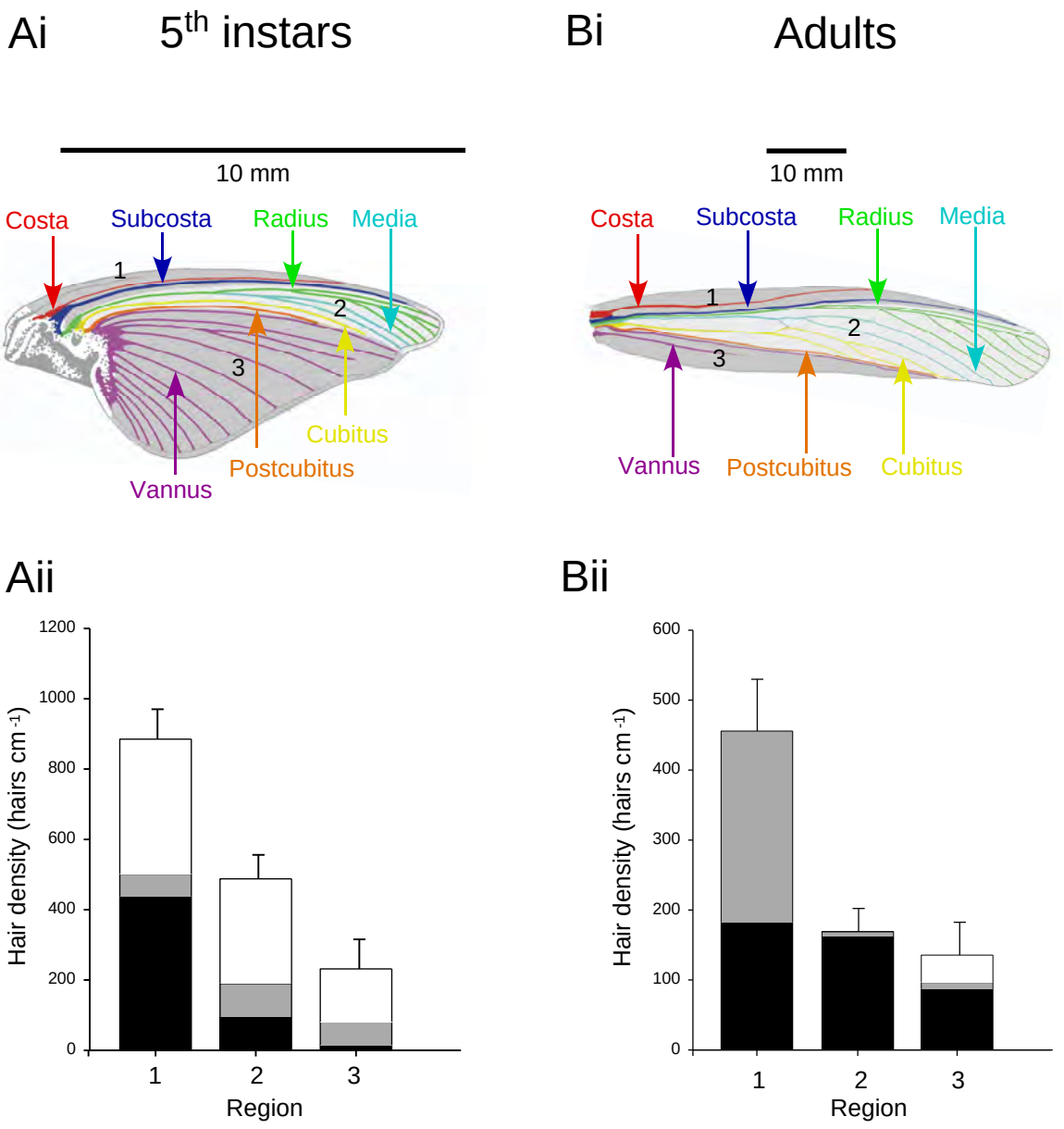


Figure 15. Hair density on the exposed wing surface of 5 th instar and adult S. gregaria. Wing regions (light and dark grey) were based on vein homology (coloured lines) between the ventral hind wing (Ai) and dorsal fore wing (Bi). Aii, Bii) Bars represent the total mean hair density for each region with SD. Column subdivisions show mean density of short hairs (black), medium hairs (grey), and long hairs (white).

instar ventral hindwing (grey bars in Fig. 15 Aii; repeated measures ANOVA, df =2, F = 0.685, $P = 0.526$).

In adults, the density of short hairs was also highest in region 1 and decreased significantly toward region 3 (black bars in Fig. 15 Bii; repeated measures ANOVA, df = 2, F = 24.672, $P < 0.001$). Adults had a significantly higher density of medium hairs in region 1 than in either region 2 or 3 (grey bars in Fig. 15 Bii; repeated measures ANOVA, df = 2, F = 113.399, P <0.001), and had a significantly higher density of long hairs in region 3 than in either region 1 or 2 (dark grey bars in Fig. 15 Bii; repeated measures ANOVA df = 2, F = 292.673, P < 0.001).

Probability of 5th instar and adult wing surfaces eliciting a scratching response

To determine the relative effectiveness of touch applied to different wing surfaces in eliciting a scratching response, the mean response frequency was calculated for each wing surface. In 5th instar *S. gregaria*, stimulating the dorsal forewing, ventral forewing or dorsal hindwing did not reliably elicit scratching (grey bars in Fig. 16). However, touching the normally exposed wing surface in 5th instars, the ventral hindwing, elicited a response significantly more often than for other wing surfaces (repeated measures ANOVA df = 1.562, F = 89.146, P <0.001, see grey asterisk in Fig. 16).

In adults, stimulating any wing surface elicited a response in 45% or more trials (see black bars in Fig. 16). Despite much variability in activity level between animals (large error bars associated with black bars in Fig. 16), wing surface had a significant effect on response rate (repeated measures ANOVA, df = 3, F = 11.671, P < 0.001). *Post-hoc t-*

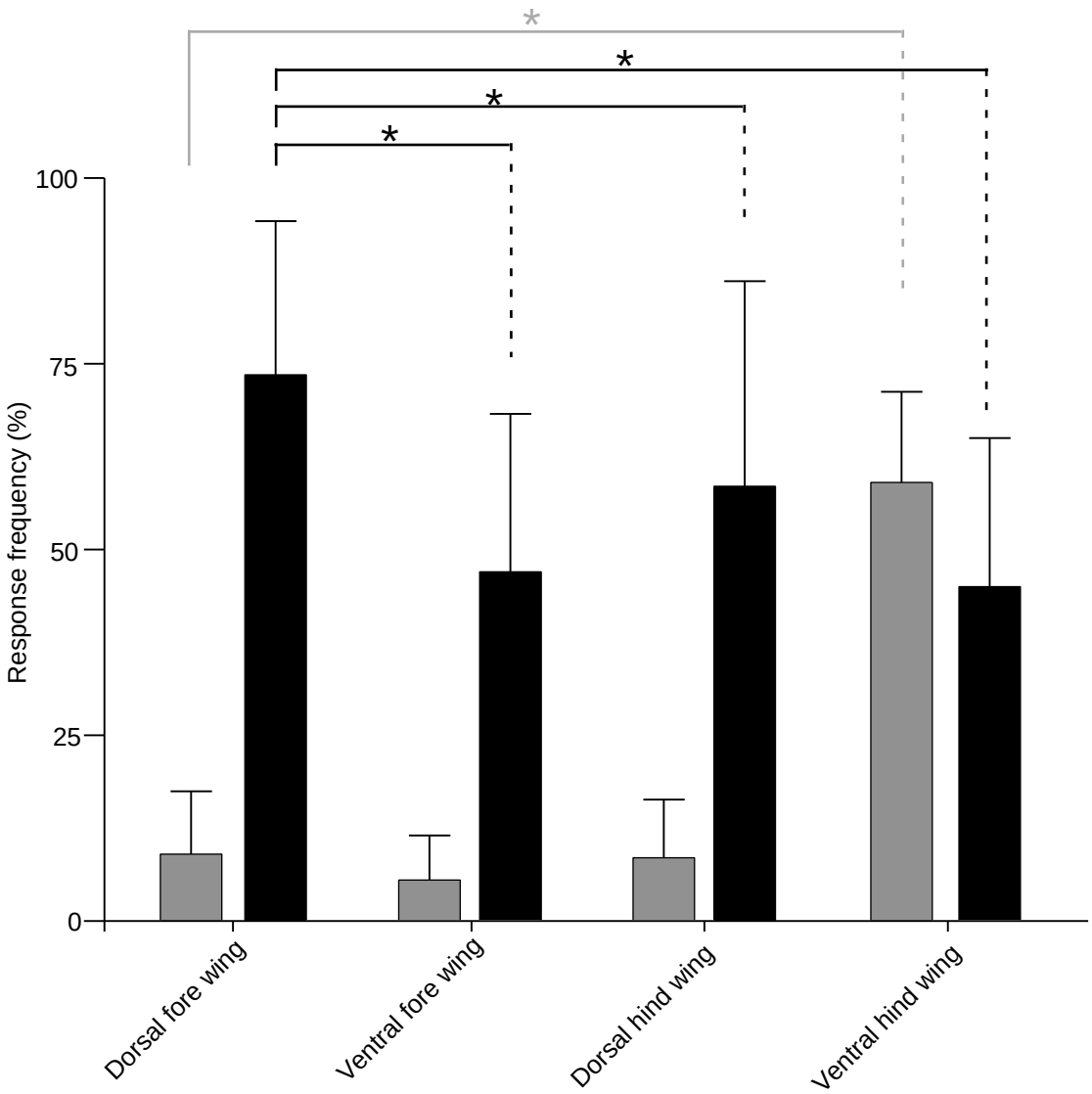


Figure 16. Response frequency to touch of different wing surfaces in 5 th instars (grey) and adult S. gregaria (black; mean and SD). Stimulation of the 5 th instar ventral hind wing elicited significantly more responses than the dorsal fore wing, ventral fore wing or dorsal hind wing. Stimulation of the adult dorsal fore wing elicited significantly more responses than the ventral fore wing, ventral hind wing or dorsal hind wing. Stimulation of the adult dorsal hind wing elicited significantly more responses than stimulation of the adult ventral hind wing. A Repeated Measures ANOVA negated interanimal variability of adults (large SD). $P < 0.05$ shown with asterisk.

tests with Bonferroni correction revealed that stimulation of the exposed wing surface in adults, the dorsal forewing, resulted in significantly more scratching movements than for any other wing surface (dorsal forewing compared to: ventral forewing: P=0.009; dorsal hindwing: P = 0.044; and ventral hindwing: P <0.001). The dorsal hindwing was significantly more responsive than the ventral hindwing (P = 0.015).

The relative effectiveness of touch applied to wing surfaces in 5^{th} instar and adult *S. gregaria* was compared to determine the effects of developmental changes in the organisation of sensory hairs. There was a significant difference in the responsiveness of 5^{th} instars and adults (two-way ANOVA, df = 3, F = 20.005, P <0.001). *Post-hoc t*-tests with Bonferroni correction confirmed that adults were more responsive than 5^{th} instars for the dorsal forewing (P < 0.001), ventral forewing (P < 0.001), and dorsal hindwing (P < 0.001), but not for the ventral hindwing (P = 0.078). The unresponsiveness of the dorsal forewing, ventral forewing and dorsal hindwing was associated with the lack of hairs on those wing surfaces in 5^{th} instars. The development of hairs on those wing surfaces in adults was associated with the wing surface becoming responsive to the tactile stimuli. In contrast, the ventral hindwing elicits similar response rates in 5^{th} instars and adults (P = 0.078), despite 5^{th} instars having a higher density of hairs than adults (381 versus 15 hairs cm^{-1}).

There was no significant difference between the response rates in a comparison of the exposed wing surfaces; the ventral hindwing in 5^{th} instars, and the dorsal forewing in adults (independent samples t-test, df = 18, t = -1.909, P = 0.072).

The retargeting of scratching behaviour toward ventral hindwing targets

During scratching of *S. gregaria*, the distal tibia (effector) makes a characteristic movement towards the target on the wing surface. A detailed comparison of 5th instar and adult scratching movements under normal conditions was presented in Chapter 1, demonstrating that movements elicited by stimulation of the ventral hindwing in 5th instars were appropriately aimed and that movements elicited by stimulation of the dorsal forewing in adults were appropriately aimed, despite the larger size of the adult wing. Here, I compared movements toward targets on the ventral hindwing in 5th instars and adults, to determine whether aiming movements to the same wing surface are retargeted. The forewing and hindwing were fixed vertically above the body to allow access to the ventral hindwing, and the consequence of raising the wings was also tested as a control.

Stimulation of either a fore wing or hind wing that was outstretched led to movements that had similar tarsal trajectories and limb kinematics as those elicited by stimulation of a wing folded against the abdomen. In response to stimulation of the wing tip of either the raised fore or hind wing, the tarsus left the start position and instead of moving toward the artificial, raised, position of the wing, moved posteriorly toward the tip of the abdomen, where the wing tip would be when folded. The tarsus typically performed a cyclic movement near to the region of space occupied by the wing tip when the wings were folded, before returning to the substratum.

Accuracy

To determine whether accuracy of scratching changes as a result of raising the wings or developmental modification, the distance between the closest point of the effector trajectory and the stimulus was measured to determine the accuracy for each scratching movement. Subsequently, mean accuracy values were calculated for each animal for movements toward wing base (hatched bars) and wing tip (black bars) stimuli on the 5th instar ventral hindwing, and the adult forewing and hindwing when folded or raised (Fig. 17).

A two-way ANOVA comparing the effect of target location (wing base, wing tip) and wing (5th instar ventral hindwing, folded adult dorsal forewing, raised adult dorsal forewing, raised adult ventral hindwing) on the minimal distance of the effector from the target demonstrated an effect of target location ($F(1, 40) = 8.994$, $P = 0.005$) and wing condition ($F(3, 40) = 6.320$, $P = 0.001$). *Post-hoc* tests revealed only two significant differences in accuracy (asterisks in Fig. 17; Table 2): (i) paired samples *t*-tests found that movements toward wing tip targets were significantly more accurate than those toward wing base targets for the adult ventral hindwing ($df = 5$, $t = 5.398$, $P = 0.003$); (ii) Tukey's comparisons found a significant difference between the 5th instar ventral hindwing and the *raised* adult dorsal forewing for wing tip ($F(3, 20) = 2.698$, $P = 0.049$) but not wing base targets ($F(3, 20) = 3.477$, $P = 0.052$). There was no difference in the accuracy of movements toward wing base or wing tip stimuli on other wing surfaces.

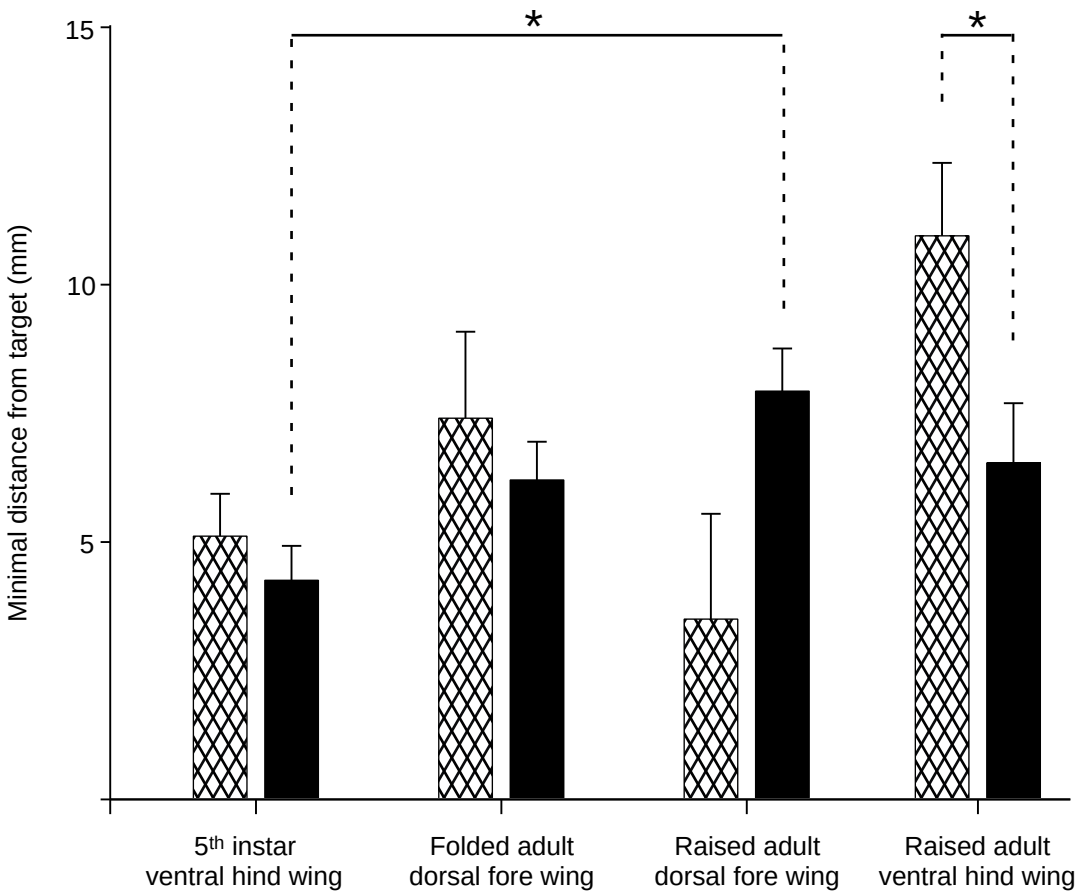


Figure 17. Accuracy of scratches aimed at different wing surfaces on 5 th instar and adult S. gregaria. Accuracy was assessed as the minimal distance between the effector and the target for each movement. Mean minimal distance from wing base (hatched) and wing tip (filled) targets for all animals is shown with SEM. All significant differences are shown (asterisk), details in Table 2.

Table 2. Post-hoc tests for accuracy of scratches aimed at different wing surfaces (Fig. 17)

		Wing base, F (3, 20) = 3.477			
		5th instar ventral hind wing	Folded adult dorsal fore wing	Raised adult dorsal fore wing	Raised adult ventral hind wing
Wing tip, F (3, 20) = 2.698	5th instar ventral hind wing	df = 5 t = 1.052 P = 0.341	P = 0.737	P = 0.052	P = 0.080
	Folded adult dorsal fore wing	P = 0.512	df = 5 t = 0.733 P = 0.497	P = 0.322	P = 0.435
	Raised adult dorsal fore wing	P = 0.049*	P = 0.512	df = 5 t = 1.481 P = 0.199	P = 0.996
	Raised adult ventral hind wing	P = 0.309	P = 0.980	P = 0.740	df = 5 t = 5.398 P = 0.003*

Two post-hoc tests were used: paired samples t-test for comparison of wing tip and wing base accuracy on the same wing surface (shaded), and Tukey's test for comparison of different wing surfaces for wing base or wing tip stimuli (unshaded). Significant differences are shown in bold and *.

Speed

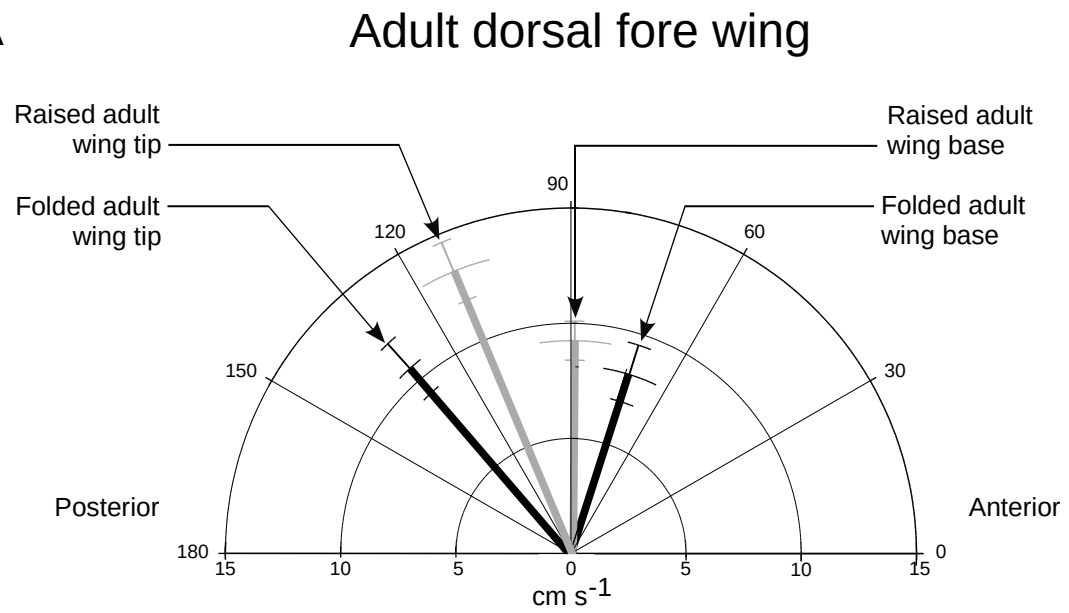
To determine whether movements aimed toward the ventral hindwing in 5th instars had different trajectories from their onset compared to those in adults, I analysed the initial 200 ms of movement that formed the out-going trajectory of the effector. Raising the wings had no significant effect on movement speed (two-way repeated measures ANOVA F (1, 5) = 1.342, P = 0.305). The mean speed of adults was 10.5 mm s^{-1} for movements toward the wing base and wing tip, and was not significantly different whether the wings were raised or folded (grey and black lines in Fig. 18 A).

Raising the wings altered the initial direction of movements elicited by stimulation of targets on the adult forewing (Fig. 18 A). For the wing base target, the initial direction of movement was rotated postero-dorsally when the wing were raised, although the difference was not significant (paired samples *t*-test, df = 5, *t* = -2.977, P = 0.31, where Bonferroni adjusted alpha level = 0.025). For wing tip targets, the initial direction of movement rotated antero-dorsally when the wing was raised (paired samples *t*-test, df = 5, *t* = 3.508, P = 0.017, where Bonferroni adjusted alpha level = 0.025).

Movements toward ventral hindwing targets are different from onset for 5th instars and adults

The mean initial directions of movement elicited by stimulation of wing base targets were not significantly different between 5th instars and adults (Fig. 18 B; independent samples *t*-test, df = 10, *t* = 0.03, P =0.768). In contrast, the initial directions of movements toward targets at the tip of the ventral hindwing were significantly different between 5th instars and adults (Fig. 18 B; independent samples *t*-test, df = 10, *t* = -4.44, P = 0.001). In 5th instars, stimulating the ventral hindwing tip elicited an anteriorly

A



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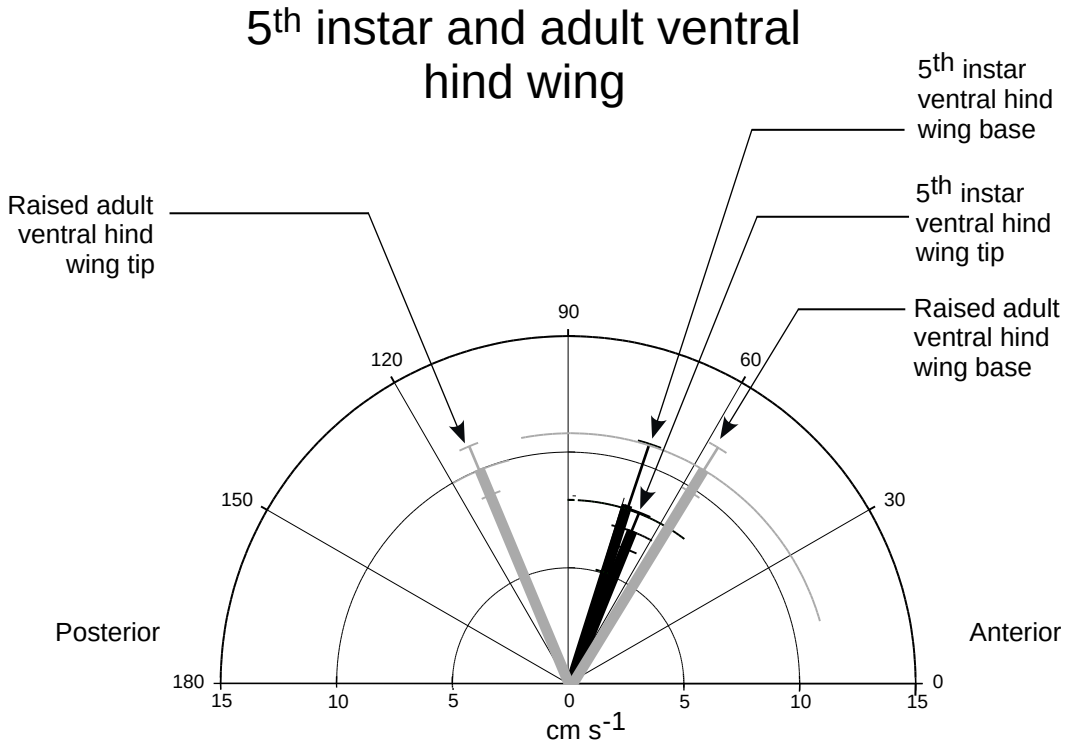

Figure 18. A) Speed and angle of effector's initial movement toward wing base and wing tip targets on the folded (black) and raised (grey) dorsal fore wing of adult S. gregaria. The mean initial movement for the first 200 ms is represented as a vector (thick line), indicating mean speed (line length) and SEM (thin orthogonal lines); and mean angle (angle of thick line) with SEM (thin line perpendicular to vector). B) Speed and angle of initial movement toward wing base and wing tip targets on the ventral hind wing for 5 th instars (wing folded) and adults (wing raised).

directed movement, like that for 5th instar and adult wing base targets. Stimulating the same wing tip location in adults, however, elicited posteriorly directed movements, which resembled closely movements toward wing tip targets on the forewing (compare Fig.18 A, B).

Scratches aimed at ventral hindwing targets differ for 5th instars and adults

The overall pattern of movement of a group of scratches was summarised as a probability distribution representing the position of the effector during movements toward a target. Regions of highest probability are darkest in Figs 19 and 20, and the weighted centre of the distribution is indicated by a circle (mean ± SEM) termed the mean centre of density that represents the centre of the cyclical part of the movement.

To determine the effect of raising the wings on the overall form of a scratch, the probability distributions for movements aimed at targets on the dorsal forewing were compared when the wings were folded or raised (Fig. 19 A, B, respectively). For wing base targets, which lay anterior to the tarsal start position, raising the wings did not alter the shape of the probability distributions (compare Fig. 19 Ai, Bi). Both distributions form arcs that curve from near the start position (black squares), dorsally and anteriorly, then ventrally to near the targets (white triangles). This shape reflects the trajectory of the effector as it moved from the start position toward the target, the cyclical scratching movements near the target, and then the return to the ground. The mean centres of density (white circles) were dorsal and posterior to the target. Similarly, for wing tip targets, which lay posterior to the tarsal start position, raising the wings also did not alter shape of the probability distributions (compare Fig. 19 Aii, Bii), both forming

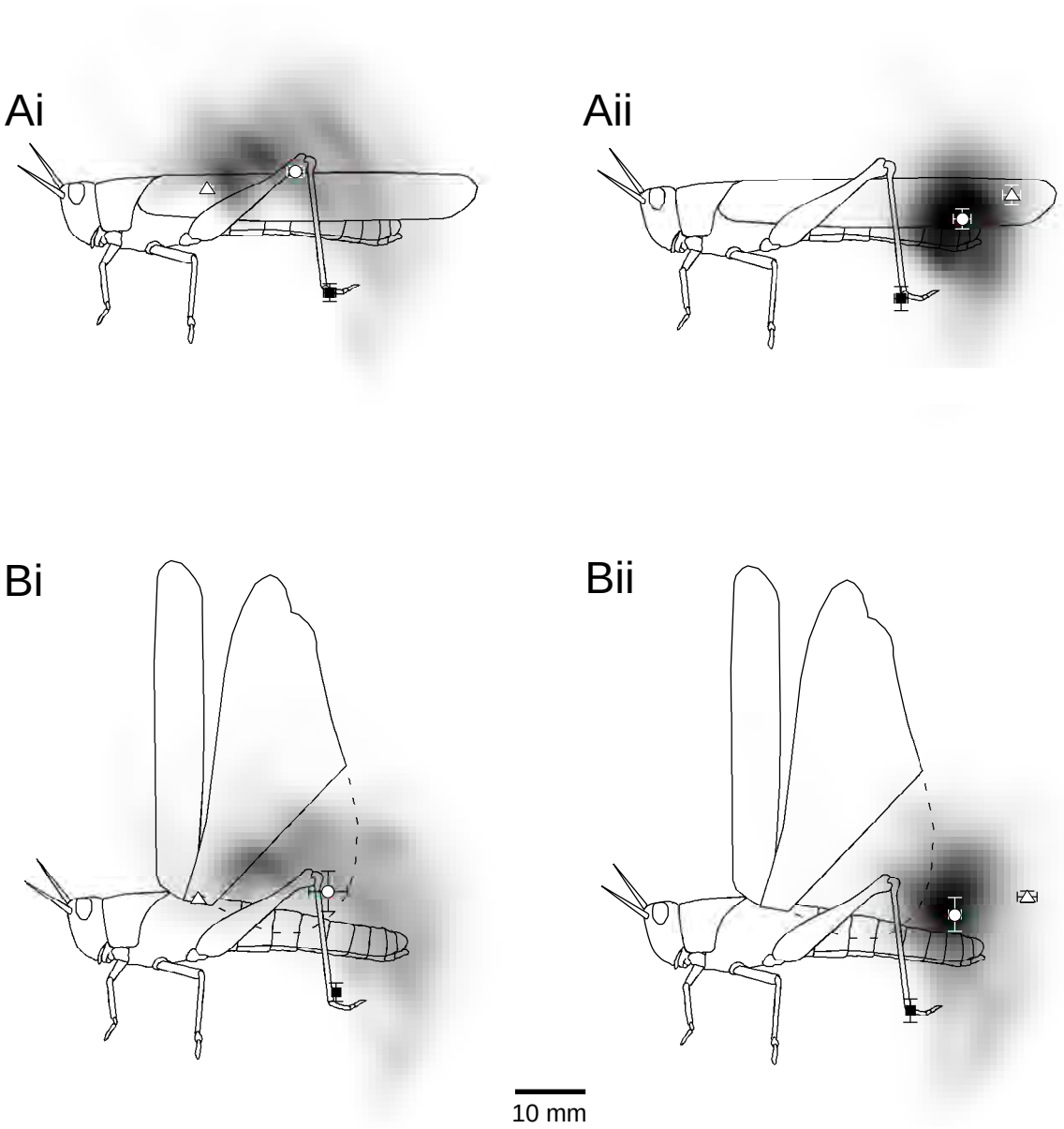

Figure 19. Effector probability distributions of movements toward wing base (i) and wing tip (ii) targets on the folded (A) or raised (B) dorsal fore wing of adult S. gregaria. Mean and SEM for target (white triangle), tarsus start position (black square) and centre of density (white circle) are shown. The grey scale codes the likelihood with which a particular point in the workspace would be groomed by the effector. The centre of density for each probability distribution (white circle, mean $\pm$ SEM) represents the effector's most probable location during a group of movements. The fore and hind wings are depicted raised in B; the posterior region of the hind wing was removed, the anterior border of which is denoted by a solid black line.

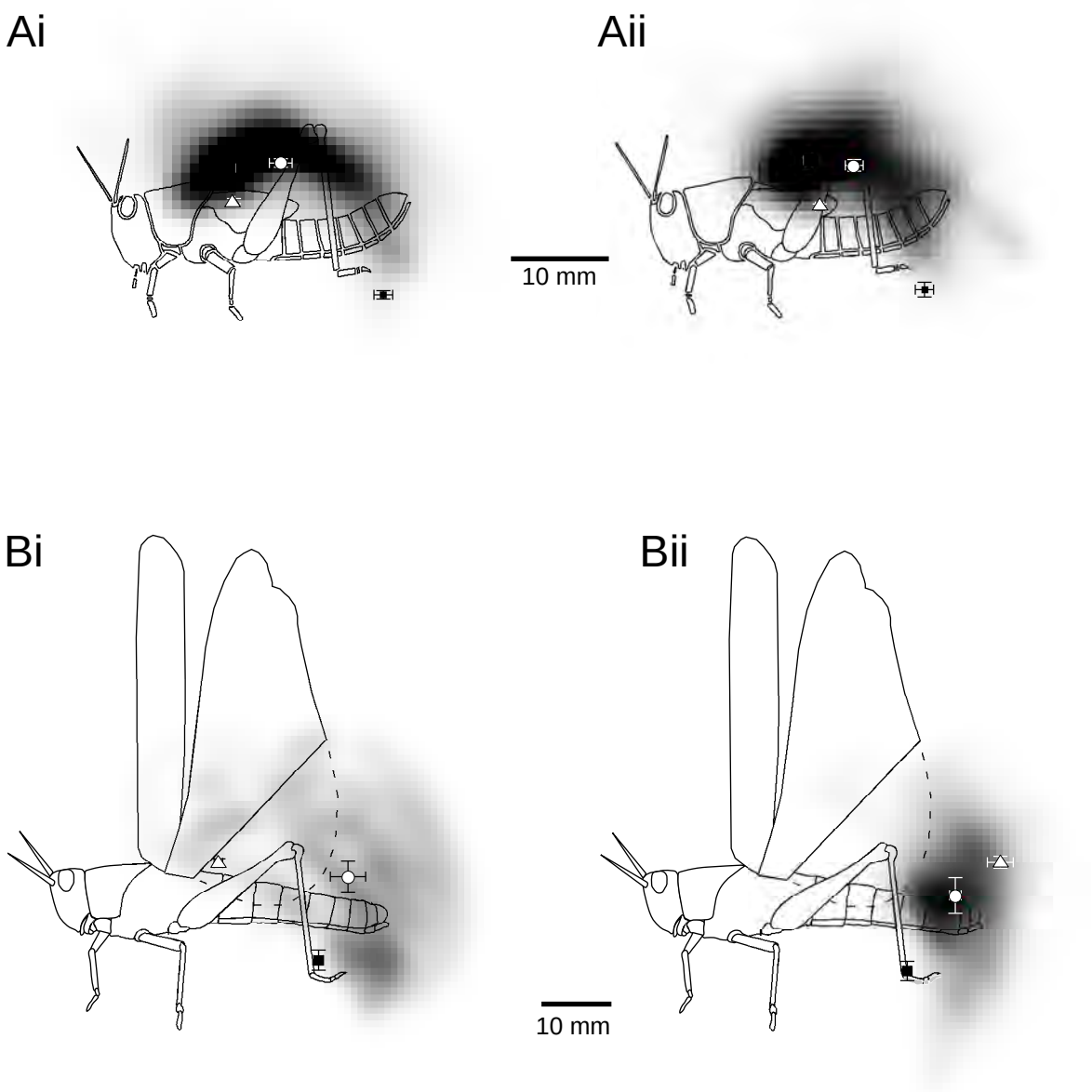

Figure 20. Effector probability distributions of movements toward wing base (i) and wing tip (ii) targets on the ventral hind wing of 5 th instars (A) and the raised ventral hind wing of adult (B) S. gregaria. Mean and SEM for target (white triangle), tarsus start position (black square) and centre of density (white circle) are shown. The grey scale codes the likelihood with which a particular point in the workspace would be groomed by the effector. The centre of density for each probability distribution (white circle, mean +_ SEM) represents the effector's most probable location during a group of movements.

Table 3 A. Overlap and discriminability of probability distributions for targets on the folded and raised dorsal forewing of adult S. gregaria (Fig. 9).

	Folded adult dorsal forewing base	Raised adult dorsal forewing tip
Folded adult dorsal forewing tip		
Overlap, %	36.8	72.0
Probability	0.265	0.445
No. required	11 *	223
Raised adult dorsal forewing base		
Overlap, %	77.7	60.9
Probability	0.456	0.383
No. required	351	51

Table 3 B. Overlap and discriminability of probability distributions for movements toward targets on the ventral hindwing in 5 th instars and when raised in adult S. gregaria (Fig.10).

	5th instar ventral hindwing base	Raised adult ventral hindwing tip
5th instar ventral hindwing tip		
Overlap, %	75.6	
Probability	0.445	
No. required	223	
Raised adult ventral hindwing base		
Overlap, %		65.3
Probability		0.425
No. required		119

compact distributions close to the target sites (white triangles). This shape indicates that the effector moved from the start position (black squares) dorsally and posteriorly toward the target and back to the ground. The mean centres of density (white circles) were ventral and anterior to the target. These control experiments showed that raising the wings had no effect on the trajectories of movements toward anterior or posterior targets in adults.

Two statistical analyses were used to test for differences between the probability distributions. The first determined how many observations (video frames) were required to deduce reliably the correct target site from pairs of probability distributions (Chapter 1 Methods). This number is related to the overlap between the probability distributions for movements toward two targets. Raising the wings did not significantly alter the distributions: probability distributions of movements following wing base stimulation overlapped by 78% and those following wing tip stimulation overlapped by 72% (Table 3 A). As a second measure to test whether the distributions were affected by raising the wings, I statistically compared the centres of density of movements when the wings were folded or raised. A two-way ANOVA comparing the effects of wing surface (5^{th} instar ventral hindwing, folded adult dorsal forewing, raised adult dorsal forewing, and raised adult ventral hindwing) and stimulus location (wing base, wing tip) demonstrated a significant interaction ($F(3, 40) = 3.417$, $P = 0.026$). *Post-hoc t*-tests with Bonferroni correction demonstrated that there was no significant difference in the centres of density between movements following stimulation of the folded adult forewing and the raised adult forewing ($P = 1.000$) or raised adult hindwing ($P = 0.144$). Thus raising the wings had no effect on movements. In contrast, the centres of density for movements toward the 5^{th} instar ventral hindwing differed significantly from those toward the folded adult

forewing ($P < 0.001$), raised adult forewing ($P < 0.001$) and raised adult hindwing ($P < 0.001$).

The probability distributions for targets on the ventral hindwing were compared for 5th instars and adults. Fifth instar probability distributions were denser than probability distributions for adults, reflecting the fact that 5th instar movements inevitably covered a smaller area because the limbs are smaller (Fig. 20). The probability distributions for movements toward wing base targets, which are anterior to the tarsal start position, were similar in shape for 5th instars and adults (Fig. 20 Ai, Bi). Both formed an arc that curved from near the start position (black squares), dorsally and anteriorly, then ventrally to near the target (white triangles). The mean centres of density (white circles) for wing base targets for both 5th instars and adults were dorsal and posterior to the targets, although for adults the mean centre of density was shifted more posteriorly. Following stimulation of the wing tip, probability distributions for movements had markedly different shapes in 5th instars and adults (Fig. 20 Aii, Bii), reflecting the anterior location of the stimulus in 5th instars and the posterior position of the stimulus in adults. The probability distribution for movements toward the hindwing tip in 5th instars was similar to that for hindwing base targets in 5th instars (Fig. 20 Ai, Aii), forming an arc that curved from the start position (black square) dorsally and anteriorly toward the target (white triangle). The probability distributions for movements toward the hindwing tip in adults, however, was different (Fig. 20 Bii), forming a compact distribution near to the target (white triangle). The centres of density (white circles) for wing tip targets on the ventral hindwing of 5th instars and adults changed in a corresponding way. In 5th instars the centre of density for movements toward the hindwing tip was in a similar, dorsal and posterior position to that for movements

toward the hindwing base in 5th instars (Fig. 20 Ai, Aii). In adults, the centre of density for movements toward the hindwing base was in a different position, anterior and ventral to the target (compare white circles in Fig. 19 Biv with Bii), respectively).

Discussion

The data presented here represent the first investigation into the development of mechanosensory and contact chemosensory hairs on the wing of a hemimetabolous insect. Comparing 5th instar and adult *S. gregaria* hair distributions for the exposed wing surface identified four findings. First, 5th instars had twice the total hair density of adults on the exposed wing surface. Second, both 5th instars and adults had the highest density of short hairs on the leading edge of the exposed wing surface, with reducing hair density toward the trailing edge. Third, medium hairs were evenly distributed across the exposed wing surface in 5th instars, but in adults medium hairs were found only on the costa and subcosta. Fourth, long hairs were distributed across the entire exposed wing surface in 5th instars, but in adults all the long hairs were located along the post-cubitus.

A low probability of eliciting scratching when touching the dorsal forewing, ventral forewing and dorsal hindwing of 5th instars was associated with a lack of hairs on those wing surfaces. The development of hairs on those wing surfaces in adults was associated with the higher probability that stimulation of that wing surface elicited scratching. In contrast, stimulating the exposed wing surface in 5th instars, the ventral hindwing, had a similar probability of eliciting a scratch as stimulating the same wing surface in adults, despite 5th instars having a far higher density of hairs than adults (381 versus 15 hairs cm^{-1}).

Touching the exposed wing surfaces of 5th instars and adults elicited similar numbers of responses, even though there was almost twice the density of hair on the ventral hindwing of 5th instars as on the adult dorsal forewing. A Repeated Measures ANOVA

suggested that, despite high variability between animals, touching the dorsal forewing of adults elicited scratching responses more frequently than touching other wing surfaces. There was no significant difference in the sensitivity of the ventral hindwing in 5th instars or adults.

Types of sensilla on the locust wing and their functions

Non-innervated hairs

There are many socket-less hairs of varying length, particularly around the base of the hindwing. Based on the lack of articulation and flexibility of the hair shaft these are unlikely to be mechanosensory, and the absence of a pore suggests they are unlikely to be chemosensory. Indeed, Page and Matheson (2004) found that these hairs were electrophysiologically unresponsive to either mechanical or chemical stimulation (Table 4). Zhou *et al*. (2008) described similar socket-less, aporous hairs located at the base of each wing in *Locusta migratoria* but failed to note that they corresponded to Page and Matheson's "non-innervated" hair class, and confusingly termed them short hairs, a term used by Page and Matheson (2004) for the shortest innervated hairs. Furthermore cross-sections of the hair did not reveal an associated dendrite (Zhou *et al.*, 2008). Albert *et al*. (1976) also noted many non-innervated hairs of various sizes, particularly at the base of the wing in the grasshopper, *Melanoplus sanguinipes*. The purpose of non-innervated hairs near to the wing base may be to reduce friction during movements of the wing. These non-innervated hairs were not assessed further in the present study.

Table 4. Lengths of contact chemosensory and mechanosensory hairs on some orthoptera.

Species	Anatomical location	Non-innervated	Length of mechanosensory hair			
			Minute	Short	Medium	Long
S. gregaria	Wing[1]	Length not given		< 50 μm[b] ("basiconic")	73 - 159 μm	316 - 511 μm
	Leg[2, 3, 4]			25 - 45 μm[b] ("gustatory")	265 k 10 μm	450 - 780 μm
	Abdomen[5]	None described		15 - 25 μm[b] ("gustatory")	30 - 300 μm	300 - 600 μm ("guard hairs")
L. migratoria	Wing[6]	30 - 60 μm ("short hairs")		20 - 40 μm[b] ("sensilla chaetica")	80 - 200 μm	300 - 510 μm
M. sanguinipes	Wing[7]	Large and small spines or microtrichia	<15 μm	20 - 35 μm	40 - 100 μm	> 100 μm
G. domesticus	Wing[8]	None described	Length not given ("short hairs")	25 - 60 μm ("medial hairs")	55 μm ("sense bristles")	112 - 115 μm
G. bimaculatus	Wing[9]	None described	10 μm ("type II")	46 μm[a] ("type III")		264 μm ("type I")

[1]Page and Matheson (2004) innervation demonstrated by neuronal recordings. [2]Chapman (1982). [3]Newland and Burrows demonstrated innervation by neuronal recordings.. [4]Newland (1991) demonstrated innervation by neuronal recordings. [5]Tousson and Hustert (2006) demonstrated innervation by Neurobiotin staining. [6]Zhou et al. (2008) innervation demonstrated by transmission electron microscopy. [7]Albert et al. (1976) demonstrated innervation by methylene blue stain. [8]Fudalewicz-Niemczyk and Rosciszewska demonstrated innervation by methylene blue stain. [9]Hiraguchi et al. (2002). [a]Innervation not confirmed. [b]Multiple innervation confirmed. Author's terminology given in brackets if different from headings.

Contact chemosensory sensilla

Short (<50 µm), blunt tipped and uniporous hairs were identified on all wing surfaces. There is some confusion in the literature regarding the naming of these hairs in locusts (Table 4). On the wing, hindleg and tarsi of *S. gregaria* they have been termed *sensilla basiconica* by Page and Matheson (2004), Burrows and Newland (1994), Newland (1998) and Kendall (1970). On the palps of *S. gregaria* they have been termed *terminal sensilla* by Blaney and Chapman (1969) and Blaney *et al.* (1971). On the antenna of *S. gregaria* and the wing of *L. migratoria* they have been termed *sensilla chaetica*, whilst another hair type was termed *sensilla basiconica* (Zhou *et al*., 2008; Ochieng *et al.*, 1998). On the antenna of *L. migratoria* they have been termed *trichoid sensilla*, whilst two other hair types were described as *sensilla basiconica* (Yamamoto-Kihara *et al*., 2004). All of these authors appear to describe the same hair type (Fig. 2), which has the following characteristics; it is blunt tipped with a terminal pore, faint longitudinal ridges, a conical shape and a straight-edged profile, inserts into a socket, and is innervated by a single mechanosensory dendrite and approximately four chemosensory neurones. The single terminal pore indicates that these hairs are contact chemosensory sensilla (Slifer, 1970). In this thesis these hairs are termed contact chemosensory sensilla, reflecting their chemosensory properties, although mechanosensory properties have also been demonstrated by these hairs (Page and Matheson, 2004).

On the antenna of *S. gregaria* another hair type was termed *sensilla basiconica* by Ochieng and co-workers. (1998), but although they are generally similar in appearance, they can be distinguished by their slightly smaller size (10 µm v. 14.5 µm), hundreds of pores in the wall of the hair instead of a single apical pore, the lack of a socket, and a curved-edged, not straight-edged profile. These hairs have between 20-50 sensory

neurones associated with them and the presence of multiple pores indicates that these sensilla are olfactory (Slifer, 1970). Antibody staining against a protein thought to be involved in chemical binding at chemoreceptors strongly labelled the contact chemoreceptors on the wing (Zhou *et al.,* 2008), tarsus, the maxillary palps (Angeli *et al.,* 1999) and the antennae (Ochieng *et al.,* 1998) but not these olfactory sensilla that Ochieng and co-workers (1998) termed *sensilla basiconica*.

In addition to those on the wings, contact chemosensory sensilla have been found on most body parts of the locusts, *S. gregaria* (Newland and Burrows, 1994) and *L. migratoria* (Chapman, 1982), and the grasshopper, *M. sanguinipes* (Slifer, 1955). Determining the function of contact chemoreceptors is challenging, due to the need to balance non-invasive behavioural experiments and the difficulty of preventing confounding airborne odorants stimulating nearby olfactory receptors. Contact chemoreceptors have been implicated as gustatory receptors and detect phago-stimulants or phago-deterrents (Chapman, 2003); and in the reflex withdrawal of the hindleg tarsus (Rogers and Newland, 2000a, b).

Page and Matheson (2004) demonstrated that mechanical stimulation of the mechanosensory hairs on the wing of *S. gregaria* elicits a scratching movement of the ipsilateral hindleg, with the distal end of the tibia aimed at the stimulus site (also see Matheson 1997, 1998). Page and Matheson concluded that the short hairs probably had a weaker influence on the neuronal networks generating scratching that did the long hairs, based on stimulation experiments of different regions of the wing. However they were unable to determine whether mechanical stimulation of the contact chemosensory sensilla alone could elicit scratching movements, because their small size and

distribution amongst medium or long mechanosensory hairs precluded independent activation. When applied to the hindleg tarsus, chemical stimulation using acetic acid vapour reliably elicited hindleg withdrawal responses in 74% of trials, but rarely elicited a response when applied to the wing (10% of trials), implying that stimulation of the chemosensory neurones of the wing sensilla does not elicit scratching on its own.

Mechanosensory sensilla

The last two hair types are medium and long mechanosensory hairs (see Fig. 2 A, B). These hairs are mechanosensory, based on the non-porous hair shaft, the articulated socket and the lack of response to chemical stimulation (Page and Matheson, 2004). Two lengths of mechanosensory hair were identified in the present study that corresponded to those already described on the forewing of *S. gregaria*; medium length hairs (73-159µm), and long hairs (316-511µm) (Page and Matheson, 2004). Several papers on insect wing sensilla describe multiple distinct groups of lengths of mechanosensory hairs (Table 2), implying a role for stimulus filtering mediated by hair length. The medium and long mechanosensory hairs described here correspond well with hair lengths in *L. migratoria* (Zhou *et al*., 2008), but *M. sanguinipes, G. bimaculatus* and *G. domesticus* all had smaller hair lengths and an additional minute hair length (Albert *et al*., 1976; Fudelewicz-Niemczyk and Rosciszewska, 1972; Hiraguchi *et al.*, 2003). No minute hairs were identified on the wings of locusts in this study or in others (Table 2; Page and Matheson, 2004; Zhou *et al.*, 2008), although an additional smaller mechanosensory hair (10µm) has been described in *S. gregaria* both on the tarsus and the antennae (Kendall *et al.*, (1970); Yakamoto-Kihara (2004) respectively). Minute hairs in *G. bimaculatus* had a distinct twisted shaft (Hiraguchi *et al.*, 2008), which should have easily distinguished them from the short hair type. The

minute hairs described by Kendall and co-workers (1970) and Yakamoto-Kihara (2004) did not have twisted shafts. Mechanosensory hairs on the abdomen of *S. gregaria* fall into three length groups of similar magnitude (Tousson and Hustert, 2006). The sternite, lateral tergite and dorsal tergite of the abdomen possess "regular tactile hairs" (30 to >300µm), and contact chemosensory hairs (15-25 µm), whilst "guard hairs" (300-600 µm) are restricted to the sternite. The hindleg of *S. gregaria* has a single long hair (450-780 µm) at the base of each spine on the dorsal tibia, whilst smaller hairs on the ventral tibia have a mean length of 265 ± 10 µm (Mean ± SEM; Newland, 1991b) and "gustatory" or contact chemosensory sensilla are dispersed across the entire surface (25 - 45 µm; Chapman, 1982, Newland and Burrows, 1994). Therefore *S. gregaria* appear to have three lengths of sensilla on the body and appendages that detect mechanical stimuli.

The length of a hair is likely to be important in detecting or distinguishing different stimuli. In the cricket cercal system, hairs of different lengths detect differences in air current speed or acceleration (Landolfa and Miller, 1995). However, cercal filiform sensilla produce a steady firing frequency without hair displacement (Landolfa and Miller, 1995), whereas mechanosensory hairs on the wing, abdomen and leg are silent in the absence of hair shaft displacement (Tousson and Hustert, 2006; Page and Matheson, 2004; Newland, 1991b). Furthermore, cercal filiform hairs detect the position of a stimulus through the direction of hair shaft displacement by an air current, and as such the specific position of a hair within the receptive field is relatively unimportant. In contrast, displacement of specific mechanosensory sensilla within a receptive field signal the position of the stimulus, so encoding the spatial representation of each hair is important.

Direct mechanical stimulation of the long hairs on the locust post-cubitus provides the strongest drive to elicit scratching behaviour. Page and Matheson (2004) concluded that long hairs have the strongest effect on eliciting a scratch, but that short and medium hairs also contribute. The results presented here provide direct evidence for the contribution of medium length mechanosensory hairs and/or contact chemosensory hairs, as stimulation of the tip of the ventral hindwing in adults, where only those hair types are present, is able to elicit scratching. Thus whilst a precedent has been set for hair length to filter stimuli in the cercal system, it appears that long and medium mechanosensory hairs and contact chemosensory hairs may all contribute to the likelihood of eliciting the same scratching behaviour.

Stimulating the medium length hairs on the hindleg of *S. gregaria* elicits an avoidance reflex, in which the hindleg is lifted by flexion or extension of several joints. Touching several hairs on the tibia is not a very powerful stimulus for avoidance movements. However, touching the cuticle (stimulating campaniform receptors or short hairs) was a better stimulus to induce the avoidance response (Pflüger, 1980). Thus, increasingly shorter hair lengths may be progressively stimulated as an object approaches the cuticle, and act to filter the response as urgent or non urgent (Burrows, 1996).

Reorganisation of sensilla during the imaginal moult

This study has shown that the distribution pattern of wing sensilla in locusts changes dramatically during the imaginal moult. Although published descriptions of wing hairs in adult locusts differ in some key ways, several generalisations can be made. First, contact chemosensory sensilla are distributed across all wing (and body) surfaces (Page and Matheson, 2004; Zhou *et al*., 2008; Albert *et al*., 1976). Second, medium length

mechanosensory hairs are distributed across all wing surfaces, but have a higher density on the leading edge of both the fore and hindwing (Page and Matheson, 2004; Zhou *et al.*, 2008; Albert *et al*., 1976, leading edge wrongly labelled). Third, long mechanosensory hairs are located on the post-cubitus in *S. gregaria*, and *M. sanguinipes*, but not in *L. migratoria* (Page and Matheson, 2004; Albert *et al.*, 1976; compared to Zhou *et al*., 2008). *L. migratoria* has a different pattern of wing venation, where the post-cubitus is markedly more ventral, and is not prominent when the wings are folded. The long hairs on the displaced post-cubitus in *L. migratoria* may have been lost during evolution, when they failed to protrude and so the functional advantage in detecting predators was lost. It would be interesting to know if the long hairs on the hindwing of *L. migratoria* (Zhou *et al*., 2008) are exposed when the wings are folded.

In contrast to the wing hair distributions in adults, 5^{th} instars have contact chemosensory, and medium and long mechanosensory sensilla dispersed across the entire exposed wing surface. To my knowledge there have been no other investigations describing the distribution of wing hairs in immature insects.

The change in distribution of hairs suggests that medium length hairs may have a role in flight, whilst long hairs might only be functional when the wings are folded (e.g. in detecting predators). First, medium hairs increase in density when flight develops in adulthood, whereas long hairs are most abundant in the flightless 5th instar. Second, on the forewing of adults, medium hairs are almost exclusively positioned on the leading edge, a prominent position during flight but not when the wings are folded, whilst long hairs are most prominent when the wings are folded. Third, medium hairs are present on

all wing surfaces in adults suggesting they are functional when the wings are open, whilst long hairs are only located on the dorsal forewing that is exposed at rest.

Mechanisms of developmental plasticity

My data suggest that there is a profound reorganisation of locust wing hairs during the imaginal moult. Fifth instars have fewer than 200 hairs, mostly on the ventral hindwing, whereas adults have approximately 1000 hairs spread across all wing surfaces (Fig. 3), demonstrating the addition of 800 innervated hairs during the imaginal moult. However, the ventral hindwing of adults had significantly fewer hairs than 5^{th} instars in region 3b, indicating that at least some of the existing sensilla are not retained after the moult (Fig. 3). There was also a reduction in the number of hairs observed in region 1, that was approaching significance ($P = 0.028$, where a Bonferroni adjusted alpha level = 0.0125). There was no significant change in hair number for regions 2 and 3a. This corresponds to the development of the wing nerves in *L. migratoria*, counted from SEM cross-sections (Altman *et al.*, (1978)). In early instars the number of neurones innervating the wing surface increases with each moult, reaching a peak of 1,950 axons from the hindwing in fledgling adults, and then decreasing to 1,000 in mature adults. Two possible mechanisms were proposed by Altman and co-workers; first, that initial overproduction of neurones in young adults and subsequent competition-dependent cell death leaves only functional connections in mature adults. Second, that there are two sets of sensory neurones, one innervating the 5th instar hairs, the other innervating the adult hairs and these temporarily co-exist in the fledgling adult before the 5th instar set degenerate. The results presented here support the concept of separate sets of 5th instar and adult neurones for the following reasons. First, almost all the hairs on the dorsal fore wing, ventral fore wing and dorsal hind wing develop solely for adulthood. Second,

almost all the existing long hairs on the ventral hind wing in 5th instars are lost by adulthood. Third, the organisation of hair type changes markedly between 5th instars and adults. Degeneration of larval sensory neurones and the emergence of new adult neurones are characteristic of metamorphosis in holometabolous insects (Levine and Weeks, 1990; Truman, 1983; 1990; Truman *et al.*, 1993). Therefore it seems that hemimetabolous wing development may live up to its meaning of a "partial metamorphosis" in that the wings alone out of the locust body undergo complete structural and neuronal metamorphosis. Indeed, Holdsworth (1942) commented on wing development of the hemimetabolous insect, *Pteronarcys proteus*:

> "During all nymphal stages, the histological appearance of the wing tissue does not differ from the many descriptions of wing epithelia in the literature. But during metamorphosis the tissue changes considerably and looks very much like the epithelia during pupation of Leptinotarsa (Tower, 1902) and Ephestria (Behrends, 1936)."

It is possible that to accommodate the changes in size and structure of the wings and to modify associated behaviours, requires metamorphosis of the wing epidermis within the wing pads of 5th instar *S. gregaria*. It is possible that the reorganisation of existing sensilla is a challenge that can only be overcome by developing a completely new set of adult sensilla.

In the leg or cerci, where there is modest increase in size and little change in shape at each moult, the new cuticle developing under the old cuticle is very closely apposed (Gnatzy, 1978) so it is easy to envisage a new hair forming under the old one. For the

wings, and particularly the hindwing, the new structure is highly folded within the wing bud. How do locations on the old 5th instar cuticle map onto the convoluted new adult wing surface? The hypothesis that the wings, but not the cerci or legs, undergo a mini-metamorphosis, with complete transformation of the sensory apparatus and cuticular structure, would not require a mechanically implausible apposition of old and new surfaces.

Insect hairs and their development have been studied extensively and are well summarised by Keil (1997a, b). However previous work has not addressed the development of the wing and its sensilla, instead focusing primarily on the development of hairs on the cricket cerci (Gnatzy and Romer, 1980; Gnatzy, 1978). In the cricket cercal system, existing hairs are maintained and modified in terms of length with each successive moult. Prior to moulting, the epidermis separates from the cuticle around the sensilla, and the trichogen and tormogen cells begin forming a new hair and socket respectively. The sensory neurones do not degenerate; instead the dendrite remains attached to the old hair shaft, elongating to cross the cavity between the old cuticle and the forming cuticle. During ecdysis the dendrite breaks from the old hair and attaches to the base of the new hair. The putative metamorphic development of the wings suggests that, in contrast, new sensory apparatus would form from undifferentiated tissue within the epidermis, based on the descriptions of development in holometabolous insects (e.g. Levine *et al.*, 1995). The differentiation of new sensory neurones from the wing allows for different synaptic connections with the motor network controlling the hindleg and thus may mediate the apparent retargeting of scratching movements toward the hindwing from 5th instar to adult *S. gregaria*.

Control of an aimed behaviour

Scratching behaviour, an aimed limb movement, is reliably elicited by mechanical stimulation of mechanosensory hairs on the wings. For movements to remain correctly targeted, some modification of the underlying neuronal pathways must occur to account for the increase in size of the locust wing (from less than 1 cm to over 6 cm). I show that this modification occurs in the wing hairs and suggest that there may be corresponding changes in the projections and synaptic connections of the associated sensory neurones. This modification could be mediated by the development of a novel adult wing sensory apparatus, and the removal of the old 5^{th} instar sensory apparatus. Thus, the subsequent network of interneurones and motor neurones comprising the hindleg motor network can remain unchanged, maintaining a single, modifiable, movement pattern during development (Dürr and Matheson, 2003).

The wing sensory apparatus of 5^{th} instars and adults differs in two important ways, implying that there should be a difference in response. First, the density of wing sensilla on the exposed wing surface of 5^{th} instars is approximately twice that in adults, implying both a greater resolution for targeting, and that a smaller stimulus could elicit a response. Second, the exposed wing surface in 5^{th} instars is covered with long hairs, whilst in adults long hairs have a limited distribution along the post-cubitus. Long hairs provided the strongest drive for scratching responses in adults (Page and Matheson, 2004) suggesting that 5^{th} instars should be more responsive than adults. Despite these differences, movements toward the exposed wing surface in 5^{th} instars and adults were very similar. There was no significant difference in the accuracy of movements aimed at the 5^{th} instar ventral hindwing or the adult dorsal forewing. There was no significant difference in response frequency elicited by touch of the 5^{th} instar ventral hindwing or

the adult dorsal forewing. One possible explanation for this might be that the synaptic gain of sensilla onto interneurones is lower in 5th instars than in adults. The initial direction and speed of movement, and the shape of trajectories themselves were similar for movements toward the 5th instar ventral hindwing and the adult dorsal forewing for posterior or anterior targets (Chapter 1), indicating that movement patterns were similar even though different types of hair detected the stimulus at the two stages.

In summary, this is the first report of the dramatic reorganisation of wing hair distributions during the imaginal moult in the locust, *S. gregaria*. Fifth instar locusts had higher densities of wing hairs, and the entire exposed wing surface was covered with contact chemosensory sensilla, medium and long mechanosensory sensilla. In contrast, adults had contact chemosensory sensilla dispersed across the entire exposed wing surface (dorsal forewing), but medium length mechanosensory sensilla concentrated on the leading edge, and long mechanosensory sensilla located only on the post-cubitus. The change in hair distribution indicated that most adult hairs develop *de novo*, and that at least some of the 5th instar wing hairs are not present in the adult. Fifth instar and adult scratching movements toward anterior or posterior targets were similar in accuracy and movement trajectory, suggesting little modification of the interneurones and motor neurones that control the movement. The change in targeting and apparent change in synaptic gain in combination with the increase in wing axon number in fledgling adults and the subsequent halving of axon number in mature adults (Altman *et al*., 1978), suggests that an entirely new set of adult wing sensilla develops to replaces the 5th instar sensory apparatus. I therefore suggest that the development of the wings is a mini-metamorphosis, a developmental mechanism to generate novel sensory apparatus associated with a transformation of wing size and structure.

CHAPTER 3

Sensory projections from hairs on the hind wing of the locust *Schistocerca gregaria*

Introduction

How sensory neurones encode the spatial position of a hair on the wing that receives a stimulus is key to understanding how aimed scratching movements are targeted toward the wing. Somatotopic maps have been described for a variety of vertebrates and invertebrates, including for legs of locusts. Is there a somatotopic map for hair position on the wings? How is this sensory information from the overlapping fore wing and hind wing wings integrated to form a comprehensive body map? This Chapter seeks to address these questions by describing for the first time the neuronal projections of long mechanosensory hairs on the wing. I shall begin by explain the importance representing the spatial position of a stimulus for scratching in co-ordinating a movement. I will then introduce topographic representation as a strategy for organising information from receptors in different systems. I shall then discuss the importance of integrating information from the forewing and hindwing to form a single spatial representation, and how this may be useful in the development of locusts.

The spatial position of a stimulus is vital for controlling a movement

Touching the wing surface of S. gregaria elicits scratching movements where the effector is aimed toward the site of stimulation (Matheson, 1997; 1998; Berkowitz and

Laurent 1996a b). Scratching movements are not reflexive, where a stimulus to a location within a receptive field would elicit a single stereotyped movement. Instead, movements are specific for each stimulus location on the wing, using a graded shift of a single movement to pattern to reach both anterior, posterior targets and intermittent (Dürr and Matheson, 2003). The scratches elicited aim the effector toward the stimulus (Matheson 1997, Dürr and Matheson 2003), implying that the spatial location of the stimulus must be encoded by sensory neurones of receptors that are activated. Objects making contact with the wing stimulate long and medium mechanosensory hairs, contact chemosensory hairs and if they distort the wing surface may also stimulate campaniform (Page and Matheson 2004). Thus for scratching to be aimed toward a stimulus, the position of the hair that is displaced must be encoded within the nervous system. Somatotopic organisation of hair afferent projections within the nervous system may underlie spatial encoding.

Topographic representations

In both vertebrates and invertebrates, the arrangement of sensory projections in the nervous system reflects qualities they are encoding, so called topographic representations. Somatotopic representations, where the external position of a receptor, such as a tactile hair, is represented by the area of branching of its sensory neurones within the nervous system have been described in vertebrates and invertebrates. In humans the cuticular receptors project to the cortex to form a map of the sensory homunculus from their arborisations. In rats, the whisker barrel is the subject of much research, as one of the most identifiable somatotopic maps. The external arrangement of whiskers is duplicated by morphologically distinct “barrels” structures, each associated with a single whisker (Woolsey and Van der Loos, 1970). In locusts too, the three

dimensional external position of tactile hairs on the hind leg are represented by a 3 dimensional map of their sensory afferent projections within the metathoracic ganglion (Newland, 1991a). Topographic representations occur for other sensory modalities, too. The position of photoreceptors in the retina are conserved by retinotopic maps of neurones in both the lateral geniculate nucleus and primary visual cortex (Hinds *et al.*, 2009). Sensory hair cells in the organ of Corti are arranged in order, from those detecting high frequency vibrations to those detecting low frequency vibrations, and this order is conserved in the brainstem, thalamus and auditory cortex (Talavage *et al.*, 2000, Kandler *et al.*, 2009). Thus, topographic representations of receptor position is well described, and may provide a mechanism by which spatial position is encoded by sensory neurones.

Integration of information from fore and hind wings

Neurones of the metathoracic ganglion are necessary and sufficient to generate the movement pattern for scratching behaviour, as the metathoracic ganglion can produce aimed scratching movements when isolated from the rest of the nervous system (Matheson, 1997). There are three indications that the an integrated somatotopic map from the fore and hind wings must exist. Firstly, sensory neurones from the hind wing enter the metathoracic ganglion directly, however, those from the fore wing enter the anterior mesothoracic ganglion (Altman *et al*., 1978). Both must converge directly or indirectly, via an intersegmental interneurone, on the interneurones and motor neurones that generate the scratching motor pattern. Secondly, the forewings lie on to of the hindwings, occupying the same region of space. Any contact in that region may stimulate receptor on both wing surfaces, which must be rationalised as a stimulus at a single spatial position. Third, wing rotation during development, means that sensory

signals from both the fore and hind wing must activate the same motor network for scratching. Thus the sensory representations of both wing surfaces must be intergrated to generate scratching movements.

This chapter provides the first description of individual hair afferents from the wing of an hemimetabolous insect. The first aim was to determine whether sensory afferents formed a somatotopic representation, that might be key to encoding spatial position in scratching. A second goal was to determine whether sensory signals from the fore and hind wing were integrated to form a single somatotopic map. To these ends, stains of individual neurones from hairs at different positions along the hind wing and nerve 1C1 that innervate the wing are presented for 5^{th} instar 5th instars and adults respectively. They demonstrate that wing hair afferents are somatotopically arranged within the aVAC of both metathoracic and mesothoracic ganglia. Furthermore, that wing hair afferents can project to the local ganglion and intersegmentally to the mesothoracic ganglion.

Methods

Experiments were performed on male and female desert locusts, *Schistocerca gregaria* (Forskål), taken from a crowded colony at the University of Leicester (the descendents of colonies from the University of Cambridge and Blades Biological Ltd.) Animals were restrained in Plasticene dorsal side up for individual neurone stains and ventral side up for nerve 1 stains that required dissection.

Neurobiotin staining

In 5th instars, a single long hair was surrounded with a well of petroleum jelly filled with distilled water. The hair was cut off at the level of the cuticle with a razor blade. After 5 minutes, the distilled water was removed and immediately replaced with a 2.5% solution of Neurobiotin Tracer (Vector laboratories, Burlingame, USA) in distilled water and the well was sealed with a layer of petroleum jelly, and incubated in a humid chamber at 4°C for 5 days.

After incubation and dissection, the metathoracic and mesothoracic ganglia were fixed in 4% paraformaldehyde for 20 min. Ganglia were then washed in 6 changes of phosphate buffered saline (PBS, pH7.2) for 6 min each and dehydrated in an ascending isopropanol series (6 min at 5%, 15%, 30%, 50%, 70%, 80%, 90%, 98%, 100%, 100%) to increase membrane permeability of the ganglia. Samples were subsequently rehydrated by a descending isopropanol series (as above), and washed 6 times with PBS. Ganglia were then transferred to a 1 mg/µl solution of collagenase (Sigma-Aldrich) in PBS for 1 h, and then washed 6 times in PBS for 6 min each. They were then incubated in 10% Normal Goat Serum in PBS for 1 h. Finally they were incubated

with a 1 µl : 2000 µl concentration of Cy3-conjugated streptavidin (Sigma-Aldrich) in PBS at room temperature for 48 h.

After incubation, ganglia were washed 6 times in PBS for 6 min each and dehydrated using an ascending isopropanol gradient (as described above). They were then placed in methyl salicylate (Sigma-Aldrich) and isopropanol (50 : 50 ratio) for 6 min before being transferred to 100% methyl salicylate until cleared. Samples were mounted in methyl salicylate in a cavity slide and covered with a coverslip and sealed with clear nail varnish.

Specimens were viewed on a Nikon D-Eclipse C1 Confocal Microscope using a Helium-Neon (excitation wavelength of 543 nm). Z-stack optical sections were taken 3.25 µm apart. EZ-C1 FreeViewer Gold Version 3.20 (Nikon Corporation) was used to render a single composite image for each ganglion, however this often resulted in the loss of fine branches, so stained afferents were also drawn from Z-stacks with the aid of a transparent acetate sheet fixed to a 19" TFT visual display unit. Nomenclature of the metathoracic and mesothoracic ganglia was based on Pflüger *et al*., (1988).

In total, 53 stains out of 419 attempts to stain wing hairs were obtained in which the ganglion morphology was well preserved, stained neurones had good contrast against background fluorescence, and axons and branch terminals were stained.

In adult locusts of the 84 wing hairs set up as described above none were successful. Altering ganglionic membrane permeability to the Cy3 antibody through a 5 min 100% methyl salicylate incubation after alcohol dehydration and subsequent enzyme (1mg/ml

Hyaluronidase, protease and collegenase, Sigma-aldrich) digestion after rehydration did not yield stains. Furthermore, successful stains from transected whole nerves were obtained, indicating that antibody penetration was sufficient. Concentrations of 2.5% and 5% Neurobiotin, and incubation times from 5 to 10 days also failed to produce any trace of stain within the ganglion, suggesting that uptake and transport of Neurobiotin marker along adult neurones within the wing was problematic. To obtain stains of afferents from adult wing hairs, the tip of nerve $1C_1$ (comprising neurones from the wing and tegula, Altman *et al.*, 1978) was placed in a petroleum jelly well filled with 2.5% Neurobiotin and incubated in a humid chamber at 4 °C for 3 days.

Data are based on 53 successful individual hair stains from 5^{th} instar locusts and 23 whole nerve stains from adult locusts.

Sectioning

Following confocal microscopy, some samples were embedded in paraffin wax and sectioned. Methyl salicylate was aspirated from around the ganglia and incubated 3 times in 100% ethanol for 1 h. They were then washed twice in Histoclear (BDH, UK) for 30 min each, before being washed 3 times in 45 ° C melting point paraffin wax under vacuum for 30 min each. Samples were then embedded in fresh paraffin wax. After cooling overnight, blocks were sectioned transversely on a microtome to give 6 µm sections and mounted on gelatine-coated slides. Samples were left to dry overnight and dewaxed the following day by 3 washes of Histoclear for 2 min each. They were then rehydrated in a descending ethanol gradient (100%, 90%, 70%, 50%, distilled water) and stained in 0.1% toluidine blue (Sigma-Aldrich) for 3 min. Subsequently

slides were rinsed in distilled water, dehydrated in an ascending ethanol series (as above) and mounted in DPX (BDH, UK).

Analysis

Drawings of one well preserved example of mesothoracic and metathoracic ganglia were used as a standard. Drawings of other ganglia were imported into Canvas Version 7 (Deneba Inc.) and scaled so that the maximum length and width were equivalent to the standard. Subsequently, transverse and sagittal midpoints of superimposed ganglia were aligned.

The average areas covered by neuronal branches for sensilla at the base of the wing, the middle of the costa, and the tip of the wing were quantified by calculating the mean most anterior, posterior, medial and lateral extents of dendrites from sensilla at each position relative to the transverse and sagittal midpoint of each ganglion. After converting measurements to a percentage of the distance between the midpoint and the anterior or lateral edge, the mean dendritic extents were presented as an ellipse, formed by connecting each of the 4 points (mean ± SD). Correlation analyses (Spearman's rho, SPSS) compared the distance of the midpoints of the arborisations (centre of ellipses) of sensory neurones from the centre of the ganglion to the positions of sensilla on the wing.

Results

Hind wing sensilla have two characteristic projections

In 5th instar *S. gregaria,* the neurones of individual long hairs were stained on the leading edge of the hind wings. Applying Neurobiotin to the cut base of a single long hair, on the left and right hind wings, typically resulted in the staining of a single neurone on each side of the metathoracic ganglion (41 out of 56 hairs stained; black bars in Fig. 21 A), although sometimes two, and in one case three, neurones were stained from a single hair (15 out of 56 hairs stained; grey bars in Fig. 21 A), demonstrating that some long mechanosensory hairs were multiply innervated. Dual innervation was especially prevalent at the wing base, but also occurred less frequently at the mid-wing and wing tip (Fig. 21 A). Two projection patterns were obtained; an intersegmental projection pattern with branches in both metathoracic and mesothoracic ganglia occurred most commonly (82 out of 90 stained neurones; black bars in Fig. 21 B), and a local projection pattern, restricted to the metathoracic ganglion, which was identified less frequently (8 out of 90 stained neurones; grey bars in Fig. 21 B). The segmental projection pattern occurred most frequently at the base of the wing, but also at the mid-wing and wing tip. Figure 22 illustrates the projections resulting from staining two long mechanosensory hairs, one on the tip of the left hind wing and another on the tip of the right hind wing (Fig. 22 D), resulting in a bilaterally symmetrical pattern (Fig. 22 A, B). A single hair on each side gave rise to two neurones on each side of the ganglia (4 in total), indicating dual innervation of both hairs. The neurones associated with a long mechanosensory hair on the tip of the left and right wings had almost identical projections.

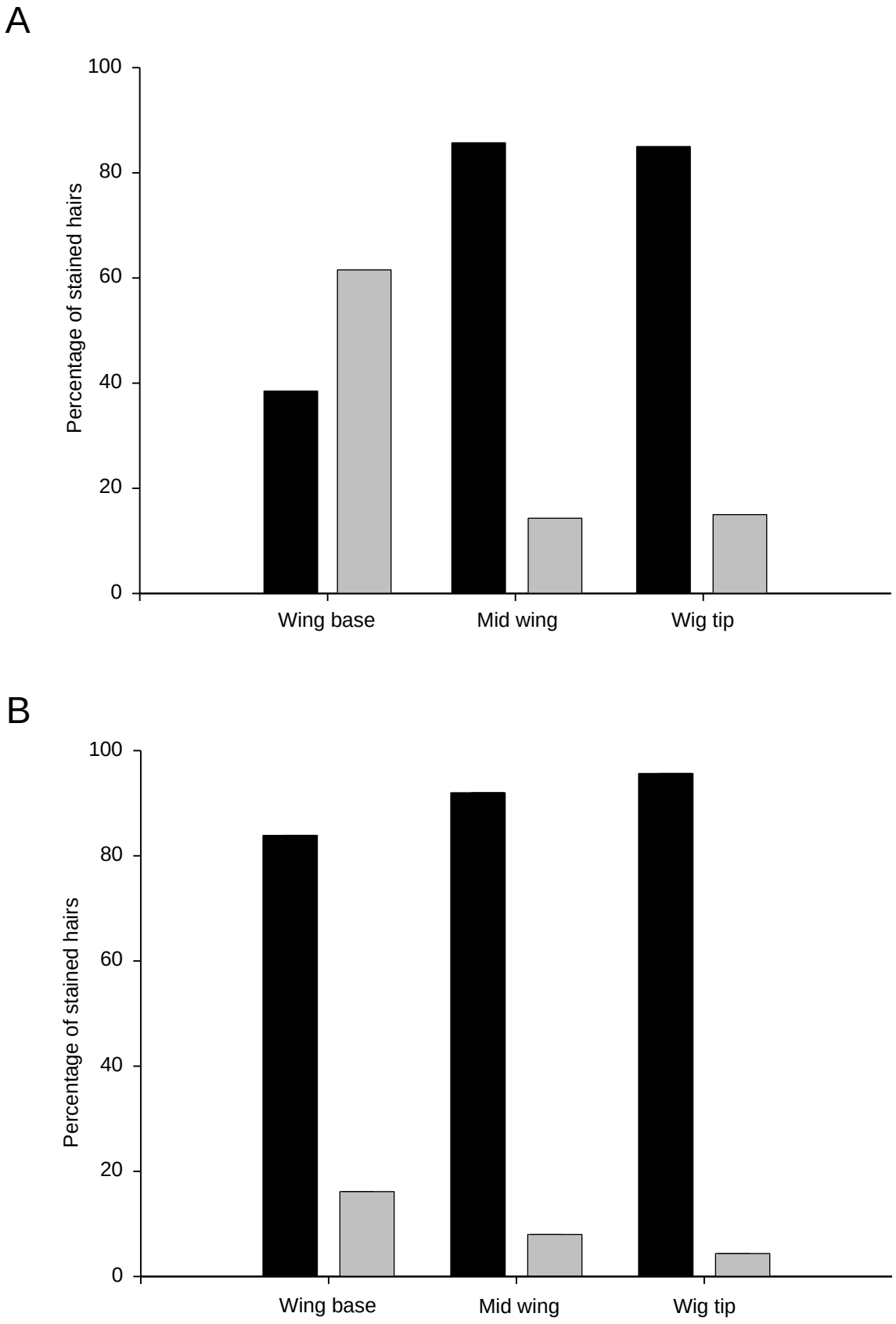

Figure 21. A) Percentage of long mechanosensory hairs that were singularly (black) or dually (grey) innervated. B) Percentage of long mechanosensory hairs at the wing base, mid wing or wing tip that when stained revealed intersegmental (black) or segmental (grey) projections.

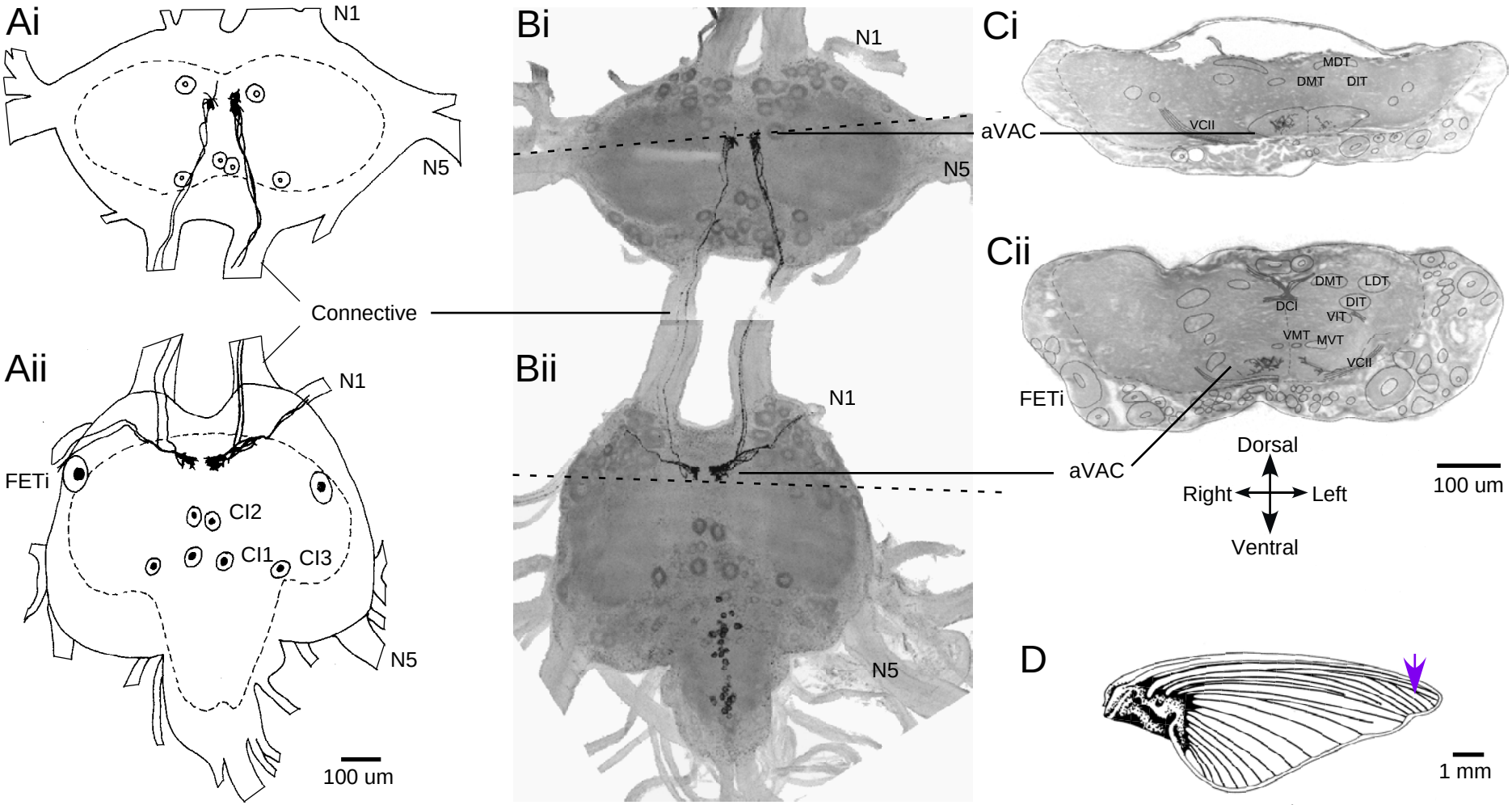

Figure 22. Sensory neurones from one long hair on the tip of the left hindwing and one long hair on the tip of the right hind wing of 5 th instar S. gregaria (A, B) and sectioned transversely (C). Diagram of the mesothoracic ganglion (Ai) and metathoracic ganglion (Aii) shown in Bi, Bii. Nerve 1 (N1); Nerve 5 (N5; Fast extensor tibia (FETi); Common inhibitors 1, 2, 3 (C1, C2, C3, respectively). Bi, Bii) Plane of sections (dashed line) for the mesothoracic ganglion (Ci) and metathoracic ganglion (Cii). Branching occurs in the anterior ventral association centre (aVAC) D) Position of stained hair on the ventral hindwing (blue arrow).

Neurones from long mechanosensory hairs on the hind wing of 5th instar *S. gregaria* entered the metathoracic ganglion through nerve 1 (N1), entering the neuropile through the root 1ii (Fig 22 Aii, Bii). After 133 ± 35 µm the main neurite bifurcated, either within the aVAC (Fig. 22 Aii, Cii) or on the anterior margin of the aVAC (Fig. 3 Bii). One branch gave rise to many projections with a mean length of 78 ± 19 µm, and the second branch continued anteriorly along the median ventral tract (MVT) of the ipsilateral connective into the mesothoracic ganglion. Within the mesothoracic ganglion, the main neurite projected through the ventral median tract (VMT) for 240 ± 60 µm to the aVAC. Transverse sections showed the region of branching to be within the aVAC of the mesothoracic ganglion (Fig. 22 Ci). Once there, minor branches either extended immediately or the main neurite turned posteriorly and branched near the posterior margin of the aVAC. Typically branches were 62 ± 17 µm in length. In only one case did the neurone cross the midline where it continued for 128 µm along the anterior margin of the contralateral metathoracic aVAC (Fig. 23 Cii); however branches within the mesothoracic ganglion remained within the ipsilateral aVAC (Fig. 23 Ci).

The second local projection pattern is shown in Fig. 24. Again, two neurones have been stained from a single hair at the base of the wing. These neurones enter the metathoracic ganglion through nerve 1 (N1), also entering the neuropile root 1ii (Fig. 24 A, B). The main neurite, after 114 ± 35 µm, gave off branches within the aVAC that extended for 100 ± 25 µm toward the midline. The main neurite turned posteriorly and left the dorso-lateral border of the aVAC, and descended almost parallel to the midline along either the MVT or the VMT for 702 ± 197 µm, as far posteriorly as the 3rd abdominal neuromere. The descending axon gave off short (<50 µm) branches that extended near

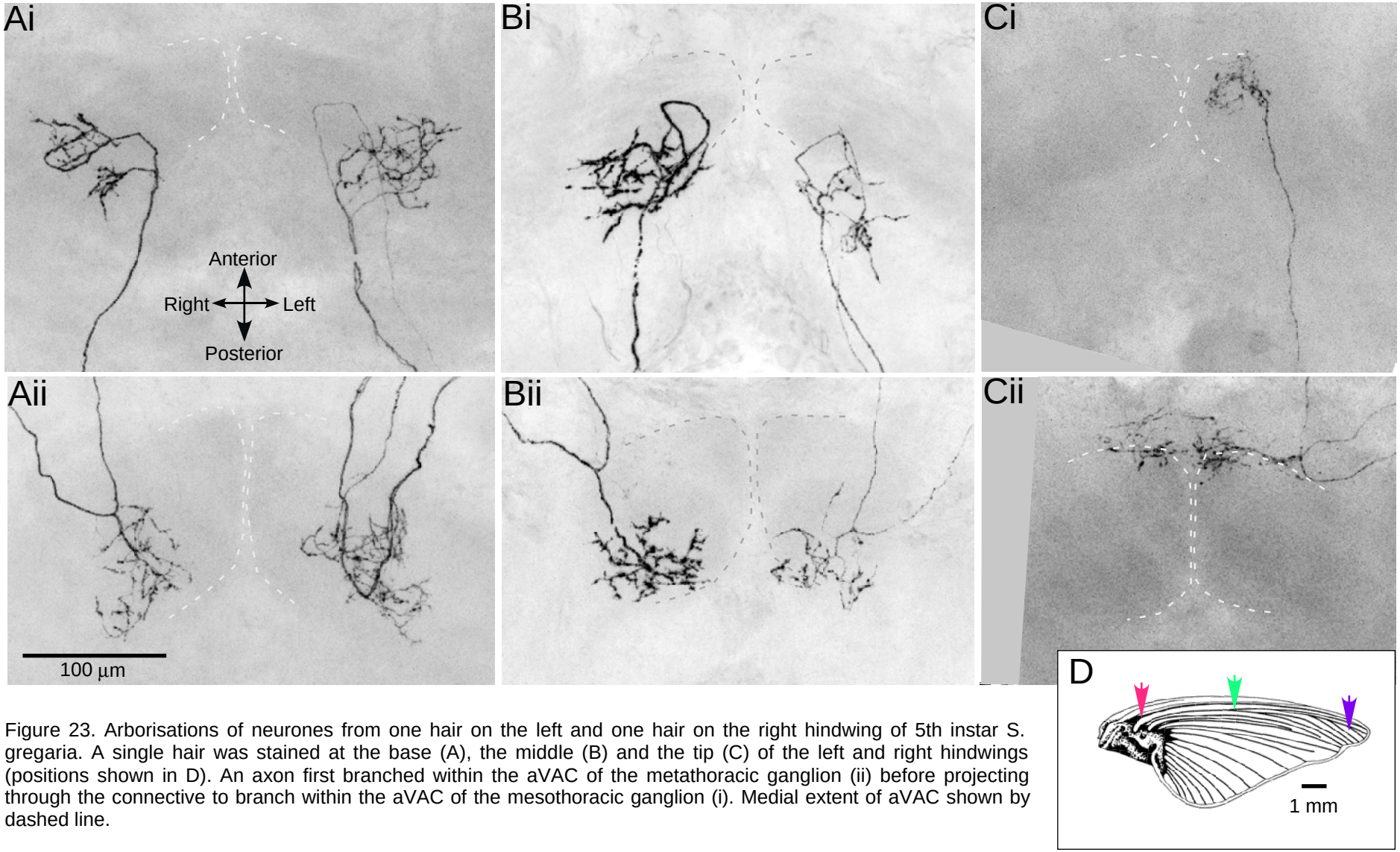

Figure 23. Arborisations of neurones from one hair on the left and one hair on the right hindwing of 5th instar S. gregaria. A single hair was stained at the base (A), the middle (B) and the tip (C) of the left and right hindwings (positions shown in D). An axon first branched within the aVAC of the metathoracic ganglion (ii) before projecting through the connective to branch within the aVAC of the mesothoracic ganglion (i). Medial extent of aVAC shown by dashed line.

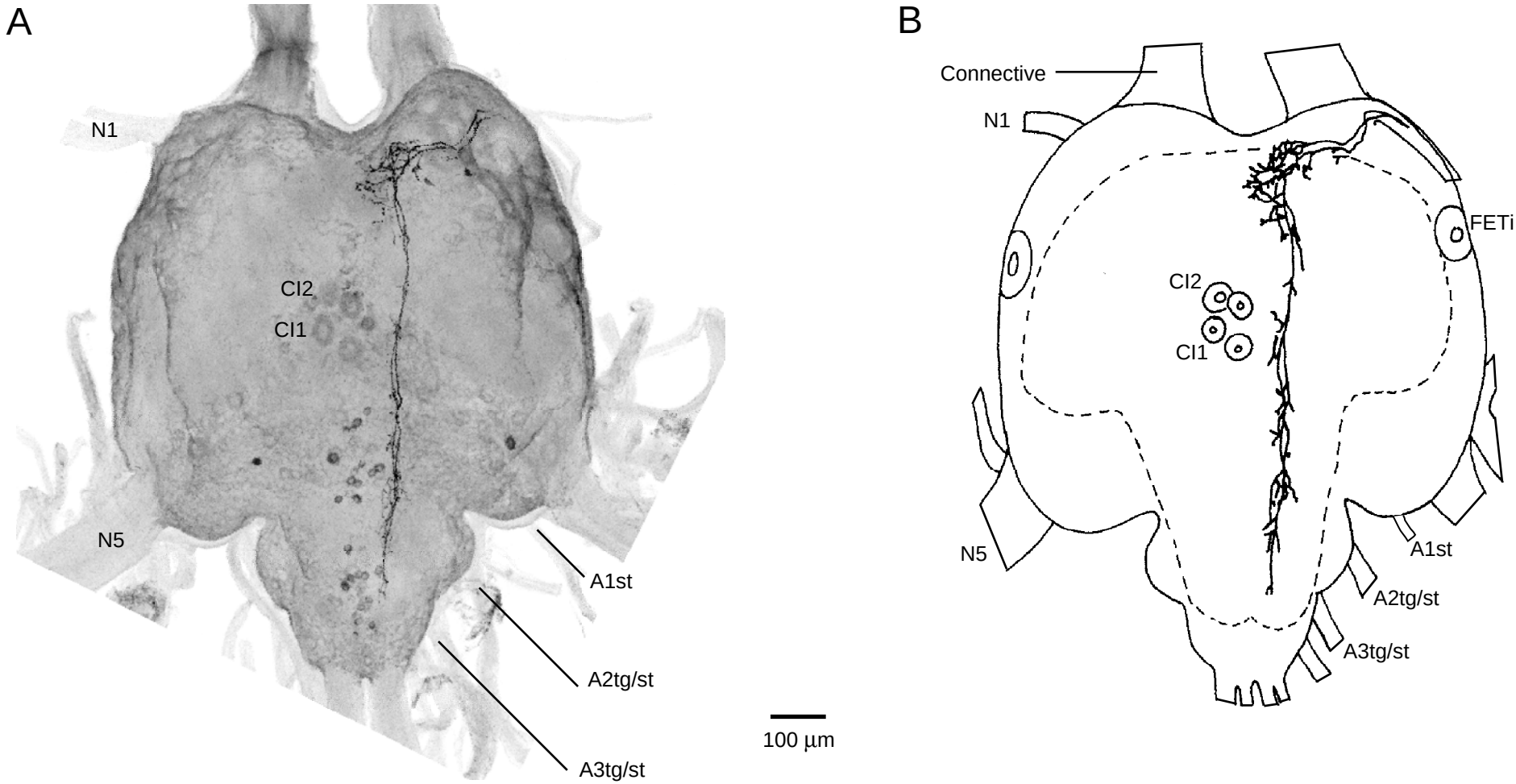

Figure 24: Projection pattern of two sensory neurones from a single long hair at the base of the wing in 5th instar S. gregaria (A, B). Both neurones enter the metathoracic ganglion through nerve 1 (N1), form arborisations in aVAC, before the main neurite descends along the dorsal median tract (DMT) to the level of the 3rd abdominal neuromere. Nerve 1 (N1); Fast extensor tibia cell body (FETi); Common inhibitors (CI); Nerve 5 (N5); sternal/tergal nerve of 1st, 2nd and 3rd fused abdominal neuromeres (A1st, A2tg/st, A3tg/st)..

to the MVT. This branch of the main neurite gave rise to minor branches that projected for 43 ± 3 µm laterally or medially.

In adults, staining of individual wing hairs proved unsuccessful, so results are based on stains of branch C of metathoracic nerve 1. Nerve 1C contains the axons of receptors on the hind wing and the tegula (Büschges *et al.*, 1992), and staining therefore revealed several partially overlapping but distinct neuronal projection patterns in the metathoracic ganglion (Fig. 25). Nerve 1C enters the metathoracic neuropile and bifurcates immediately into axons that project into medial and lateral axon bundles (Fig. 25 A). The medially projecting bundle of axons crossed the midline along the anterior margin of the neuropile, extended through the ipsilateral connective to the mesothoracic ganglion, and descended posteriorly, parallel to the midline along at least two tracts. The lateral bundle projected to the dorso-lateral margin of the anterior lateral association centre (aLAC; white arrow, Fig. 25 Bii). Transverse sections of the metathoracic ganglion demonstrated the medial bundle was further divided into dorsal axons that projected posteriorly along the dorsal median tract (DMT) and more posteriorly, along the median dorsal tract (MDT) white arrows, Fig. 25 Bii, Biii), and ventral axons that branched within the aVAC before descending along the along the median ventral tract (black arrow, Fig. 25 Bii, Biii). The projections of N1C1 within the dorsal neuropile (along the DMT and toward the aLAC) did not have projections to the mesothoracic ganglion, and corresponded to the tegula projections described by Büschges *et al*. (1992, Fig. 25 Ci). The remaining ventral projections (Fig. 25 Cii, Fig. 26 Ai, Aii) had branches within the aVAC of the metathoracic (Fig. 26 Aii, Bii) and mesothoracic ganglia (Fig. 26. Ai, Bi), axons that ran toward the posterior of the metathoracic ganglion parallel to the midline along the MVT (Fig. 25, Bii, Fig. 26, Aii,

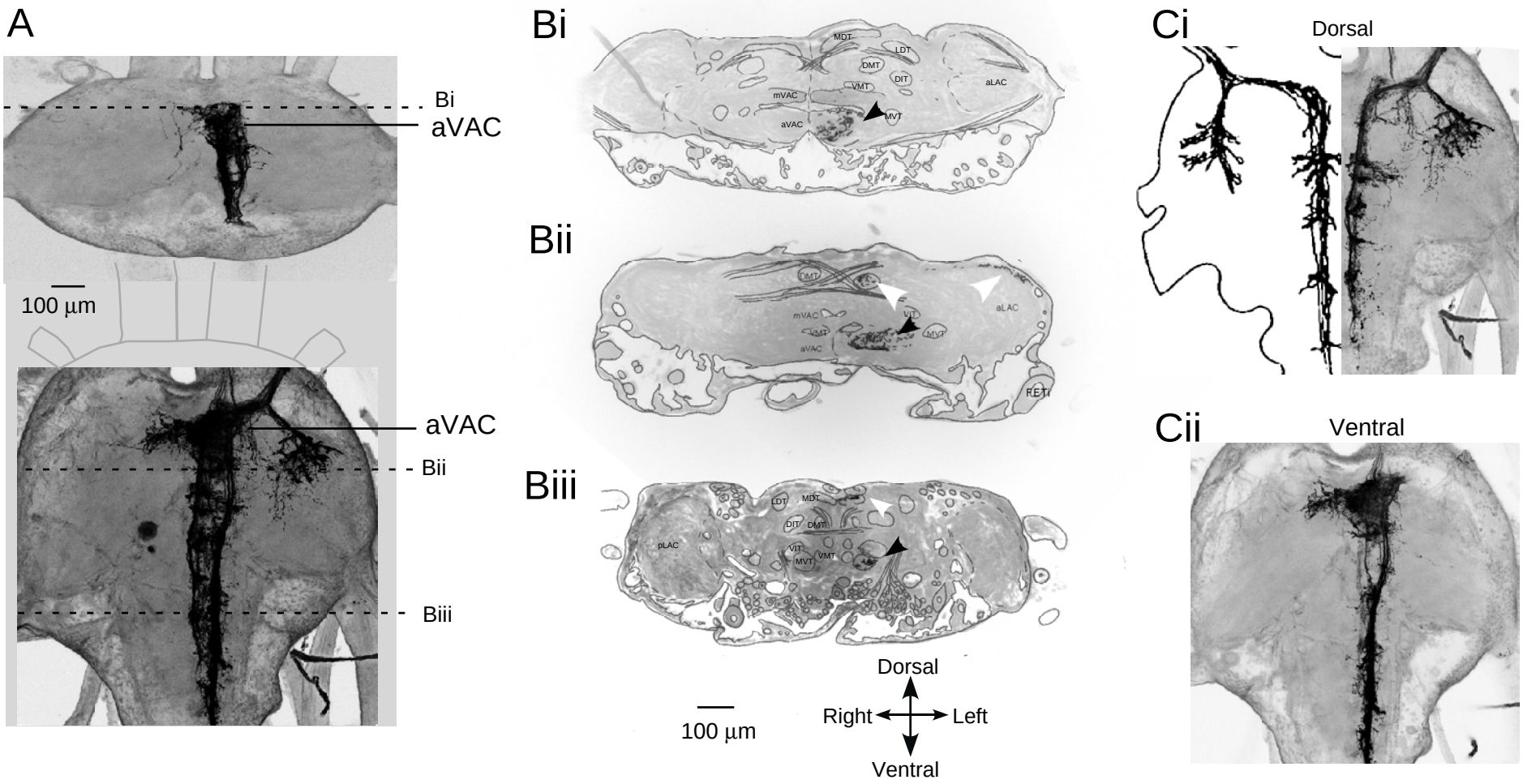

Figure 25. Neuronal projections from the adult hindwing of S. gregaria. Stains of metathoracic nerve 1C1 revealed at least two overlapping projection patterns within the metathoracic and mesothoracic ganglia (A). Transverse sections of the metathoracic ganglion showed projections could be separated as dorsal or ventral, projecting to either the dorsal median tract (DMT) and anterior lateral association centre (white arrows, Bii),) or the anterior ventral association centre (aVAC, black arrow Bii). Thus, the two types of projection can be viewed separately as two focal planes within the metathoracic ganglion (Ci, ii). The dorsal projections are similar to stains of from the hindwing tegula by Buschges et al. 1992 (left side of Ci). The ventral projections are likely to be from the hindwing sensilla.

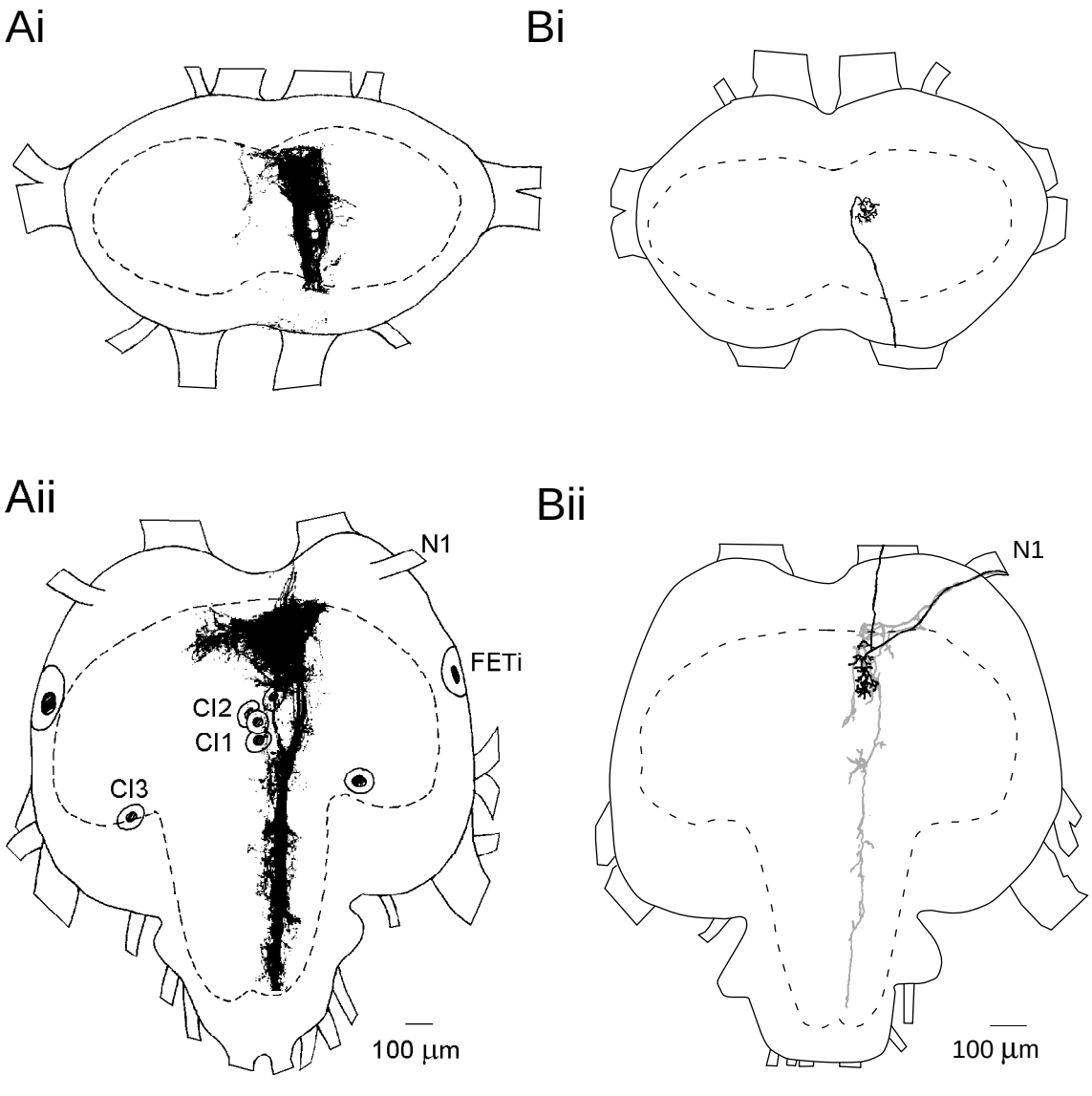

Figure 26. Projections of adult S. gregaria hind wing sensilla from a stain of metathoracic Nerve 1C1 (A) and from a single long mechanosensory hair on the hind wing of a 5 th instar (B). Nerve branch N1C was stained in adults and revealed neuronal projections that branched within the aVAC of both mesothoracic (Ai) and metathoracic ganglia (Aii), projections also extended toward the posterior of the ganglion along the median ventral tract and ventral midline tract. B) 5th instar stains of single hairs on the hindwing demonstrated two neuronal projection pattern types, one branched in the aVAC of both mesothoracic and metathoracic ganglia with no posterior metathoracic projections (black neurone, Bi, Bii), whilst the other projects posteriorly with no mesothoracic projections (grey neurone Bi)

Bii), and some axons that projected through the ipsilateral connective to the mesothoracic ganglion (Fig. 26 Aii, Bii). In 5th instars, a similar distribution was observed which was composed of two projections types; one projecting posteriorly along the MVT (grey neuronal projection in Fig. 26 Bii), and one that formed branches within the aVAC of both metathoracic and mesothoracic ganglia (black neurone in Fig. 26 Bi, Bii). Thus both adult and 5th instar locusts are likely to have neurones from mechanosensory wing hairs that project either intersegmentally or segmentally.

Somatotopic representation

Neurobiotin stains were performed on long mechanosensory hairs along the costal vein, at the base of the hind wing (n = 9), the tip of the hind wing (n = 8) and at a mid-point between the two, defined as ventral to the anterior fork of the radial vein (n = 15) (see pink, blue and green arrows respectively in Fig. 27 E). Figure 7 shows examples of projection patterns from each of these areas. Branches from individual neurones occupied only a small part of the total area of aVAC (shaded area in Fig. 27 A-C). Neurones from sensilla at the base of the hind wing occupied the more lateral and posterior aVAC, whilst neurones from sensilla in the middle of the hind wing, and the tip of the hind wing branch progressively more medial and anterior in aVAC (Fig. 26 D). Thus the position of sensilla on the hind wing is conserved by the spatial projections of their neurones within the segmental metathoracic ganglion.

In the mesothoracic ganglion of 5th instars the same neurones also extended axons to the aVAC to form a somatotopic representation of hair position (Fig. 28). Neurones from the base of the hind wing projected to the lateral and posterior part of the aVAC, and neurones from sensilla on the middle of the wing and on the tip of the wing projected to

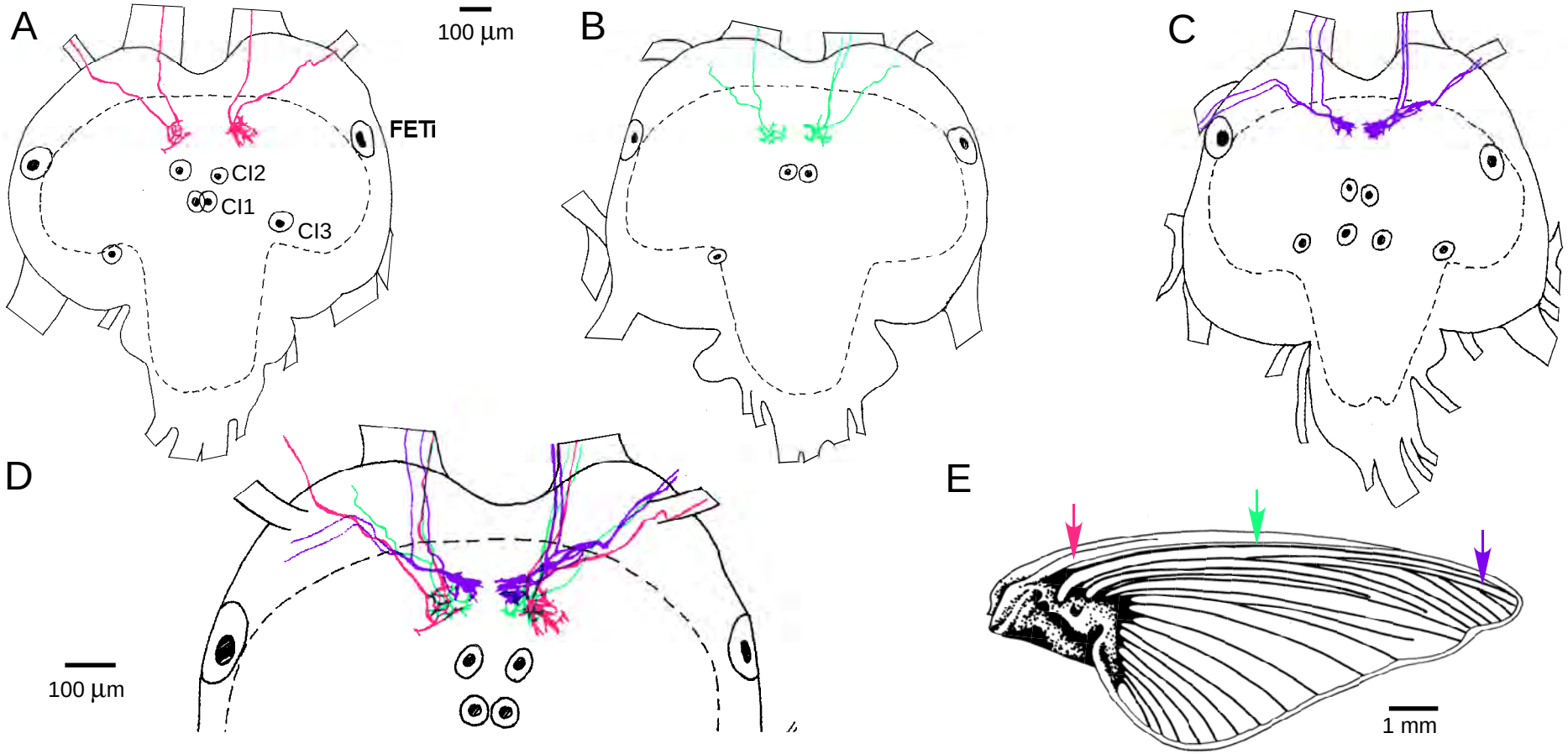

Figure 27. Central projections of sensory neurones from long tactile hairs along the proximo-distal wing axis of a 5th instar S. gregaria. A) Sensory neurones from a single hair on the base of the right and left hind wings project to the anterior ventral association centre of the metathoracic ganglion. B) A sensory neurone from a hair on the middle of the wing overlaps with that from the base of the wing, although some of its branches are more anterior and medial. C) Two sensory neurones from a single hair on the tip of the right and left wings overlap with the mid-wing hair projections but are again generally more anterior and medial. D) Superimposing the sensory neurones from the different positions along the wing (E) shows that there is a systematic change in projection position based on the spatial position of the hair on the wing such that proximally located hairs send their sensory projections most posteriorly and laterally in the ganglion, whereas distally located hairs have their projections located most anterior-medially in the ganglion.

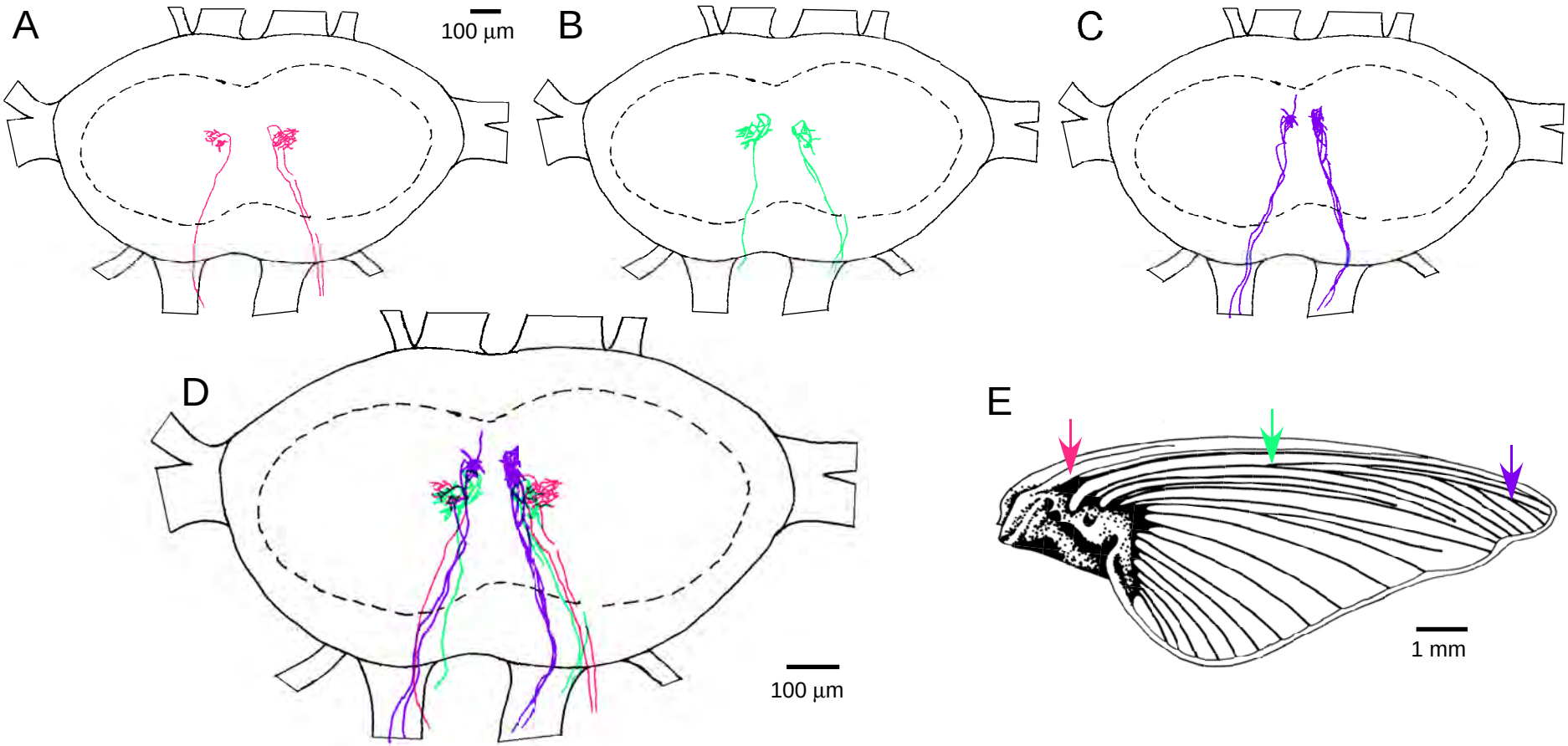

Figure 28. Mesothoracic projections of sensory neurones from long mechanosensory hairs along the proximo-distal wing axis. A) In addition to branches within the anterior ventral association centre (aVAC) of the metathoracic ganglion, a single sensory sensory neurone from a hair on the base of the wing also projects to the aVAC of the mesothoracic ganglion. B) A sensory neurone from a hair on the middle of the hind wing overlaps with that from the base of the wing, although some of its branches are more anterior and medial. C) A sensory neurone from a hair on the tip of the wing overlaps with the mid-wing hair projections but is again generally more anterior and medial. D) Superimposing the sensory neurones from the different positions along the wing (E) shows that there is a systematic change in projection position based on the spatial position of the hair on the wing such that proximally located hairs send their sensory projections most posteriorly and laterally in the ganglion, whereas distally located hairs have their projections located most anterior-medially in the ganglion. Shaded area represents the total area occupied by the projections of neurones from mechanosensory hairs.

progressively more medial and anterior positions within the aVAC respectively (Fig. 28 A-C). Therefore the locations of hairs on the hind wing are represented in two separate, parallel somatotopic maps within the metathoracic and mesothoracic ganglia.

The mean area of neuronal branching from mid-hind wing hair afferents was medial and anterior to the mean area of neuronal branching of sensilla at the base of the hind wing. Medial and anterior to both of these mean regions of branching were that of afferents from sensilla on the tip of the hind wing. The relationship between the region of hair afferent branching and the position of the hair on the wing was determined by correlation analyses (Fig. 29). In the metathoracic ganglion, the positions of hairs at the wing base, mid-wing and wing tip were significantly related to the distance of the midpoint of arborisations (centre of ellipse, Fig. 29) from the centre of the ganglion (Spearman's rho correlation, X-axis: $r = -0.628$, p (2-tailed) $= 0.004$, Y-axis, $r = 0.698$, $p = 0.001$). In the mesothoracic ganglion, hair position was also significantly related to the distance of the midpoint arborisation from the centre of the ganglion for the Y-axis ($r = 6.34$, $p = 0.004$) but not the x-axis ($r = -0.392$, $p = 0.097$).

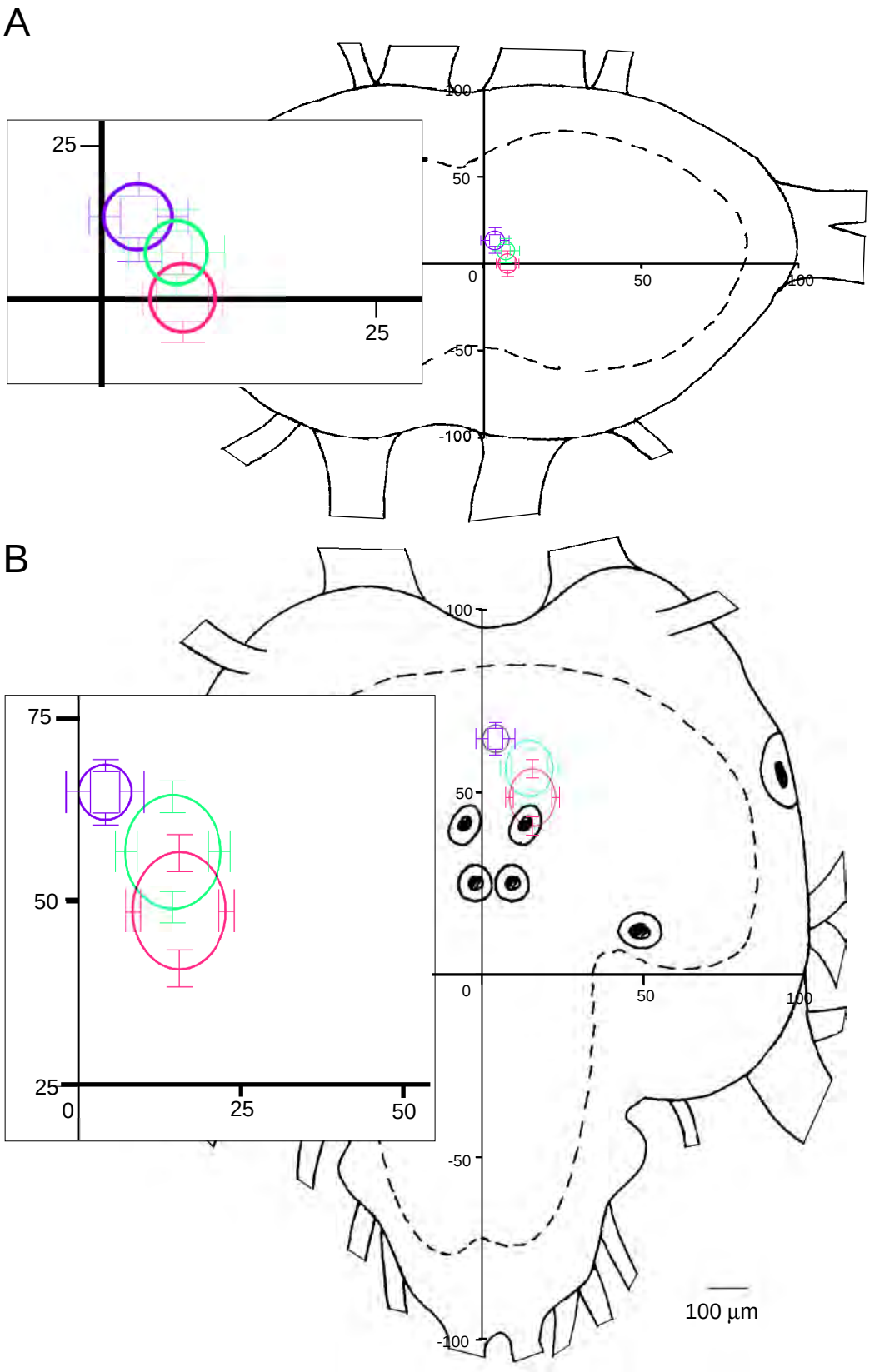

Figure 29. Average position of the most anterior, posterior, medial and lateral extents of the arborisations from long tactile hairs from the base (pink), middle (green) and tip (purple) of the hindwing of 5th instar S. gregaria, as shown by ellipses connecting each of the four points. Each position was calculated as a percentage of the distance from the centre of the ganglion to the maxiumum width of the hemiganglion. Distances are were calculated as a percentage of the distance from the midpoint to the edge of the ganglion. Average positions of neuronal branches were calculated from 9 and 7 base, 15 middle and 8 and 7 tip stains respectively.

Discussion

Comparisons between the projections of wing sensilla of 5th instar and adult *S. gregaria* were confounded by the difficulty in staining neurones from individual wing sensilla in adults. Previously published results of metathoracic nerve 1C have also been unsuccessful in staining wing hair afferents (*Chortoicetes terminifera* Tyrer and Altman, 1974; *L. migratoria* Wolf and Büschges, 1997). This may be due to the axons of wing sensilla having diameters that are mostly less than 1 µm (Altman *et al.* 1978), which could restrict the diffusion or transport of some stains. To determine the projections of adult wing hairs, the entire branch of metathoracic nerve 1C1 was stained with Neurobiotin. Nerve 1C1 contains approximately 800 axons from hairs on the wing, 85 axons from campaniform sensilla at the base of the wing and about 80 neurones from the tegula at the wing hinge (40 axons from sensory hairs on the tegula, and 30 from the chordotonal organ within the tegula, and 10 very fine axons of unknown origin) (Altman *et al.*, 1978; Tyrer and Altman, 1974). By discounting the dorsal projections known to belong to the tegula neurones (Büschges *et al*., 1992, Tyrer and Altman, 1974), the ventral projections of the adult wing sensilla were shown to project to the same regions as 5th instar wing hairs; i.e. to the metathoracic aVAC, toward the posterior of the metathoracic ganglion, and to the mesothoracic aVAC.

Dual Innervation of mechanosensory hairs on the wing and body

Some of the stains of individual long mechanosensory hairs revealed two sensory neurones associated with a single hair. Stains of abdominal tactile hairs in *S. gregaria* have also revealed dual innervation, and this was confirmed by TEM sections of the hair base, which located two sensory neurones attached to the base of a single hair (Tousson

and Hustert, 2006). These dually innervated hairs could not be reliably morphologically distinguished from singly innervated tactile hairs. Dual innervation of wing mechanosensory hairs in 5th instar locusts may help to explain the increase in number of wing neurones in fledgling adults and the subsequent decrease during maturation (Altman *et al.*, 1978, discussed in chapter 2). For example during the imaginal moult, the tormogen, thecogen and trichogen cells of the hair complex may be conserved, whilst a 5th instar sensory cell is replaced by an adult sensory cell. This, however, would not explain the dual innervation observed in abdominal hairs.

Multiple innervations is also seen in contact chemosensory hairs, with a single neurone detecting mechanical displacement of the hair shaft, and approximately four neurones that respond to chemical qualities, such as sugar, salt, water. For the legs and abdomen of *S. gregaria*, the mechanosensory and chemosensory neurones of contact chemosensory sensilla projected to the same region of the ganglion, and for the hind leg sensilla were found to have parallel somatotopic representations (Newland *et al.*, 2000a, b). The absence of a pore, however, in long mechanosensory hairs indicates they are unlikely to be chemosensory (Slifer, 1970). Two types of tactile hair on the locust hindleg have been described based not on hair length, but on response threshold (Newland, 1991a). High threshold hair afferents respond phasically to imposed deflections of the hair shaft at a speed of 21.1 ± 4.2 ^{o} s^{-1} and adapt rapidly to repeated stimulation. Low-threshold hairs respond phasotonically to imposed deflections of the hair shaft at a speed of less than 3 ^{o} s^{-1}, and they show some reduction in firing rate, but still continue to fire in response to repetitive stimuli. It is therefore possible that sensory neurones that dually innervate hairs on the wing or abdomen, may respond to different stimuli. Tousson and Hustert (2006) recorded phasic responses to hair shaft

displacement from one of the sensory neurones of dually innervated hairs, but did not identify the sensitivity of the other sensory neurones. Further work should be done to establish whether these neurones respond to different stimuli, and to determine whether post-synaptic interneurones also preserve these differences.

Two projection patterns were associated with long mechanosensory hairs on the wing

Stains of individual hair afferents from the hind wing in 5th instars identified two projection patterns. The first projects to the metathoracic ganglion and intersegmentally to the mesothoracic ganglion and the second projects to the metathoracic ganglion alone.

Intersegmental projections

After entering the metathoracic ganglion through nerve 1, the majority (85%) of mechanosensory hairs branch within aVAC before the main neurite extends anteriorly through the connective to the mesothoracic ganglion, branching within aVAC also. Sensory information from the mechanosensory hairs on the hind wing is likely to be integrated with that from the fore wing within the mesothoracic ganglion. This would be useful because the wings overlap, and so tactile hairs on both the forewing and hindwings would encode similar external positions. A stimulus on the uppermost wing surface (fore wing in adults) is likely to stimulate tactile hairs and campaniform sensilla on the underlying wing.

In locusts and crickets, mechanosensory hair afferents from the thorax and abdomen (Hustert, 1985; Tousson and Hustert, 2006), but not from the hind leg (Newland,

1991a), also extend posteriorly or anteriorly to the homologous regions of the next adjacent ganglion. In the cricket, *G. bimaculatus*, most hair afferents from the sternum are restricted to the VAC of the local segmental ganglion; however one pair of long mechanosensory hairs from the sternum has afferents that branch in VAC of both the metathoracic and mesothoracic ganglia, and a pair of pronotal hairs also project intersegmentally to the prothoracic and mesothoracic ganglia (Hustert, 1985). In *S. gregaria*, mechanosensory hairs on the 6th abdominal segment have afferents that project to the same homologous VAC region of one or more adjacent ganglia (Tousson and Hustert, 2006). This suggests that the projection patterns of fore wing hair afferents may also project intersegmentally, entering the mesothoracic ganglion through N1, forming arborisations within aVAC before descending through the ipsilateral connective to form arborisations in the metathoracic aVAC. Tyrer and Altman (1974) stained mesothoracic N1C and N1D demonstrating that neurones from this group do project to both the mesothoracic and metathoracic ganglia, and consist of a dorsal bundle of axons (probably from the tegula, N1C) and a ventral bundle (probably from tactile hairs on the wing, N1C or the thorax, N1D). It is evident that both dorsal and ventral neuronal bundles project intersegmentally, branching first in an anterior region of the mesothoracic ganglion, before extending to an anterior region of the metathoracic ganglion and toward the fused abdominal neuromeres of the metathoracic ganglion. These projection patterns do not exclude the neuronal projections from sensory hairs on the thorax (from N1D), and so although there is an implication that some of the ventral projections are from wing hair sensory neurones, this cannot be assumed without further research.

Intersegmental projections of wing hair afferents to both metathoracic and mesothoracic aVACs could accommodate rotation of the wings during development (Uvarov, 1977): during development, the wings rotate such that the hindwing is uppermost in 5th instars, and the forewing is uppermost in adult. As such different wing surfaces and exteroceptors receive a given stimulus during development. The projections of wing hair afferents to both metathoracic and mesothoracic aVACs mean that sensory information from both wings is likely to converge on the same group of postsynaptic interneurones, maintaining the subsequent neuronal pathway.

Certain local and intersegmental interneurones with their cell bodies within the metathoracic ganglion have branches with aVAC and respond to stimulation of wing nerves (Matheson, 2002). Local interneurones forming a subtype of midline spiking interneurones defined by Burrows and Siegler (1982, 1984) and Siegler and Burrows (1983, 1984) have input branches within aVAC and branches within the either VAC (area containing terminations of hind leg hair afferents) or lateral association centre (LAC) (containing terminations of hind leg proprioceptors) (Matheson, 2002). These interneurones respond to both wing stimulation and tactile stimulation or movement of the legs. The convergence of information about stimulus location from wing hair afferents with information about the starting position of the hindleg is necessary to generate the hind leg scratching movements following stimulation of the wing in locusts. These local interneurones, that also have a role in reflex movements of the hind leg (see Burrows, 1996), are involved in integrating information about stimulus location and hind leg position.

Some intersegmental interneurones have branches in aVAC and receive inputs from wing afferents. Type A intersegmental interneurones have additional branches in the anterior lateral association centre (aLAC) and lateral VAC and respond to stimulation of both fore and hind wings, and touching or moving the ipsilateral hind leg. However the branching pattern of these intersegmental interneurones within the mesothoracic ganglion has not been described, so it is not clear whether the synaptic connections with fore wing hair afferents occur within the mesothoracic or metathoracic ganglia. Type B intersegmental interneurones have bilateral patterns of branching, belong to a population of 35 ascending interneurones that respond to stimulation of tactile hairs on the hindleg (ipsilateral VMT or contralateral DIT), or stimulation of the femoro tibial chordotonal organ (ipsilateral MDT or contralateral VIT) (Laurent and Burrows, 1988). Some Type B intersegmental interneurones also respond to stimulation of the forewing and hind wing nerves, or by tactile stimulation of hairs on the wings surface (corresponding to dense arborisations in aVAC). Ascending axons of Type B intersegmental interneurones do not branch densely in the ventral neuropile of the mesothoracic ganglion. Thus, the branches of wing hair sensory neurones in metathoracic aVAC appear to input onto several types of postsynaptic interneurone. Generally speaking, information from sensory hairs on the both wings converges with sensory information from mechanosensory hairs on the hindleg, or from hind leg proprioceptors in both local and intersegmental interneurones.

Local projections

After entering the metathoracic ganglion through nerve 1, neuronal projections from a proportion of (15%) long mechanosensory hairs on the hind wing of 5th instars formed arborisations within aVAC before the main neurite extended posteriorly. This projection

extended toward the fused abdominal neuropiles, and the short branches at intervals along the length of the main neurite may have been toward the fused abdominal aVACs (Pflüger *et al*., 1988). None of these neurones had projections to the mesothoracic ganglion or to the posterior free abdominal ganglia.

Type C ascending intersegmental interneurones had, amongst others, bilateral projections to aVAC and descending branches along MVT. It responded to depolarisation of nerve branches within the metathoracic wing, and more strongly to depolarisation of metathoracic N1 itself. Type C interneurones are similar is morphology and response to Type 404 interneurones in the mesothoracic ganglion (Matheson, 2002), that have been implicated in the initiation of flight, although they do not themselves make direct output connections on to flight motor neurones (Pearson *et al.,* 1985). I propose that projections along MVT to the fused abdominal neuromeres are involved in the control of abdomen posture both during scratching and flight. Stimulation of long mechanosensory hairs may elicit avoidance movements of the abdomen, instead of or in addition to scratching movements of the hind leg that have been previously described (Page and Matheson, 2004; Chapter 1 and 2). Indeed, 5th instars, more so than adults, were observed to raise their abdomens during stimulation of the hind wing (personal observation). Type 404 or Type C interneurones, may instead be involved in raising the abdomen during flight in adults (Cooter, 1973), a position also adopted by flightless 5th instar locusts (Altman, 1975) in response to lack of tarsal contact and suspension in an airstream. During flight itself, movement of the abdomen and hind legs change the drag, shift the centre of mass, and change the moment of inertia of the body, and during turns the abdomen is curled to the left or right, acting like a rudder (Camhi, 1971). The abdomen vibrates vertically in time with the wing beat

frequency. This is not due to mechanical vibrations, but bursts of spikes from the motor neurones controlling the abdominal muscles, that are in synchrony with the wing beat rhythm, (Camhi and Hinkle, 1972). Thus, displacement of long mechanosensory hairs with this posterior projection pattern maybe involved in movement of the abdomen seen in flight, in addition to abdominal movements associated with scratching.

Somatotopic map

The sensory neurones of contact chemosensory and mechanosensory hairs on the hind leg of *S. gregaria* project locally to the ventral association centre of the metathoracic ganglion without intersegmental projections to adjacent ganglia (Newland, 1991a; Newland *et al.*, 2000a, b; Newland and Burrows, 1994). Within the VAC of the metathoracic ganglion, afferents of both mechanosensory and gustatory (contact chemosensory) hairs on the hind legs of *S. gregaria* conserve external hair position within somatotopic maps. The external location of mechanosensory hairs is represented three dimensionally within the ventral neuropile of the metathoracic ganglion, so that the proximo-distal, antero-posterior and dorso-ventral position of the hair is represented within the central nervous system. The afferents of hairs on proximal segments of the leg (femur) project to more anterior regions of lateral ventral association centre (lVAC) than do those from hairs on distal leg segments (tarsus). The afferents of hairs on the posterior side of the leg project more medially within lVAC than do afferents from the anterior side of the leg. Lastly, afferents from hairs on the ventral surface of the leg project to more ventral areas of lVAC and also the ventralmost VAC, than do afferents from dorsal hairs (Newland, 1991a). Thus the region of VAC in which the hair afferents have arborisations is dependent on the peripheral position of the hair.

The somatotopic representation of mechanosensory receptors on the hind leg is further conserved by the partially overlapping receptive fields of postsynaptic interneurones (Burrows and Newland, 1993). Specific interneurones have receptive fields that overlap with regions of the somatotopic map of hind leg hair afferents. Whilst some spiking local interneurones have extensive ventral branches and corresponding receptive fields that stretch from the proximal femur to the tip of the tarsus, some spiking local and intersegmental interneurones have restricted branches that correspond to small receptive fields. Interneurones with branches in the posterior part of the ventral neuropile have receptive fields on the tarsus, whilst those with branches in the anterior neuropile have receptive fields on the tarsus. Interneurones with branches in the lateral neuropile have receptive fields on the anterior surface of the hind leg, and those with branches in the medial neuropile have receptive fields on the posterior surface of the hindleg. Thus the spatial position of an array of hairs is conserved in the somatotopic representation of their afferents and the postsynaptic pattern of activation across different interneurones (Burrows and Newland, 1993).

The central projections of hind wing hairs form somatotopic representations within aVAC of the metathoracic and mesothoracic ganglia. This map does not overlap with the projections of hind leg mechanosensory hairs (Burrows and Newland 1993), but is adjacent to it, extending it. Despite the hind leg and wing both being metathoracic appendages, the orientation of afferent arborisations that represent the proximo-distal axes of the appendages are in opposite orientations. The proximo-distal axis of the hind leg follows the convention of proximal hair receptors having arborisations near the midline of the ganglion and increasingly lateral receptors having arborisations that are progressively more lateral (Newland, 1991a; Hustert, 1985; Pflüger *et al.* 1981). In

contrast, the proximo-distal axis of the hind wing is represented by hair afferents from the proximal wing base having branches at the lateral border of the aVAC, and hair afferents from the wing tip having branches near the midline of the aVAC. The anterior-posterior axis for the mechanosensory hair afferents from the wing does, however, correspond to that for the hind leg, with anterior hairs having afferents that branch in more lateral areas of the VAC to those from posterior wing hair afferents. If this somatotopic representation is used by the nervous system to determine the spatial location of targets, how is the map altered by growth of the wing? Sensory neurones from the wing tip in 5th instars project to the midline, so it is unclear where adult neurones from a more posterior wing tip would project to. Attempts to determine the projections of wing hair afferents from the wing base and wing tip of adults by removing successive portions of the wing and allowing neurones from those regions to die off was unsuccessful, confounded by potential changes of neurone number between fledgling adults and mature adults (Altman *et al*., 1978).

In summary, my work represents the first description of sensory neurone projections arising from mechanosensory hairs on the wings of a hemimetabolous insect. I have identified two types of projection pattern. One, as suggested by Matheson (2002), did indeed have neurones that project to the aVAC of the metathoracic ganglion. In addition, the same neurones also project to the aVAC of the mesothoracic ganglion. This intersegmental projection allows sensory information from the fore wing and hind wing to be integrated, which is functionally relevant as the wings overlap in 5th instars and adults, and the same stimulus may be detected by the underlying wing as well as by the uppermost wing. Furthermore, integrating wing sensory information so that both wings converge on the same group of postsynaptic interneurones may accommodate the

rotation of the wings that occurs during development. Several local and intersegmental interneurones integrate sensory information from wing hairs with that from either hind leg hairs, or hind leg proprioceptors, but not both. The second projection pattern had arborisations in the metathoracic aVAC, and a descending projection along MVT. I propose that this is involved in control of abdomen movements during scratching. Finally, I demonstrated that wing hair afferents are arranged somatotopically within aVAC in both the metathoracic and mesothoracic ganglia, and as such may be key to encoding spatial information for scratching.

GENERAL DISCUSSION

The purpose of this thesis is to develop our understanding of how an aimed limb movement might adapt to developmental growth. This is one of the first investigations of the plasticity of an aimed limb movement associated with growth in an insect. I have described changes in the wing-directed scratching behaviour of 5th instar and adult *S. gregaria*, before and after the imaginal expansion of the wings, and have identified changes in the organisation of the mechanosensory hairs that, when stimulated, elicit scratching behaviour.

In hemimetabolous insects, the adult wings emerge and expand from small pads on the thorax during the imaginal moult. Whilst body and hind leg growth are allometric, the increase in size of the wings is non-allometric. Furthermore, in some species such as *S. gregaria*, the wing pads rotate during development so that in late instars the ventral surface of the hind wing pad is exposed, but in adults the dorsal surface of the fore wing is exposed (Uvarov, 1977; Ivanova, 1947). The data presented in this thesis demonstrate that scratching movements aimed at the exposed wing surface adapt to these morphological changes, maintaining appropriately aimed behavioural responses during development.

In **Chapter 1,** I presented the first description of scratching in 5th instar *S. gregaria***.** Like adult *S. gregaria*, 5th instars make scratching movements of the hind leg in response to tactile stimulation of hairs on the wing surface. Movements change to follow the change in position of the tip of the wing from near to the hind leg coxa in 5th instars, to posterior to the abdomen tip in adults. The initial trajectory of the effector,

and the hind leg angles used whilst approaching wing tip targets change from those appropriate for anterior targets in 5th instars to those appropriate for posterior targets in adults. However the speed, accuracy and effector used remain similar. I suggest that dynamic joint stiffness and proprioceptive reflexes compensate for changes in limb mass, muscle force and musculo-skeletal resistance and result in movement kinematics being conserved. In adults, continually graded combinations of hind leg angles are used to reach targets along the wing surface, indicating that the use of a single movement pattern is modifiable for different targets (Dürr and Matheson, 2003). My data demonstrate that the same movement pattern is used by 5th instars, and that it is modified for targets on a different and larger wing surface. This gives support to the equilibrium point hypothesis of movement control, wherein limb position is achieved by a balance of muscle force and musculo-skeletal resistance, which are encoded internally, and movements arise through a shift in this equilibrium. I proposed that putative "preferred states" of different proprioceptive receptors might reach equilibrium by the interaction of their respective proprioceptive reflex pathways. Movement trajectories could therefore arise from temporarily graded depression of reflexes opposing the desired movement and the graded potentiation of proprioceptive reflexes assisting the desired movement.

In **Chapter 2**, I compared for the first time the organisation of wing hairs in juvenile instars and adults of a hemimetabolous insect and demonstrated that changes in their organisation are involved in eliciting scratching behaviour. In 5th instar *S. gregaria*, long mechanosensory hairs cover the entire exposed wing surface (ventral hind wing), but in adults such hairs are located only along the postcubital vein of the exposed wing surface (dorsal fore wing), meaning they are prominent when the wings are folded at rest. The

distribution of medium length mechanosensory hairs on the exposed wing surface also changes, from sparsely dispersed across the exposed wing of 5th instars to a higher density along the leading edge of the wing in adults. These data suggest that long mechanosensory hairs are involved in eliciting scratching movements, supporting previous findings (Page and Matheson 2004), but that medium length mechanosensory hairs may be involved in flight. Few hairs are present on the other wing surfaces (dorsal fore wing, ventral fore wing, and dorsal hind wing) in 5th instars, meaning that most hairs develop *de novo* for adulthood. Although there are many long mechanosensory hairs on the ventral hind wing of 5th instars, few remain in adults. It is possible that some hair shafts may change in length to form medium length mechanosensory hairs. One region of the hind wing, however, still demonstrated a significant loss in the total number of sensilla, indicating that the 5th instar compliment of hairs may degenerate and be replaced with adult sensilla as suggested by Altman and colleagues (1978). Stimulation of hairs on the tip of ventral hind wing of 5th instars elicited movements aimed at a different location to movements elicit by stimulation of hairs on the tip of the ventral hind wing in adults. I suggest that the wings undergo a mini-metamorphosis, where sensory neurones within the hind wing pad of 5th instars are replaced with an entirely new adult set of sensory neurones. I further suggest that this is a mechanism to accomplish a reorganisation of sensory apparatus needed for the retargeting of movements aimed at the wing.

In **Chapter 3**, I described for the first time the projection pattern of individual hairs on the wing of a hemimetabolous insect. Sensory neurones from long mechanosensory hairs on the hind wing of 5th instar *S. gregaria* form a somatotopic representation of external receptor position within the anterior ventral association centre (aVAC) of the

metathoracic ganglion. The same neurones also ascend intersegmentally to form another somatotopic representation within aVAC of the mesothoracic ganglion. A second, less prevalent group of neurones project to aVAC of the metathoracic ganglion before descending to the level of the fused abdominal neuromeres. Although staining of individual neurones in adults proved unsuccessful, whole nerve stains of N1C1 in adults present a similar pattern to individual sensilla stains in 5th instars. A bundle of axons form branches within aVAC of the metathoracic and mesothoracic ganglia, and a bundle of axons descended to the level of the fused abdominal neuromeres.

Do movements adapt to developmental growth, and how?

Wing directed scratching movements adapt to both changes in the musculo-skeletal system and changes in wing size and orientation, using different mechanisms that shall be discussed below.

Scratching movements maintain similar characteristics (speed, initial direction, combinations of joint angles used together), implying that potential changes in muscle strength or limb mass may be compensated for. This may be achieved by mechanisms that compensate for loads added to the limb or movement perturbations. In locusts, these mechanisms may be dynamic joint stiffness (Matheson and Dürr, 2003) or proprioceptive reflexes (Burrows, 1987, 1996; Field and Burrows, 1982). Similar load compensation mechanisms exist in vertebrates (e.g. Lackner and Dizio, 1994; Shadmehr and Mussa-Ivaldi, 1994), and so may play a role in maintaining movements through muscle and limb growth. Although changes in muscle strength and limb mass in locusts is probable during development (Queathem and Full, 1995), they were beyond the scope of this research and shall not be discussed further.

Scratching movements are accurately aimed at both the small wing surface in 5th instars, and also the much larger wing surface of adults. The same single movement pattern is used by 5th instars and adults, suggesting that the motor network generating scratching movements is conserved during development. Instead, there are changes in the wing surface receiving the stimuli due to the rotation of the wings, and the development of new and different tactile hairs, and therefore changes in sensory input. Thus hairs on the tip of the wing in 5th instars have different synaptic connections onto the motor network that generate scratching, than hairs from the adult wing tip. These sensory neurones in 5th instars and adults are able to encode different spatial locations. The rotation of the wings, where the ventral forewing receives input in 5th instars and the dorsal forewing receives input in adults, creates problems in it's self, and which may be solved by the intersegmental projections of wing tactile hairs forming an integrated map within both metathoracic and mesothoracic ganglia. Resulting in the overlap of both wings being represented internally, and inputting into a single motor network. Thus it appears that the adaptation of behaviour is associated with the development of different sensilla on the fore and hind wing and their rotation during development, and thus is hard-wired.

The proposal of a mini-metamorphosis and wing rotation as mechanisms that allow behavioural adaptation favours hard wired developmental changes over neuronal plasticity. This is not unprecedented, as the work by Maldonado and colleagues (1970; 1974) demonstrated that structural changes of the visual sensory system allows aimed striking movements to adapt to limb growth. This suggests that in insects developmental growth may be compensated for in other systems by predetermined developmental changes of the sensory system. This does not negate the role of neuronal plasticity and learning, as there are many examples of learning. For example, in *S. gregaria*,

shortening the femoral chordotonal organ causes altered proprioceptive feedback about the position of leg, leading to displaced movements. Over a course of several days, however, locusts learn to compensate for the altered signals and functional movements are restored (Page and Matheson, 2009). In addition, locusts can learn to hold their hindlegs at a particular fixed angle to avoid an aversive stimulus (Forman, 1984). There may be a distinction between the situations where the two mechanisms are employed. *Automatic adaptation* occurs in relation to predetermined developmental changes in morphology, where changes in the sensory system evolve to compensate for growth of the body. Learning occurs in response to novel situations and stimuli. Thus in terms of the control of movement, any predetermined changes, such as body growth, would be automatically adapted for, whilst any novel changes, such as limb amputation, would require learning.

It seems, therefore, that whilst sensory input that elicits scratching changes, the motor network that generates the scratching movement is unchanged. This is important in locusts, as the motor neurones that innervate the hindleg for scratching are used for walking and jumping (Burrows, 1996). Components of motor networks may be shared in vertebrates too. D'Avello and colleagues (2005) proposed that movements in frogs were composed of shared or specific muscle synergies (building blocks), each of which could be scaled temporally. Whilst several muscle synergies were specific for behaviours such as jumping, swimming and running, many were shared between these behaviours. Stein and colleagues (1986) found that hindleg movements of the turtle could be electrically induced to change between scratching and swimming movements. Thus demonstrating shared neuronal circuitry for scratching and swimming behaviour.

The work presented here suggests that different sensory input into that conserved motor network can shift the movement toward a target. This lends support to hypotheses where limb trajectories are calculated for specific movements (Kawato, 1999; Schenau, 1995), as opposed to being assembled from reflex-like building blocks (d'Avella *et al.*, 2003; Poppele and Bosco, 2003; Tresch *et al.*, 1999). The role of proprioceptive feedback appears to be central in controlling movements as assumed changes in limb mass and muscle strength are compensated, paralleling compensation of loads and forces applied to the limb. This gives support to the equilibrium position hypothesis, where an internal representation of limb position is shifted to produce a trajectory (Feldman, 1966). A consenus on the control of movement is far from being reached at this time, but the simplicity of the locust system and its ability to accurately aim movements provides a useful model to further research movement control.

Mini-metamorphosis of the wings

The reorganisation of wing sensory structures occurs only for the ventral hindwing, which has in 5th instars a full set of sensory hairs. Wing growth would require many of sensory neurones to encode one spatial location of their tactile hairs in 5th instars, and a different spatial location of their tactile hair in adults. If somatosensory representations are indeed how spatial information is conserved by the nervous system, then a change in receptor position would require a change in branching pattern of the associated sensory neurones. I propose that in locusts a different mechanism has evolved. Instead of existing sensory neurones becoming respecified, a new adult set of sensilla replace the 5th instar set, and as such have new afferent branches that form an enlarged somatotopic representation of the adult wing. Studies in drosophila indicate that this may be under genetic control, by the differentiation of an epithelial cell into the tormogen, trichogen,

thecogen and sensory neurone that generate the tactile hair and neurone (Mitchell *et al.*, 1990, 1983). Three possible routes of enquiry are evident to test the hypothesis that the 5th instar wing sensilla are replaced by adult sensilla. First, to make use of the marker of cell division, 5'-bromo-deoxy-uridine (BrdU) colocalised with toluidine blue to identify dividing neuronal cells in the 5th instar wing pad (Urbach *et al.*, 2003; Shepherd and Bate, 1990). Their number and location could be linked to the wing hair distribution described here, to show that a new set of adult sensilla are formed. Second, to selectively inhibit the division of the differentiated epithelial cells that give rise to the new adult hair complexes, by using hydroxyurea to block DNA-synthesis. This experiment would involve determining a time when the differentiated epithelial cells are sensitive to hydroxyurea but undifferentiated epithelial cells are not. This has successfully been used in *M. sexta* to prevent growth of adult-specific sensory neurones, without altering functional larval neurones (Truman and Booker, 1986). New adult sensilla would not develop, leaving only respecified 5th instar sensilla. Third, transmission electron microscopy of the wing nerves of *S. gregaria,* to chart axon number development during the 5th instar, immature adult and mature adult, replicating the work of Altman and colleagues (1978) on *Locusta migratoria*. The number of wing hair axons in an immature adult should be the sum of those in the 5th instar and those in the mature adult. A mechanism such as the replacement of larval sensilla with adult sensilla is likely to be specific to insects, and may be related to the full metamorphosis of holometabolous insects, such as the Hawk moth *Manduca sexta* (e.g. Levine *et al.*, 1995*).*

How is the spatial location of a target encoded by tactile hairs?

I have demonstrated that the position of hairs on the ventral hind wing of locusts are represented by the position of sensory neurone branches within nervous system. In insects the spatial location of wind-sensitive and tactile hairs are represented by somatotopic maps in flies and crickets (Murphey *et al.* 1980; Newland, 1991a). Tonotopic and retinotopic maps exist for the auditory system (Römer, 1983) and visual system respectively (Strausfield, 1971). Topographic representations of receptor position are found in the also found in the visual system, somatosensory system, and auditory system of vertebrates (Hinds *et al.*, 2009; Udin and Fawcett, 1998; Talavage *et al.*, 1999). Therefore understanding the function of somatotopic maps is relevant to both vertebrates and invertebrates. For the locust hind leg, the spatial arrangement of tactile hairs is preserved by their sensory afferents but also by the receptive fields of the postsynaptic interneurones (Newland, 1991a; Burrows and Newland, 1993). This suggests that interneurones postsynaptic to wing hair afferents may also conserve this arrangement. How might a somatotopic map encode spatial position? Rall's cable theory (1962; 1969; 1973) suggests that the position of a synaptic input within a receptive field determines the attenuation of that synaptic input. Furthermore, varying expression of ion channels in different regions of a dendritic tree can modify synaptic inputs (Schwindt and Crill, 1995; Magee and Johnston, 1997; Williams and Stuart, 2000). In other words, a sensory neurone with branches at the limit of an interneurone's receptive field creates a different activation pattern in the post-synaptic interneurone than a sensory neurone with branches at the centre of the interneurone's receptive field. In movement control, forcefields (another form of building block) have been suggested, stimuli in different regions of a receptive field cause differential activity patterns in one or more post-synaptic interneurones (Mussa-Ivaldi *et al.*, 1994). The locust scratching

model provides a unique opportunity to investigate whether somatotopic maps encode spatial position of receptors. Is the region within the aVAC of metathoracic ganglion enlarged to accommodate the increased length of the wing? An increased somatotopic map size indicates spatial position is encoded by the area a sensory neurone occupies within the somatotopic map. The use of other stains may solve the problems I encountered using Neurobiotin on sensory neurones on the adult wing. Vertebrate growth is gradual not saltatory (at intervals) and would not require adaptation to such dramatic morphological changes. However, given this difference, the neuronal pathways of locust scratching provide a unique opportunity to investigate whether somatotopic maps do encode spatial location.

In summary, scratching movements do accommodate developmental changes that occur between the 5^{th} instar and adulthood of S. gregaria. Changes in target position caused by body growth may be facilitated by the development of new mechanosensory hairs and different synaptic connections onto postsynaptic interneurones. Wing rotation that occurs in Acrididaea is likely to be accommodated by the intersegmental projections of wing hair afferents, and the convergence of hind wing and fore wing sensory signals onto the same group of post-synaptic interneurones. Thus *automatic adaptation* occurs, where development of the sensory system and wings automatically compensates for growth of the wing surface. Wing hair afferents form a somatotopic representation within the segmental ganglion, suggesting that they may form an internal representation of the wing surface, on to which movements can be mapped.

REFERENCE LIST

Albert,P.J., Zacharuk,R.Y., & Wong,L. (1976) Structure, Innervation, and Distribution of Sensilla on Wings of A Grasshopper. *Canadian Journal of Zoology-Revue Canadienne de Zoologie*, **54**, 1542-1553.

Albrecht,F.O. (1953) The thorax, *The Anatomy of the Migratory Locust*, p. 12-29. The Athlone Press, London.

Altman,J.S. (1975) Changes in Flight Motor Pattern During Development of Australian Plague Locust, Chortoicetes-Terminifera. *Journal of Comparative Physiology*, **97**, 127-142.

Altman,J.S., Anselment,E., & Kutsch,W. (1978) Post-Embryonic Development of An Insect Sensory System - Ingrowth of Axons from Hindwing Sense-Organs in Locusta-Migratoria. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **202**, 497.

Angeli,S., Ceron,F., Scaloni,A., Monti,M., Monteforti,G., Minnocci,A., Petacchi,R., & Pelosi,P. (1999) Purification, structural characterization, cloning and immunocytochemical localization of chemoreception proteins from Schistocerca gregaria. *European Journal of Biochemistry*, **262**, 745-754.

Balderrama,N. & Maldonado,H. (1973) Ontogeny of Behavior in the Praying Mantis. *Journal of Insect Physiology*, **19**, 319-336.

Ball,E. & Young,D. (1974) Structure and Development of Auditory-System in Prothoracic Leg of Cricket Teleogryllus-Commodus (Walker) .2. Postembryonic

Development. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie,* **147**, 313-324.

Barros-pita,J.C. & Maldonado,H. (1970) A Fovea in Praying Mantis Eye .2. Some Morphological Characteristics. *Zeitschrift fur Vergleichende Physiologie,* **67**, 79.

Bassler,U. (1974) Femoral Chordotonal Organ Controlled Reaction in Grasshopper Carausius Morosus - Measurement of Tibial Force in Active and Inactive Animal. *Kybernetik,* **16**, 213-226.

Bassler,U. (1976) Reversal of A Reflex to A Single Motoneuron in Stick Insect Carausius-Morosus. *Biological Cybernetics,* **24**, 47-49.

Bate,C.M. (1976) Embryogenesis of An Insect Nervous-System .1. Map of Thoracic and Abdominal Neuroblasts in Locusta-Migratoria. *Journal of Embryology and Experimental Morphology,* **35**, 107-123.

Bate,C.M. (1978) Development of sensory systems., *Handbook of sensory physiology* (ed. by M.Jacobson), p. 1-53. Springer-Verlag, New York.

Behrends,J. (1935) Ueber die entwicklung des lakunen, ader- und tracheen-systems während der puppenruhe in flügel der mehlmotte, Ephestia kuhniella. *Zeller. Zeit. Morph. Oekol Tiere.*, **30**, 575-596.

Bentley,D. & Toroianraymond,A. (1981) Embryonic and Post-Embryonic Morphogenesis of A Grasshopper Interneuron. *Journal of Comparative Neurology,* **201**, 507-518.

Berkowitz,A. & Laurent,G. (1996a) Central generation of grooming motor patterns and interlimb coordination in locusts. *Journal of Neuroscience,* **16**, 8079-8091.

Berkowitz,A. & Laurent,G. (1996b) Local control of leg movements and motor patterns during grooming in locusts. *Journal of Neuroscience,* **16**, 8067-8078.

Bizzi,E., Mussaivaldi,F.A., & Giszter,S. (1991) Computations Underlying the Execution of Movement - A Biological Perspective. *Science,* **253**, 287-291.

Blaney,W.M. & Chapman,R.F. (1969) The fine structure of the terminal sensilla on the maxillary palps of Schistocerca gregaria (Forskal) (Orthoptera, Acrididae). *Z Zellforsch Mikrosk Anat,* **99**.

Blaney,W.M., Chapman,R.F., & Cook,A.G. (1971) Structure of Terminal Sensilla on Maxillary Palps of Locusta-Migratoria (L), and Changes Associated with Moulting. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie,* **121**, 48.

Bläsing,B. & Cruse,H. (2004a) Mechanisms of stick insect locomotion in a gap-crossing paradigm. *Journal of Comparative Physiology A-Neuroethology Sensory Neural and Behavioral Physiology*, **190**, 173-183.

Bläsing,B. & Cruse,H. (2004b) Stick insect locomotion in a complex environment: climbing over large gaps. *Journal of Experimental Biology*, **207**, 1273-1286.

Burnett,G.F. (1951) Observatopns on the life-history of the red locust, Nomadacris septemfasciata (Serv.) in the solitary phase. *Bulletin of Entomological Research,* **42**, 478-490.

Burrows,M. (1996) *The neurobiology of an insect brain*, Oxford University Press Inc., New York.

Burrows,M. (1987) Parallel Processing of Proprioceptive Signals by Spiking Local Interneurons and Motor Neurons in the Locust. *Journal of Neuroscience,* **7**, 1064-1080.

Burrows,M. (1989) Processing of Mechanosensory Signals in Local Reflex Pathways of the Locust. *Journal of Experimental Biology*, **146**, 209-227.

Burrows,M. & Newland,P.L. (1993) Correlation Between the Receptive-Fields of Locust Interneurons, Their Dendritic Morphology, and the Central Projections of Mechanosensory Neurons. *Journal of Comparative Neurology,* **329**, 412-426.

Burrows,M. & Newland,P.L. (1994) Convergence of Mechanosensory Afferents from Different Classes of Exteroceptors Onto Spiking Local Interneurons in the Locust. *Journal of Neuroscience,* **14**, 3341-3350.

Burrows,M. & Siegler,M.V.S. (1982) Spiking Local Interneurons Mediate Local Reflexes. *Science,* **217**, 650-652.

Burrows,M. & Siegler,M.V.S. (1984) The Morphological Diversity and Receptive-Fields of Spiking Local Interneurons in the Locust Metathoracic Ganglion. *Journal of Comparative Neurology,* **224**, 483-508.

Burrows,M., Watson,A.H.D., & Brunn,D.E. (1989) Physiological and Ultrastructural Characterization of A Central Synaptic Connection Between Identified Motor Neurons in the Locust. *European Journal of Neuroscience,* **1**, 111-126.

Büschges,A., Ramirez,J.-M., Driesang,R., & Pearson,K.G. (1992) Connections of the Forewing Tegulae in the Locust Flight System and Their Modification Following Partial Deafferentation. *Journal of Neurobiology,* **23**, 44-60.

Camhi,J.M. (1971) Flight Orientation in Locusts. *Scientific American,* **225**, 74.

Camhi,J.M. & Hinkle,M. (1972) Attentiveness to Sensory Stimuli - Central Control in Locusts. *Science,* **175**, 550.

Camhi,J.M. & Tom,W. (1978) Escape Behavior of the Cockroach Periplaneta-Americana .1. Turning Response to Wind Puffs. *Journal of Comparative Physiology,* **128**, 193-201.

Camhi,J.M., Tom,W., & Volman,S. (1978) Escape Behavior of the Cockroach Periplaneta-Americana .2. Detection of Natural Predators by Air Displacement. *Journal of Comparative Physiology,* **128**, 203-212.

Chapman,R.F. (2003) Contact chemoreception infeeding by phytophagous insects. *Annual Review of Entomology*, **48**, 455-484.

Chapman,R.F. (1982) Chemoreception - the Significance of Receptor Numbers. *Advances in Insect Physiology*, **16**, 247-356.

Chiba,A., Kamper,G., & Murphey,R.K. (1992) Response Properties of Interneurons of the Cricket Cercal Sensory System Are Conserved in Spite of Changes in Peripheral Receptors During Maturation. *Journal of Experimental Biology,* **164**, 205-226.

Chiba,A., Shepherd,D., & Murphey,R.K. (1988) Synaptic Rearrangement During Postembryonic Development in the Cricket. *Science,* **240**, 901-905.

Cooter,R.J. (1973) Visual and Multimodal Interneurones in Ventral Nerve Cord of Cockroach, Periplaneta-Americana. *Journal of Experimental Biology,* **59**, 675-696.

Cruse,H. (1979) The control of the anterior extreme position of the hind leg of a walking insect, Carauius morosus. *Physiological Entomology,* **4**, 121-124.

Cruse,H. & Schmitz,J. (1983) The Control-System of the Femur-Tibia Joint in the Standing Leg of A Walking Stick Insect Carausius-Morosus. *Journal of Experimental Biology,* **102**, 175-185.

d'Avella,A. & Bizzi,E. (2005) Shared and specific muscle synergies in natural motor behaviors. *Proceedings of the National Academy of Sciences of the United States of America,* **102**, 3076-3081.

d'Avella,A., Saltiel,P., & Bizzi,E. (2003) Combinations of muscle synergies in the construction of a natural motor behavior. *Nature Neuroscience,* **6**, 300-308.

Dagan,D. & Volman,S. (1982) Sensory Basis for Directional Wind Detection in 1St Instar Cockroaches, Periplaneta-Americana. *Journal of Comparative Physiology,* **147**, 471-478.

Dangles,O., Pierre,D., Magal,C., Vannier,F., & Casas,J. (2006) Ontogeny of air-motion sensing in cricket. *Journal of Experimental Biology,* **209**, 4363-4370.

Dean,J. (1992) Is Equilibrium-Point Control All There Is to Coding Movement and do Insects do It, Too. *Behavioral and Brain Sciences,* **15**, 731-732.

Deliagina,T.G., Feldman,A.G., Gelfand,I.M., & Orlovsky,G.N. (1975) On the role of central program and afferent inflow in the control of scratching movements of the cat. *Brain Research,* **100**, 297-313.

Doe,C.Q. & Goodman,C.S. (1985) Early Events in Insect Neurogenesis .1. Development and Segmental Differences in the Pattern of Neuronal Precursor Cells. *Developmental Biology,* **111**, 193-205.

Drew,T. (1993) Motor Cortical Activity During Voluntary Gait Modifications in the Cat .1. Cells Related to the Forelimbs. *Journal of Neurophysiology,* **70**, 179-199.

Drew,T., Andujar,J.E., Lajoie,K., & Yakovenko,S. (2008) Cortical mechanisms involved in visuomotor coordination during precision walking. *Brain Research Reviews,* **57**, 199-211.

Dürr,V. & Krause,A. (2001) The stick insect antenna as a biological paragon for an actively moved tactile probe for obstacle detection. *Climbing and Walking Robots,* 87-95.

Dürr,V. & Matheson,T. (2003) Graded limb targeting in an insect is caused by the shift of a single movement pattern. *Journal of Neurophysiology (Bethesda),* **90**, 1754-1765.

Easter,S.S. (1983) Postnatal Neurogenesis and Changing Connections. *Trends in Neurosciences,* **6**, 53-56.

Edwards,J.S. (1969) Postembryonic development and regeneration of the insect nervous system. *Advances in Insect Physiology,* **6**, 97-137.

Feldman,A.G. (1966) Functional tuning of the nervous system with control of movement or maintenance of a steady posture. *Biophysics,* **11**, 667-675.

Field,L.H. & Burrows,M. (1982) Reflex Effects of the Femoral Chordotonal Organ Upon Leg Motor Neurons of the Locust. *Journal of Experimental Biology,* **101**, 265-285.

Flanagan,J.R. & Wing,A.M. (1997) The role of internal models in motion planning and control: Evidence from grip force adjustments during movements of hand-held loads. *Journal of Neuroscience,* **17**, 1519-1528.

Flash,T. (1987) The Control of Hand Equilibrium Trajectories in Multijoint Arm Movements. *Biological Cybernetics,* **57**, 257-274.

Forman,R.R. (1984) Leg Position Learning by An Insect .1. A Heat Avoidance-Learning Paradigm. *Journal of Neurobiology,* **15**, 127-140.

Fudalewicz-Niemczyk,W. & Rosciszewska,M. (1972) The innervation and sense organs of the wings of Gryllus domesticus L. (Orthoptera). *Acta Biologica Cracoviensia Series Zoologia,* **15**, 35-51.

Fukson,O.I., Berkinblit,M.B., & Feldman,A.G. (1980) The Spinal Frog Takes Into Account the Scheme of Its Body During the the Wiping Reflex. *Science,* **209**, 1261-1263.

Gee,C.E. & Robertson,R.M. (1994) Effects of Maturation on Synaptic Potentials in the Locust Flight System. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology,* **175**, 437-447.

Gelfand,I.M., Orlovsky,G.N., & Shik,M.L. (1988) Locomotion and scratching in tetrapods, *Neural control of rhythmic movements in vertebrates* (ed. by A.H.Cohen, S.Rossignol, & S.Grillner), p. 167. Wiley, New York.

Giszter,S.F., Mcintyre,J., & Bizzi,E. (1989) Kinematic Strategies and Sensorimotor Transformations in the Wiping Movements of Frogs. *Journal of Neurophysiology,* **62**, 750-767.

Gnatzy,W. (1978) Development of Filiform Hairs on Cerci of Gryllus-Bimaculatus Deg (Saltatoria, Gryllidae). *Cell and Tissue Research,* **187**, 1-24.

Gnatzy,W. & Romer,F. (1980) Morphogenesis of Mechanoreceptor and Epidermal-Cells of Crickets During the Last Instar, and Its Relation to Molting-Hormone Level. *Cell and Tissue Research,* **213**, 369-391.

Gray,J.R. & Robertson,R.M. (1994) Activity of the Forewing Stretch-Receptor in Immature and Mature Adult Locusts. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology*, **175**, 425-435.

Hinds,O., Polimeni,J.R., Rajendran,N., Balasubramanian,M., Amunts,K., Zilles,K., Schwartz,E.L., Fischl,B., & Triantafyllou,C. (2009) Locating the functional and anatomical boundaries of human primary visual cortex. *Neuroimage*, **46**, 915-922.

Hiraguchi,T., Yamaguchi,T., & Takahata,M. (2003) Mechanoreceptors involved in the hindwing-evoked escape behaviour in cricket, Gryllus bimaculatus. *Journal of Experimental Biology*, **206**, 523-534.

Holdsworth,R.P. (1942) The wing development of Pteronarcys Proteus Newman (Pteronarcidae Plecoptera). *Journal of Morphology*, **70**, 431-462.

Honegger,H.W., Reif,H., & Muller,W. (1979) Sensory Mechanisms of Eye Cleaning Behavior in the Cricket Gryllus-Campestris. *Journal of Comparative Physiology*, **129**, 247-256.

Hoyle,G. (1978) Distributions of Nerve and Muscle-Fiber Types in Locust Jumping Muscle. *Journal of Experimental Biology*, **73**, 205.

Hustert,R. (1985) Multisegmental Integration and Divergence of Afferent Information from Single Tactile Hairs in A Cricket. *Journal of Experimental Biology*, **118**, 209-227.

Ivanova,T.S. (1947) Development of the wing base in *Calliptamus italicus. (In Russian) Doklady Akademii Nauk, USSR*, **56**, 885-887.

Jan,Y.N., Ghysen,A., Christoph,I., Barbel,S., & Jan,L.Y. (1985) Formation of Neuronal Pathways in the Imaginal Disks of Drosophila-Melanogaster. *Journal of Neuroscience,* **5**, 2453-2464.

Jordan,M.I. & Rumelhart,D.E. (1992) Forward Models - Supervised Learning with A Distal Teacher. *Cognitive Science,* **16**, 307-354.

Joyce,J. Bayes' Theorem. Zalta, E. N. The Stanford Encyclopedia of Philosophy . 2008.

Kamper,G. (1992) Development of Cricket Sensory Hairs - Changes of Dynamic Mechanical-Properties. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology*, **170**, 49-55.

Kandler,K., Clause,A., & Noh,J. (2009) Tonotopic reorganization of developing auditory brainstem circuits. *Nature Neuroscience*, **12**, 711-717.

Kanou,M., Osawa,T., & Shimozawa,T. (1988) Ecdysial Growth of the Filiform Hairs and Sensitivity of the Cercal Sensory System of the Cricket, Gryllus-Bimaculatus. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology*, **162**, 573-579.

Kargo,W.J. & Giszter,S.F. (2008) Individual premotor drive pulses, not time-varying synergies, are the units of adjustment for limb trajectories constructed in spinal cord. *Journal of Neuroscience,* **28**, 2409-2425.

Kawato,M. (1999) Internal models for motor control and trajectory planning. *Current Opinion in Neurobiology*, **9**, 718-727.

Kawato,M. & Wolpert,D. (1998) Internal models for motor control. *Sensory Guidance of Movement*, **218**, 291-307.

Keil,T.A. (1997a) Comparative morphogenesis of sensilla: A review. *International Journal of Insect Morphology & Embryology*, **26**, 151-160.

Keil,T.A. (1997b) Functional morphology of insect mechanoreceptors. *Microscopy Research and Technique*, **39**, 506-531.

Kendall,M.D. (1970) Anatomy of Tarsi of Schistocerca-Gregaria Forskal. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie*, **109**, 112.

Khattar,N. (1972) A description of the adult and nymphal stages of Schizodactylus monstrous (Drury)(Orthoptera). *Journal of Natural History*, **6**, 589-600.

Knyazev,A.N. & Popov,A.V. (1981) Functional organization of the cercal mechanoreceptor system of larvae and adults of the cricket, *Gryllus bimaculatus*. *Zh. Evol. Biokhim Fiziol.*, **17**, 503-511.

Kral,K. (1998) Spatial vision in the course of an insect's life. *Brain Behavior and Evolution*, **52**, 1-6.

Kuhta,P.C. & Smith,J.L. (1990) Scratch Responses in Normal Cats - Hindlimb Kinematics and Muscle Synergies. *Journal of Neurophysiology*, **64**, 1653-1667.

Kutsch,W. (1971) Development of Flight Pattern in Desert Locust, Schistocerca-Gregaria. *Zeitschrift fur Vergleichende Physiologie*, **74**, 156.

Kutsch,W. (1985) Pre-Imaginal Flight Motor Pattern in Locusta. *Journal of Insect Physiology*, **31**, 581-586.

Kutsch,W. & Hemmer,W. (1994) Ontogenic Studies of Flight Initiation in Locusta-Migratoria - Flight-Muscle Activity. *Journal of Insect Physiology,* **40**, 519-525.

Kutsch,W. & Murdock,L.L. (1973) Locust Flight Oscillator - Changes in Motor Output by Certain Pharmacological Agents. *Journal of Insect Physiology*, **19**, 1519-1525.

Lackner,J.R. & Dizio,P. (1994) Rapid Adaptation to Coriolis-Force Perturbations of Arm Trajectory. *Journal of Neurophysiology,* **72**, 299-313.

Land,B.B. & Seeley,T.D. (2004) The grooming invitation dance of the honey bee. *Ethology,* **110**, 1-10.

Landolfa,M.A. & Miller,J.P. (1995) Stimulus-Response Properties of Cricket Cercal Filiform Receptors. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology,* **177**, 749-757.

Lange,W. (1975) Cell number and cell density in the cerebellar cortex of man and some other mammals. *Cell and Tissue Research,* **157**, 115-124.

Lawrence,P.A. & Hayward,P. (1971) Development of Simple Pattern - Spaced Hairs in Oncopeltus-Fasciatus. *Journal of Cell Science,* **8**, 513.

Levine,R.B., Morton,D.B., & Restifo,L.L. (1995) Remodeling of the insect nervous system. *Current Opinion in Neurobiology,* **5**, 28-35.

Levine,R.B. & Weeks,J.C. (1990) Hormonally Mediated Changes in Simple Reflex Circuits During Metamorphosis in Manduca. *Journal of Neurobiology,* **21**, 1022-1036.

Magee,J.C. & Johnston,D. (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science,* **275**, 209-213.

Maldonado,H. & Barros-pita,J.C. (1970) A Fovea in Praying Mantis Eye .1. Estimation of Catching Distance. *Zeitschrift fur Vergleichende Physiologie,* **67**, 58.

Maldonado,H., Rodrigue,E., & Balderra,N. (1974) How Mantids Gain Insight Into New Maximum Catching Distance After Each Ecdysis. *Journal of Insect Physiology,* **20**, 591-603.

Matheson,T. (1997) Hindleg targeting during scratching in the locust. *Journal of Experimental Biology*, **200**, 93-100.

Matheson,T. (1998) Contralateral coordination and retargeting of limb movements during scratching in the locust. *Journal of Experimental Biology*, **201**, 2021-2032.

Matheson,T. (2002) Metathoracic neurons integrating intersegmental sensory information in the locust. *Journal of Comparative Neurology*, **444**, 95-114.

Matheson,T. & Durr,V. (2003) Load compensation in targeted limb movements of an insect. *Journal of Experimental Biology,* **206**, 3175.

Matheson,T. & Field,L.H. (1995) An Elaborate Tension Receptor System Highlights Sensory Complexity in the Hind Leg of the Locust. *Journal of Experimental Biology,* **198**, 1673-1689.

Michel,K. & Petersen,M. (1982) Development of the Tympanal Organ in Larvae of the Migratory Locust (Locusta-Migratoria). *Cell and Tissue Research,* **222**, 667-676.

Michelmore,A.P.G. & Allan,W. (1934) Observations on phases of the Red-winged Locust in Northern Rhodesia. *Bulletin of Entomological Research,* **25**, 101-128.

Mortin,L.I., Keifer,J., & Stein,P.S.G. (1985) 3 Forms of the Scratch Reflex in the Spinal Turtle - Movement Analyses. *Journal of Neurophysiology*, **53**, 1501-1516.

Murphey,R.K., Jacklet,A., & Schuster,L. (1980) A Topographic Map of Sensory Cell Terminal Arborizations in the Cricket Cns - Correlation with Birthday and Position in A Sensory Array. *Journal of Comparative Neurology*, **191**, 53-64.

Mussaivaldi,F.A., Hogan,N., & Bizzi,E. (1985) Neural, Mechanical, and Geometric Factors Subserving Arm Posture in Humans. *Journal of Neuroscience*, **5**, 2732-2743.

Newland,P.L. (1998) Avoidance reflexes mediated by contact chemoreceptors on the legs of locusts. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology*, **183**, 313-324.

Newland,P.L. (1991b) Morphology and Somatotopic Organization of the Central Projections of Afferents from Tactile Hairs on the Hind Leg of the Locust. *Journal of Comparative Neurology*, **312**, 493-508.

Newland,P.L. (1991a) Physiological-Properties of Afferents from Tactile Hairs on the Hindlegs of the Locust. *Journal of Experimental Biology*, **155**, 487-503.

Newland,P.L. & Burrows,M. (1994) Processing of Mechanosensory Information from Gustatory Receptors on A Hind Leg of the Locust. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology*, **174**, 399-410.

Newland,P.L., Rogers,S.M., Gaaboub,I., & Matheson,T. (2000a) Parallel somatotopic maps of gustatory and mechanosensory neurons in the central nervous system of an insect. *Journal of Comparative Neurology*, **425**, 82-96.

Newland,P.L., Rogers,S.M., Matheson,T., & Gaaboub,I. (2000b) Overlapping chemosensory and mechanosensory maps in the thoracic ganglia of the locust. *European Journal of Neuroscience,* **12**, 498.

Ochieng,S.A., Hallberg,E., & Hansson,B.S. (1998) Fine structure and distribution of antennal sensilla of the desert locust, Schistocerca gregaria (Orthoptera : Acrididae). *Cell and Tissue Research,* **291**, 525-536.

Page,K.L. & Matheson,T. (2009) Functional Recovery of Aimed Scratching Movements after a Graded Proprioceptive Manipulation. *Journal of Neuroscience,* **29**, 3897-3907.

Page,K.L. & Matheson,T. (2004) Wing hair sensilla underlying aimed hindleg scratching of the locust. *Journal of Experimental Biology,* **207**, 2691-2703.

Palka,J. & Edwards,J.S. (1974) Cerci and Abdominal Giant Fibers of House Cricket, Acheta-Domesticus .2. Regeneration and Effects of Chronic Deprivation. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **185**, 105.

Pearson,K.G. (1993) Common Principles of Motor Control in Vertebrates and Invertebrates. *Annual Review of Neuroscience,* **16**, 265-297.

Pearson,K.G., Reye,D.N., Parsons,D.W., & Bicker,G. (1985) Flight-Initiating Interneurons in the Locust. *Journal of Neurophysiology*, **53**, 910-925.

Pelisson,D., Prablanc,C., Goodale,M.A., & Jeannerod,M. (1986) Visual Control of Reaching Movements Without Vision of the Limb .2. Evidence of Fast Unconscious Processes Correcting the Trajectory of the Hand to the Final Position of A Double-Step Stimulus. *Experimental Brain Research,* **62**, 303-311.

Pflüger,H.J. (1980) The Function of Hair Sensilla on the Locusts Leg - the Role of Tibial Hairs. *Journal of Experimental Biology,* **87**, 163-175.

Pflüger,H.J., Braünig,P., & Hustert,R. (1988) The Organization of Mechanosensory Neuropiles in Locust Thoracic Ganglia. *Philosophical Transactions of the Royal Society of London Series B - Biological Sciences,* **321**, 1.

Poppele,R. & Bosco,G. (2003) Sophisticated spinal contributions to motor control. *Trends in Neurosciences,* **26**, 269-276.

Pryde,K.M., Roy,E.A., & Patla,A.E. (1997) Age-related trends in locomotr ability and obstacle avoidance. *Human Movement Science,* **16**, 507-516.

Queathem,E.J. & Full,R.J. (1995) Variation in Jump Force Production Within An Instar of the Grasshopper Schistocerca-Americana. *Journal of Zoology,* **235**, 605-620.

Rall,W. (1969) Time Constants and Electrotonic Length of Membrane Cylinders and Neurons. *Biophysical Journal,* **9**, 1483.

Rall,W. & Rinzel,J. (1973) Branch Input Resistance and Steady Attenuation for Input to One Branch of A Dendritic Neuron Model. *Biophysical Journal,* **13**, 648-688.

Rall,W.I.L.F. (1962) Electro-physiology of a dendritic neuron model. *Biophysical journal Jour,* **2**, 145-167.

Richardson,A.G., Slotine,J.J.E., Bizzi,E., & Tresch,M.C. (2005) Intrinsic musculoskeletal properties stabilize wiping movements in the spinalized frog. *Journal of Neuroscience,* **25**, 3181-3191.

Ridgel,A.L., Frazier,S.F., & Zill,S.N. (2003) Post-embryonic development of cuticular caps of campaniform sensilla of the cockroach leg: potential implications in scaling force detection. *Arthropod Structure & Development,* **32**, 167-173.

Robertson,G.A., Mortin,L.I., Keifer,J., & Stein,P.S.G. (1985) 3 Forms of the Scratch Reflex in the Spinal Turtle - Central Generation of Motor Patterns. *Journal of Neurophysiology,* **53**, 1517-1534.

Robertson,R.M. & Pearson,K.G. (1983) Interneurons in the Flight System of the Locust - Distribution, Connections, and Resetting Properties. *Journal of Comparative Neurology,* **215**, 33-50.

Rodriguez,E. & Maldonado,H. (1974) Allometric Growth in Praying Mantis Stagmatoptera-Biocellata. *Journal of Zoology,* **173**, 487-503.

Rogers,S.M. & Newland,P.L. (2000a) Behavioural and physiological responses to chemosensory stimulation of the leg of the locust. Schistocerca gregaria. *European Journal of Neuroscience,* **12**, 497.

Rogers,S.M. & Newland,P.L. (2000b) Local movements evoked by chemical stimulation of the hind leg in the locust Schistocerca gregaria. *Journal of Experimental Biology,* **203**, 423-433.

Romer,H. (1983) Tonotopic Organization of the Auditory Neuropil in the Bushcricket Tettigonia-Viridissima. *Nature,* **306**, 60-62.

Roonwal,M.L. (1940) Preliminary note on some directional changes among locusts and other acrididae, and the importance of the third instar. *Indian Journal of Entomology,* **2**, 137-144.

Rose,U. (2004) Morphological and functional maturation of a skeletal muscle regulated by juvenile hormone. *Journal of Experimental Biology*, **207**, 483-495.

Sabes,P.N. (2000) The planning and control of reaching movements. *Current Opinion in Neurobiology*, **10**, 740-746.

Sbrenna,G. (1971) Postembryonic growth of the ventral nerve cord in Schistocerca gregaria (Orthoptera: Acrididae). *Bollettino di Zoologia*, **38**, 49-74.

Schafer,R. (1973) Postembryonic Development in Antenna of Cockroach, Leucophaea-Maderae - Growth, Regeneration, and Development of Adult Pattern of Sense Organs. *Journal of Experimental Zoology*, **183**, 353-363.

Schenau,G.J.V., Vansoest,A.J., Gabreels,F.J.M., & Horstink,M.W.I.M. (1995) The Control of Multijoint Movements Relies on Detailed Internal Representations. *Human Movement Science*, **14**, 511-538.

Schenk,O. (1903) Die antennalen Hautsinnesorgane elniger Lepidopteren und Hymenopteren. *Zoologische Jahrbuch far Anatomie*, **17**, 573-618.

Schwindt,P. & Crill,W. (1995) Persistent sodium conductance in apical dendrite of neocortical neurons aids transmission of synaptic current. *Society for Neuroscience Abstracts*, **21**, 585.

Sehnal,F., Svacha,P., & Zrzavy,J. (1996) Evolution of insect metamorphosis, *Metamorphosis* (ed. by L.I.Gilbert, J.R.Tata, & B.G.Atkinson), p. 3-58. Academic Press Inc., San Diego.

Seymour,K.J. The neural control of oviposition in the locust Scistocerca gregaria. 1990. England, Univerity of Cambridge.

Shadmehr,R. & Mussaivaldi,F.A. (1994) Adaptive Representation of Dynamics During Learning of A Motor Task. *Journal of Neuroscience*, **14**, 3208-3224.

Shepherd,D. & Bate,C.M. (1990) Spatial and Temporal Patterns of Neurogenesis in the Embryo of the Locust (Schistocerca-Gregaria). *Development*, **108**, 83-96.

Shepherd,D. & Smith,S.A. (1996) Central projections of persistant larval sensory neurones prefigure adult sensory pathways in the CNS of Drosophila. *Development*, **122**, 2375-2384.

Sherrington,C.S. (1906) Observations on the scratch-reflex in the spinal dog. *Journal of Physiology*, **34**, 1-50.

Sherrington,C.S. (1910) Notes on the scratch-reflex of the cat. *Experimental Physiology*, **3**, 213-220.

Siegler,M.V.S. & Burrows,M. (1983) Spiking Local Interneurons As Primary Integrators of Mechanosensory Information in the Locust. *Journal of Neurophysiology*, **50**, 1281-1295.

Siegler,M.V.S. & Burrows,M. (1984) The Morphology of 2 Groups of Spiking Local Interneurons in the Metathoracic Ganglion of the Locust. *Journal of Comparative Neurology*, **224**, 463-482.

Siegler,M.V.S. & Pousman,C.A. (1990a) Distribution of Motor Neurons Into Anatomical Groups in the Grasshopper Metathoracic Ganglion. *Journal of Comparative Neurology,* **297**, 313-327.

Siegler,M.V.S. & Pousman,C.A. (1990b) Motor Neurons of Grasshopper Metathoracic Ganglion Occur in Stereotypic Anatomical Groups. *Journal of Comparative Neurology,* **297**, 298-312.

Slifer,E.H. (1970) Structure of Arthropod Chemoreceptors. *Annual Review of Entomology,* **15**, 121-&.

Slifer,E.H. (1955) The distribution of permeable sensory pegs on the body of the grasshopper (Orthoptern: Acridldae). *Entomological News,* **66**, 1-5.

Soechting,J.F. & Lacquaniti,F. (1983) Modification of Trajectory of A Pointing Movement in Response to A Change in Target Location. *Journal of Neurophysiology,* **49**, 548-564.

Stein,P.S.G. & Johnstone,K.M. (1986) Motor Patterns During Rostral Scratching and Forward Swimming in the Turtle Pseudemys-Scripta-Elegans. *Society for Neuroscience Abstracts,* **12**, 790.

Strausfield,N.J. (1971) Organization of Insect Visual System (Light Microscopy) .1. Projections and Arrangements of Neurons in Lamina Ganglionaris of Diptera. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie,* **121**, 377.

Svidersky,V.L. (1969) Receptors on the forehead of the locust *Locusta migratoria* in ontogenesis. *Journal of Evolutionary Biochemistry and Physiology,* **5**, 482-490.

Talavage,T.M., Ledden,P.J., Benson,R.R., Rosen,B.R., & Melcher,J.R. (2000) Frequency-dependent responses exhibited by multiple regions in human auditory cortex. *Hearing Research,* **150**, 225-244.

Thompson,K.J. (1986) Oviposition Digging in the Grasshopper .2. Descending Neural Control. *Journal of Experimental Biology,* **122**, 413-425.

Thompson,K.J. & Roosevelt,J.L. (1998) Comparison of neural elements in sexually dimorphic segments of the grasshopper, Schistocerca americana. *Journal of Comparative Neurology,* **394**, 14-28.

Tissot,M. & Stocker,R.F. (2000) Metamorphosis in Drosophila and other insects: the fate of neurons throughout the stages. *Progress in Neurobiology*, **62**, 89-111.

Tousson,E. & Hustert,R. (2006) External sensilla of the locust abdomen provide the central nervous system with an interganglionic network. *Cell and Tissue Research,* **325**, 151-162.

Tower,W.L. (1903) The origin and development of the wings of Coleoptera. *Z. Jahrb. Abth. f. u. Ontogenie,* **17**, 517-572.

Tresch,M.C., Saltiel,P., & Bizzi,E. (1999) The construction of movement by the spinal cord. *Nature Neuroscience,* **2**, 162-167.

Truman,J.W. (1990) Metamorphosis of the Central-Nervous-System of Drosophila. *Journal of Neurobiology,* **21**, 1072-1084.

Truman,J.W. (1996) Steroid receptors and nervous system metamorphosis in insects. *Developmental Neuroscience,* **18**, 87-101.

Truman,J.W. & Booker,R. (1986) Adult-Specific Neurons in the Nervous-System of the Moth, Manduca-Sexta - Selective Chemical Ablation Using Hydroxyurea. *Journal of Neurobiology,* **17**, 613-625.

Truman,J.W. & Reiss,S.E. (1995) Neuromuscular Metamorphosis in the Moth Manduca-Sexta - Hormonal-Regulation of Synapse Loss and Remodeling. *Journal of Neuroscience,* **15**, 4815-4826.

Truman,J.W., Taylor,B.J., & Awad,T.A. (1993) Formation of the adult nervous system., *The development of Drosophila melanogaster*. (ed. by M.Bate & A.Martinez Arias), p. 1245-1275. Cold Spring Harbour Laboratory Press, Cold Spring Harbour.

Tyrer,N.M. & Altman,J.S. (1974) Motor and Sensory Flight Neurons in A Locust Demonstrated Using Cobalt Chloride. *Journal of Comparative Neurology,* **157**, 117-138.

Udin,S.B. & Fawcett,J.W. (1988) Formation of Topographic Maps. *Annual Review of Neuroscience,* **11**, 289-327.

Urbach,R., Technau,G.M., & Breidbach,O. (2003) Spatial and temporal pattern of neuroblasts, proliferation, and Engrailed expression during early brain development in Tenebrio molitor L. (Coleoptera). *Arthropod Structure & Development*, **32**, 125-140.

Uvarov,B. (1977) *Grasshoppers and Locusts*, Centre for Overseas Pest Research, London.

Van Sonderen,J.F., Gielen,C.C.A.M., & Ier Van Der,G.O.N. (1989) Motor Programs for Goal-Directed Movements Are Continuously Adjusted According to Changes in Target Location. *Experimental Brain Research,* **78**, 139-146.

Walker,E.D. & Archer,W.E. (1988) Sequential Organization of Grooming Behaviors of the Mosquito Aedes-Triseriatus. *Journal of Insect Behavior,* **1**, 97-110.

Watson,J.T., Ritzmann,R.E., & Pollack,A.J. (2002a) Control of climbing behavior in the cockroach, Blaberus discoidalis. II. Motor activities associated with joint movement. *Journal of Comparative Physiology A-Neuroethology Sensory Neural and Behavioral Physiology,* **188**, 55-69.

Watson,J.T., Ritzmann,R.E., Zill,S.N., & Pollack,A.J. (2002b) Control of obstacle climbing in the cockroach, Blaberus discoidalis. I. Kinematics. *Journal of Comparative Physiology A-Neuroethology Sensory Neural and Behavioral Physiology,* **188**, 39-53.

Weeks,J.C. & Levine,R.B. (1990) Postembryonic Neuronal Plasticity and Its Hormonal-Control During Insect Metamorphosis. *Annual Review of Neuroscience,* **13**, 183-194.

Widajewicz,W., Kably,B., & Drew,T. (1994) Motor Cortical Activity During Voluntary Gait Modifications in the Cat .2. Cells Related to the Hindlimbs. *Journal of Neurophysiology,* **72**, 2070-2089.

Williams,S.R. & Stuart,G.J. (2000) Site independence of EPSP time course is mediated by dendritic I-h in neocortical pyramidal neurons. *Journal of Neurophysiology,* **83**, 3177-3182.

Wolf,H. (1990) Activity Patterns of Inhibitory Motoneurons and Their Impact on Leg Movement in Tethered Walking Locusts. *Journal of Experimental Biology,* **152**, 281-304.

Wolf,H. & Buschges,A. (1997) Plasticity of synaptic connections in sensory-motor pathways of the adult locust flight system. *Journal of Neurophysiology,* **78**, 1276-1284.

Wolpert,D.M., Ghahramani,Z., & Jordan,M.I. (1995) Are Arm Trajectories Planned in Kinematic Or Dynamic Coordinates - An Adaptation Study. *Experimental Brain Research,* **103**, 460-470.

Woolsey,T.A. & Vanderlo,H. (1970) Structural Organization of Layer-Iv in Somatosensory Region (Si) of Mouse Cerebral Cortex . Description of A Cortical Field Composed of Discrete Cytoarchitectonic Units. *Brain Research,* **17**, 205-&.

Yamamoto-Kihara,M., Hata,T., Breuer,M., & Tanaka,S. (2004) Effect of [His7]-corazonin on the number of antennal sensilla in Locusta migratoria. *Physiological Entomology,* **29**, 73-77.

Zhou,S.H., Zhang,J., Zhang,S.G., & Zhang,L. (2008) Expression of chemosensory proteins in hairs on wings of Locusta migratoria (Orthoptera : Acrididae). *Journal of Applied Entomology,* **132**, 439-450.