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Sensory Morphology, Investment and Plasticity in the Black Soldier Fly (*Hermetia illucens*)

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Abstract

The importance of sensory traits, such as the development of the antennae and compound eyes, can be seen in the wide range of reproductive life history strategies employed by insects. Despite this, few studies have investigated the scaling relationships of sensory traits and body size which may give insight into the selective pressures driving this diversity. The commercially important species *Hermetia illucens*, or black soldier fly, was used to investigate the following: (1) the ultrastructural morphology of the antennae in *H. illucens* through the use of scanning electron microscopy; (2) the allometry of sensory traits and a dispersal trait (wing size) in the black soldier fly and a comparison of the allometries of the sexes and between two separate populations, (3) the effects of larval density on developmental phenotypic plasticity of sensory traits. I discovered a wide range of allometries that shed light on potential strategies utilised by the species to improve fitness. Some traits also showed sexual dimorphism and were explained by the unusual lek-like mating behaviour exhibited by adult males. Finally, I found that lower larval densities resulted in larger antennae suggesting that this species predicts future environmental conditions based on larval rearing conditions and adapt their adult morphology to maximise fitness.

Preface/Acknowledgements

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Chapter 1: Introduction

2 THE ROLE OF SENSORY STRUCTURES IN INSECT REPRODUCTIVE BEHAVIOUR

2.1 SENSORY TRAITS IN INSECTS

The insects are an incredibly diverse taxa, host to over half of the worlds described animal species and estimated to contain a total of 5.5 million species (Stork, 1988, 2018; Stork, McBroom, Gely, & Hamilton, 2015). One possible reason for the successful radiation of the insects is the development of sensory traits enabling insects to utilise information from their surroundings.

The importance of sensory traits, such as the development of the antennae and compound eyes, can be seen in the wide range of reproductive life history strategies employed by the insects. For example, the use of pheromones in finding a mate (e.g. female moths releasing volatile odours that attract males over great distances (Cardé, 2016)), visual cues to attract mates (e.g. courtship flashing in fireflies (Woods Jr., Hendrickson, Mason, & Lewis, 2007)), courtship songs to attract a mate (e.g. male mosquitos serenading nearby females (Knight, 2017)) to detecting chemical or thermal cues to find suitable oviposition sites (e.g. Odonata species detecting oviposition sites with conspecific larvae present (Frati, Piersanti, Rebora, & Salerno, 2016), *Merimna atrata* detecting infrared radiation from forest fires to find mating and ovipositioning sites (Schneider & Schmitz, 2014)).

Whilst there is a wide array of sensory traits utilised by insects, I will focus on those used for reproductive behaviour. Specifically, the traits that deal with olfaction, tactile and auditory, and visual sensory information.

Olfaction, a form of chemoreception, is a physiological trait which enables the detection of volatile and soluble chemical stimuli in the surrounding environment. Olfaction allows for intraspecific communication, the location of oviposition sites, avoidance of danger and finding of quality food sources (Dahanukar, Hallem, & Carlson, 2005; Städler, 1984). The smallest functional unit of an olfactory morphological trait is a chemoreceptive sensillum. In many orders of insects, these sensilla are found in higher densities on the antennae which have become specialised sensory organs (Colgoni & Vamosi, 2006; D. Schneider, 1964).

Mechanoreception is a physiological sensory trait that is found across taxa. Being able to gain information from mechanical stimuli provides a variety of functions for an individual including; position in relation to themselves (proprioception) and their environment, the position or approach of a predator and the location and/or condition of a mate (Lakes-Harlan, Stölting, & Stumpner, 1999; Skals, Anderson, Kannevorff, Löfstedt, & Surlykke, 2005; Stumpner & Helversen, 2001). The smallest functional unit of an insect auditory system are the mechanoreceptors which translate the mechanical energy of sound waves into an electrical impulse. They are found across the insect body plan, including the antennae. Most arthropod taxa have mechanoreceptors enabling the detection of vibrations from the surrounding substrate, yet, the ability to detect pressure waves of sound is unique to insects and has arisen independently across different orders (Stumpner & Helversen, 2001).

At their simplest, visual sensory traits allow for the detection of light from dark. At their most complex, visual traits allow for the detection of specific wavelengths of light, perception of images and detection of movement. This is possibly due to photosensitive pigments which may occur within ocelli (single pigment pits with a simple lens), or be incorporated into an ommatidium, the smallest functional unit of the compound eye. The visual traits of insects are particularly diverse suggesting great potential for evolutionary adaptation (Briscoe & Chittka, 2001). Insects have developed vision that enables incredibly complex feats such as the tracking of a moving object whilst in flight (Olberg, 2012), the identification of specific flowers based on colour (Chittka & Menzel, 1992) and some insects have even mimicked the visual characteristics of flowers to predate on pollinators to the extent that they are more attractive than the original flowers (O'Hanlon, Holwell, & Herberstein, 2014).

2.2 SELECTION PRESSURES ON SENSORY TRAITS

Selective pressures are abstract forces that act upon natural variation within a population to maximise fitness. Whilst fitness can have several subtly different meanings (Orr, 2009), I will work with Schoener's definition that fitness is the probability that offspring will pass on genes to future generations (Schoener, 2011). There can be more than one type of selection pressure acting upon a population, such as natural selection and sexual selection. It was Darwin who first suggested that natural selection could play a role in the evolution of a species by favouring individuals that have any traits that give them an advantage in the "struggle for life" (Darwin, 1859). It was also Darwin who proposed that sexual selection, the preference

of one sex for a trait in the other, may be responsible for some of the seemingly bizarre traits that appear in the animal kingdom that would appear at odds to being adapted for survival in an environment, such as the “gorgeous plumage and strange antics” of male birds to attract the attention of the female (Darwin, 1859). Whilst there was criticism and rejection of the concept of sexual selection in the early 20th century, interest grew with the publication of the second edition of Ronald Fisher’s work, *The Genetical Theory of Natural Selection* in 1958 (Andersson, 1994; Fisher, 1930).

An example of natural selection acting upon an invertebrate sensory system can be seen in the amphipod, *Gammarus minus*. Populations of *G. minus* that live in springs with high abundances of fish predators have significantly larger eye size than populations that have low abundances of fish predators (Glazier & Deptola, 2011). The larger eyes increase the ability of the amphipod to identify and evade an approaching predator increasing its fitness. Over several generations the phenotype of larger eyes would be selected for with individuals showing the smaller eye phenotype being predated upon. Given the benefits of having larger eyes in avoiding predation, we might ask why large eyes are not ubiquitous in these amphipod populations.

Investigations into fly photoreceptors suggest that there is a law of diminishing returns that penalises increased sensory capacity with increased energetic costs) (Niven, Anderson, & Laughlin, 2007). For example, the retina of the blowfly, *Lucilia cuprina*, accounts for 10% of its oxygen consumption and 8% of its resting metabolic rate (RMR) (Howard, Blakeslee, & Laughlin, 1987; Niven & Laughlin, 2008). Thus, there is another, competing, selective pressure occurring on sensory systems – energy limitation (Niven & Laughlin, 2008) – which likely leads to a developmental trade-off.

As sensory systems are energetically costly to maintain and utilise, I would expect to see trade-offs between investment in sensory structures and other traits for example, body size.

In some cases, there may be more than one selective pressure interacting with sensory traits. An example of two competing selective pressures interacting with two individual insect sensory traits can be seen in male noctuid moths (Skals et al., 2005). Males use their antenna to detect odours of females and also use a pair of tympanal ears that allow them to detect the echolocation of predatory bats. Whilst these are two separate sensory systems, the intensity of each stimulus affects their behaviour. When males are exposed to high concentrations of female pheromone, they effectively become deaf to the sound of a bat's echolocation until a certain intensity is reached and an avoidance behaviour is elicited. Despite there being two sensory systems involved, the signals are both processed by the central nervous system which then determines a response based on the intensity of the two incongruous signals (Skals et al., 2005). There is a trade-off between the opportunity to mate and predation; if the intensity of the odour (i.e. proximity to female) is great enough, the risk of predation may be outweighed by the opportunity to mate. In contrast, if the intensity of the bat's echolocation (i.e. proximity to predator) is great, then the risk of predation outweighs the potential to (Skals et al., 2005). It appears that the threshold at which one sensory system gains primacy over the other is likely to be the equilibrium point between the two selective pressures.

3 ANTENNAE IN INSECTS

In insects, the role of the antennae is primarily sensory and may contain sensilla for the purposes of chemoreception, olfaction, gustation, hygroreception, thermoreception and mechanoreception (D. Schneider, 1964). The scape, pedicel and flagellomeres are the basic components of the antennae and protrude from the head of the insect. There are a wide range of antennal morphologies in insects with cockroaches exhibiting long filiform antennae that are utilised as tactile feelers (Okada & Toh, 2006) and moths with feathered antennae that increase the surface area available for detecting chemical scents (Steinbrecht, 1987). Morphological variation among antennae of different insect groups are likely to be a reflection of environmental constraints rather than chemical constraints (Hansson & Stensmyr, 2011). For instance, the large, feathered antennae of a moth (Lepidoptera) would create aerodynamic issues for an agile, predator like the dragonfly (Odonata).

The sensilla are the innervated, sensory ultrastructures which are found on most insect antennae. These ultrastructures are used for sensory detection and are defined as a specialised area containing formative cells, sensory nerve cells and occasionally auxiliary cells (Hallberg & Hansson, 1999; D. Schneider, 1964). Whilst it is not possible to state the function of a sensillum without experimental evidence (D. Schneider, 1964), it is sometimes possible to examine the anatomy of the sensillum to give an indication of what role they might play.

3.1 CHEMORECEPTION IN ANTENNA

In insects, chemoreceptors are found in many places on the body but are generally concentrated in areas such as the antennae, maxillary palps, and ovipositor (Hallem, Dahanukar, & Carlson, 2006). Chemoreceptors can be used for gustation and olfaction, with antennal chemoreceptors primarily being utilised for olfaction. A chemoreceptive sensillum is comprised of three parts (1) a porous cuticle, (2) sensory neurons and (3) a sheath cell (Zacharuk, 1980). Chemosensilla are traditionally characterised by their morphology with the presence of pores on the surface of the sensillum indicating a possible role in chemoreception (Dahanukar et al., 2005; Zacharuk, 1980). Uniporous sensilla (sensilla with one pore) are usually, but not always, associated with gustation and multiporous sensilla (sensilla with multiple pores) are associated with olfaction (Zacharuk, 1980). Multiporous sensilla can have pores that vary in size and number and allow chemical molecules in the air to come in contact with dendrites via interacting with odorant binding proteins, ultimately triggering an electrical signal to the insect central nervous system in response to specific chemical compounds (Dahanukar et al., 2005; Hallem et al., 2006; D. Schneider, 1964; Zacharuk, 1980).

3.2 MECHANORECEPTION IN ANTENNA

Mechanoreception allows an individual to be aware of sensations such as distortion of the body, gravity, pressure, touch, vibrations and sound through the mechanical stimulation of a receptor (Keil, 1997). Mechanoreceptors can be categorised into four groups (1) bristles (micro- and macrochaetae), (2) trichobothria (“listening hairs”), (3) campaniform sensilla and (4) scolopidia (Keil, 1997). Bristles are often found around

the joints of segments in the antennae and provide information on relative position in relation to the body (known as proprioception) but can also be used to indicate touch (Keil, 1997). Trichobothria may be found on the antennae of insects and utilised to determine movement and vibrations in air currents (Keil, 1997). Campaniform sensilla measure deformation of the cuticle (via indentation) which can provide information for regulation of movement and have been found around the pedicel of antennae in Diptera (Gnatzy, Grünert, & Bender, 1987; Keil, 1997). Scolopidia, which are found within the body, also detect passive mechanical deformations (via stretching) but have frequently been adapted for hearing for example, in the Johnston's organ found on some insect antennae (Jeram & Pabst, 1996; Keil, 1997).

4 COMPOUND EYES IN INSECTS

Compound eyes are comprised of multiple units called ommatidia which each function as an individual photoreceptor. The ommatidia in insects are all made up of the same functional components: a cornea, crystalline cone, rhabdom and pigmented cells of varying complexity (Goldsmith & Philpott, 1957; Nilsson, 1989). The structure and layout of ommatidia gives rise to the different types of compound eyes found in insects. The compound eyes of “primitive” insects have an apposition structure, the structural layout of which has led to the comment that “evolution seems to be fighting a desperate battle to improve a basically disastrous design” (Nilsson, 1989). This perhaps indicates why there has been selection for rearranged designs in compound eyes such as optical superposition, the open rhabdom and neural superposition (Land, 1997; Nilsson, 1989).

The density and size of ommatidia is not always uniform across the eye, with some species having certain areas of the eye with smaller more densely arranged ommatidia (Land, 1989). These acute zones allow an insect to have a small region of improved resolution in a location where it will be most useful. For example in the robber fly, a frontally flattened patch of ommatidia are significantly larger in diameter, have a narrower interommatidial angle (0.28°) and longer crystalline cones all of which lead to greatly increased spatial acuity and sensitivity in a very small focal area (Warrant, 2017). Having small zones with higher performance helps to minimise energetic costs and reduce the space that would otherwise be required (Land, 1989).

5 ALLOMETRY

5.1 HISTORY OF ALLOMETRY

Allometry is the scaling relationship between the size of a part and the size of the whole (Lane, 1981). Early allometric studies focused on the change in size of individual organs in relation to overall body size (Thompson, 1917). The mathematical expression $y = bx^a$, a power function, was first proposed in 1924 by the biologist Sir Julian Huxley (Julian S. Huxley, 1924). The function relates the size of a feature, y , to the overall body size of the organism, x , with the a value being of particular interest to biologists as a measurement of the growth ratio of y in relation to x . b is the intercept on the Y-axis and has been the subject of debate as to its biological relevance (Lane, 1981). The term allometry was coined later in 1932 (J. Huxley, 1932) and as the

technique was applied to a wide variety of fields the vocabulary used became increasingly diverse (J. S. Huxley & Teissier, 1936).

Despite several attempts at clarifying the terminology (J. S. Huxley & Teissier, 1936; Needham & Lerner, 1940; Julian S. Huxley, Needham, & Lerner, 1941), there has been a confusing array of terminology used in the study of allometry even today. For the purposes of this discussion, I will be working with Gould's (1966) definition of allometry, outlined below, as it provides a good synthesis of prior work.

Allometry is defined as the study of proportion changes correlated with variation in size of either the total organism or the part under consideration. The variates may be morphological, physiological or chemical; the size differences may arise in ontogeny, phylogeny or the static comparison of related forms differing in size; the term is not confined to any one form of mathematical expression, such as the power function (Gould, 1966)

I will be using the power function for my investigation of insect sensory traits due to its wide spread use which enables easy comparisons with other studies, mathematical simplicity and ease of communicating biological relevance. However, as Gould hints in his definition, there are other techniques available for investigating allometry.

Multivariate approaches and morphometrics offer a diverse toolbox for investigating allometric relationships ((Adams, Rohlf, & Slice, 2004; Klingenberg, 1996, 2016; Rohlf & Marcus, 1993)).

5.2 ISOMETRY, NEGATIVE AND POSITIVE ALLOMETRY

In traditional allometric studies, biologists are interested in the slope of the line (a) and the y-intercept (b). The slope of the line dictates whether there is positive allometry, negative allometry or isometry as illustrated in Figure 5.1.

Figure 5.1: Example of a (A) positive allometric slope, (B) isometry, (C) negative allometry. (D) & (E) show identical positive allometries but with a different y-intercept. Isometry has a slope of 1:1, i.e. as body size increases, the trait of interest increases proportionally. Positive allometry would have a slope of greater than one, i.e. as the body size increases, the trait of interest would increase at a greater rate. Negative allometry would have a slope of less than one, i.e. as body size increases, the trait of interest decreases. Gould states that the y-intercept should only be used as an independent taxonomic indicator when slope is equal between the relationships being considered (Gould, 1966). This is particularly true for insects which frequently have a high degree of plasticity in body and trait sizes. (Adapted from Shingleton, 2010)

5.3 ALLOMETRY OF GROWTH AND ALLOMETRY OF SIZE

Traditional allometry can be broadly grouped into two main categories (Lane, 1981; Teissier, 1960), (1) allometry of growth (ontogenetic allometry); the change of size of a structure as an organism grows and (2) allometry of size (static allometry); the change in size of a structure between organisms in a population, between populations or, between species or higher taxonomic groups. In insects, allometry of growth is particularly well suited to hemimetabolous insects which have external features that

change in size with each moult. In comparison, this is less useful for holometabolous insects which undergo dramatic changes in body plan between the larval and adult stages via pupation. For this reason, I will be focussing on allometry of size to study the adult life stage of the holometabolous black soldier fly, *Hermetia illucens*.

5.4 INSECTS AND ALLOMETRY

In insects, allometry has been used to investigate a variety of questions, including, sexual selection (Eberhard, 2002b), artificial selection (Wilkinson, 1993), weaponry (Eberhard, 2002a; Kelly, 2005), larval movement (Berrigan & Pepin, 1995) and physical constraints on eggs (Berrigan, 1991; Legay, 1977).

An example of the use of allometry of size in insects can be seen in studies of the influence of sexual selection. It has been put forward that sexual traits commonly exhibit a positive allometric slope for example a trait that is under strong sexual selection will increase disproportionately in contrast to body size such as male forceps in earwigs or eye-spans in the male stalk-eyed fly.

However, the allometric investigation of sexually selected traits has been criticised for a bias towards “bizarre traits” (Bonduriansky, 2007) and a disinterest in sexual traits that do not show extreme exaggeration. This sampling bias may account for the supposed universality of positive allometry in sexual traits (Bonduriansky, 2007). In a review of the literature, Bonduriansky found that there is a wide plethora of different allometric slopes (positive, negative and isometric) associated with sexual traits which contradicts the assumed universality of positive allometry for sexual traits. It is

therefore pertinent to examine allometry of morphological traits in combination with behavioural observations.

For example, male genitalia in arthropods are a type of sexual trait that departs from positive allometry, and hence demonstrates the diversity of allometric patterns that sexual traits can show. Male genitalia are considered to be sexually selected and show a wide range of divergence between species (hence their frequent use in taxonomic keys) (Hosken, Minder, & Ward, 2005). Despite this, a negative allometric slope is often seen in male invertebrate genitalia which has been attributed to a stabilising selection. This has led to the “one-size-fits-all” hypothesis (Eberhard et al., 1998).

6 PREDICTIONS ON ALLOMETRY OF SENSORY TRAITS

In insects we might expect to find that the allometry of sensory traits would reflect the life history of the individual with positive or negative allometries suggesting that selective pressures are acting upon those traits. For instance, if the female of a species needs to search for suitable oviposition sites using olfaction, I would expect there to be positive allometry associated with antennae length. If the males of the species are not actively searching for females, instead guarding territories and waiting for females to come to them in a lek-like mating system, then we might expect to see isometry or perhaps negative allometry for olfactory sensory traits and a positive allometry for traits that maximise success of competing against other males.

The only paper investigating allometries of sensory traits and the pressures acting upon them is a study of two species of Coleoptera, *Calosobruchus chinensis* and *C.*

maculatus. They found that the width of antennae when compared to the length of antennae in *C. chinensis* males were positively allometric (Colgoni & Vamosi, 2006). This reflected the importance of detecting female pheromones in the mating success of the males with the positive allometry suggesting that there is sexual selection occurring upon the males to increase the available surface area of the antennae for detecting female pheromones (Colgoni & Vamosi, 2006).

7 PHENOTYPIC PLASTICITY

West-Eberhard (1989) defines phenotypic plasticity as “the ability of a single genotype to produce more than one alternative form of morphology, physiological state, and/or behavior in response to environmental conditions” (West-Eberhard, 1989). Phenotypic plasticity provides adaptability to a range of environmental conditions which allows organisms to maximise their fitness. A classic example of phenotypic plasticity in an insect can be seen with the horned beetles. The males show size and reproductive strategy dimorphism, with adult phenotypes determined by larval nutrition. If a nutritional threshold is met male larvae will grow to be a large, horned, mate guarding phenotype whilst if the threshold is not met then male larvae will grow to be a small, hornless, sneaker phenotype (Moczek & Emlen, 2000). This phenotypic plasticity maximises the reproductive fitness of the individual based on anticipated male competition.

As individuals do not exhibit infinite plasticity, there must be limitations and costs associated with phenotypic plasticity. De Witt et al. (1998) suggest that there are nine types of costs: (1) maintenance costs, the metabolic cost of the sensory mechanisms

associated with plasticity, (2) production cost, the cost involved when a phenotypic trait is induced, (3) information acquisition cost, the energy and risk of acquiring the information, (4) developmental instability, phenotypic imprecision resulting in reduced fitness, (5) genetic costs, including linkage, pleiotropy and epistasis (6) information reliability limit, misinterpreting the environmental cue or the environment changes, (7) lag-time limit, where the individual must enter into development to alter phenotypes and the time between an environmental change and phenotypic response reduces fitness, (8) developmental range limit, a trade-off between having a phenotype that works across a variety of environments and a fixed phenotype where extremes can be selected for, (9) epiphenotype problem, where a change from one phenotype to another is not as good as either phenotype would be if it was produced from the beginning (DeWitt, Sih, & Wilson, 1998). The difficulty in identifying these costs is the challenge in creating empirical studies to investigate them which would require tightly controlled genetic experimental designs (DeWitt et al., 1998).

I hypothesise the incredible diversity of the holometabolous insects may be due to the decoupling of the larval and adult life-stages which allows for the utilisation of different niches at different stages of their life. It also allows for developmental plasticity where the conditions of the larvae determine the phenotype of the adult. This allows the adult stage to maximise its fitness based on anticipated conditions. An excellent experimental example of this was presented by Johnson et al. (2017) in the moth, *Uraba lugens*. They found that density of larvae affected the sexual and sensory traits of the adults. High densities of larvae resulted in adult males that invested more in testes size and less in olfaction and dispersal traits (antennae and wing size) whilst

low densities of larvae resulted in the opposite. This suggests that the larvae anticipate either high levels of competition for females or large distances to find females and change their adult reproductive strategies to suit (Johnson, Symonds, & Elgar, 2017).

8 PREDICTIONS REGARDING PHENOTYPIC PLASTICITY OF SENSORY TRAITS

In a holometabolous species, we might expect to see the environment of the larvae play a significant role in determining the level of investment in sensory traits in the adult. Factors during the larval lifecycle such as nutritional availability, larval density, temperature, day length, humidity and chemical stimuli are likely to play a role in determining the phenotypic outcome of the adult. In the dipteran house fly, *Musca domestica*, it has been observed that high larval densities cause adults to be smaller in body size with lower numbers of olfactory sensilla than flies raised in higher densities (Smallegange, Kelling, & Otter, 2008).

In the Acrididae (grasshoppers), which are hemimetabolous, it has been shown that high chemical complexity of the food (irrespective of nutritional value) resulted in a higher number of gustatory and olfactory sensilla in grasshoppers (Chapman, 2002). Solitary locusts and grasshoppers raised in isolation had higher numbers of antennal sensilla than gregarious locusts and grasshoppers reared in groups (Chapman, 2002; Greenwood & Chapman, 1984). Unlike the Dipteran *Musca domestica*, in the Orthoptera, the numbers of sensilla were not related to body size. It would be

interesting to see if this difference holds across all hemimetabolous and holometabolous insects.

An understanding of the adult behaviour of insects may help predict phenotypic plasticity of sensory traits. For example, in a holometabolous species of insect where the adult life stage is short-lived and non-feeding, we can assume that traits that optimise reproductive success would be selected for. Optimal traits as an adult may vary depending on the conditions experienced as larvae. In high larval densities, eventual competition for a mate is likely to be more locally confined, so sensory traits related to mate finding after dispersal are less likely to be useful whilst traits that increase competitive advantage e.g. sperm production would increase fitness of the individual. In low larval densities, mate searching is more likely to be an issue so investment in sensory traits that increase ability to find mates is more likely to be useful, for example larger eyes or more sensilla. As the species is non-feeding (Sheppard, Tomberlin, Joyce, Kiser, & Sumner, 2002), energy is a significant constraint, therefore the ability to invest in traits that maximise fitness at the expense of other traits would give a significant advantage to those individuals.

9 ENTOMOPHAGY AND THE BLACK SOLDIER FLY

The focus of this study will be on a candidate of great interest to the waste management and sustainable protein industry, *Hermetia illucens* Linnaeus, commonly known as the black soldier fly. A large amount of research effort has focussed on larval development and rearing optimisation however there is a clear need for a better

understanding of the adult biology. The importance of this species stems from concerns over global food security in the face of an expanding human population.

9.1 ENTOMOPHAGY AND A GROWING POPULATION

The world's rapidly growing population, expected to reach over 9.7 billion by 2050 (United Nations, Department of Economic and Social Affairs, 2015), along with an increase in the wealth of developing countries, will increase the demand for animal proteins for food (Boland et al., 2013) and raises concerns for food security (Godfray et al., 2010). Insects have been highlighted by the United Nations Food and Agriculture Organisation (FAO) (Van Huis et al., 2013) as an important source of dietary protein and, due to aspects of their biology, some insect species are a more sustainable option than sources of protein from livestock, poultry or seafood. Insects are poikilothermic (Byrd & Castner, 2002) which means energy gained from food is allocated to growth rather than maintaining body temperature (Bartley, Carlberg, Olst, & Ford, 1980). This allows for a feed conversion efficiency higher than poultry and much higher than mammalian livestock (Nakagaki & Defoliart, 1991; Rumpold & Schlüter, 2013). Generally, most insects produce lower levels of greenhouse gasses than mammals and some species have the potential to convert organic waste into valuable products. However, as Western perceptions surrounding consumption of insects pose a challenge towards mass uptake (Caparros Megido et al., 2016; Deroy, Reade, & Spence, 2015; Hamerman, 2016) one potential solution is to 'launder' insect protein through the human food chain (Tabassum-Abbasi, Abbasi, & Abbasi, 2016). Insect protein can be fed to livestock and aquaculture species as a sustainable source of

feed that reduces our reliance on traditional sources of protein such as soya meal and fish-meal. Several larval stages of insect species have been investigated for use in animal feed, including *Tenebrio molitor* Linnaeus (yellow meal worm) (Ramos-Elorduy, González, Hernández, & Pino, 2002), *Musca domestica* Linnaeus (housefly) (Zuidhof et al., 2003) and *Acrida exaltata* Walker (grasshopper) (Anand, Ganguly, & Haldar, 2008).

9.2 HABITAT & LIFE HISTORY

H. illucens is a cosmopolitan species of Stratiomyidae (McCallan, 1974). Post-world war two trade is noted as having been the primary means of spreading the species from the Americas around the globe, where it is now found ranging from 45°N to 40°S (McCallan, 1974), on all continents other than Antarctica. The species was first reported in New Zealand in February, 1956 and likely to have one to two generations per year, overwintering in the larval stage (May, 1961).

The species is holometabolous with the adult living for approximately four days, utilising the body fat and protein acquired in their juvenile stage. They are generally reported to be non-feeding with non-functional mouth parts, however this has been called into question with one study showing that their life span can be increased if sugar water is made available to them (Nakamura, Ichiki, Shimoda, & Morioka, 2015). Adult females are highly fecund and lay between 200 and 1000 eggs (Booth & Sheppard, 1984; J. K. Tomberlin, Sheppard, & Joyce, 2002) which hatch into neonate larvae that undergo six instars (Pujol-Luz, Francez, Ururahy-Rodrigues, & Constantino, 2008). The time spent in the larval stage can range from 22 to 40+ days

depending on feed type and temperature (Tomberlin et al., 2002; J. Tomberlin, Sheppard, & Joyce, 2005). There appears to be some plasticity in the size of adult *H. illucens* with a wild population from Rotorua producing adults that were noticeably larger than those raised in captivity in the lab (personal observations) despite being from the same genetic lineage. This suggests that larval conditions have some impact on the morphology of the adults.

The larvae of *H. illucens* is of particular interest to the waste management and agricultural industry as it is a voracious consumer of organic waste which it converts into protein and lipids stored within its body (Boland et al., 2013; Li et al., 2011). The utilisation of *H. illucens* larvae serves a dual purpose of reducing organic waste going to landfill whilst providing a source of protein and oil for feeding growing populations.

Many studies have focused on *H. illucens* as a value added manure processing tool for swine and poultry farms where it is seen as an efficient way of minimising excess nutrient waste whilst providing a source of protein and lipids for input back onto the farm (Diener, Zurbrugg, & Tockner, 2009; Jr, Newton, Hale, & Barker, 1977; Li et al., 2011; Myers, Tomberlin, Lambert, & Kattes, 2008; G. L. Newton et al., 2005; L. Newton, Sheppard, Watson, Burtle, & Dove, 2005; D. Craig Sheppard, Newton, Thompson, & Savage, 1994). However, since the turn of the century more consideration has been given to the use of *H. illucens* as a tool for up-cycling of municipal organic waste into valuable products such as dried larvae/pre-pupae, protein meal, oil, chitin and frass (Choi et al., 2009; Diener et al., 2009; Leong, Kutty, Malakahmad, & Tan, 2016; Li et al., 2011). This is likely to be due to the increasing interest in sustainability by governments as well as increasing oil prices causing

conventional protein sources, such as fish meal, to become too expensive for the agricultural sector. The nutritional value of *H. illucens* larvae makes them an ideal replacement for these conventional protein sources.

H. illucens larvae are a rich source of protein and lipids, containing approximately 42% protein and 35% lipids by dry weight (Sheppard et al., 1994). However, the diet of the larvae has been shown to have a significant impact on the crude protein content of *H. illucens*, with crude protein by dry weight values of 37.2, 44.6 and 52.3% being derived from a control group diet, high protein diet and high fibre diet respectively (Tschirner & Simon, 2015). Similarly, the crude lipid content and the fatty acid profile of the larvae can be manipulated by using diet (St-Hilaire et al., 2007). The lipids of *H. illucens* can be separated from the pupae and are a rich source of omega-6 fatty acids, lauric acid and, with enrichment, can also be a source of omega-3 fatty acids (St-Hilaire et al., 2007). The manipulation of larval diet to induce different amino acid and fatty acid contents provides opportunities for tailoring *H. illucens* larvae to meet the dietary needs of specific livestock. One example of this principle being applied is through the feeding of fish offal to larvae to increase the omega-3 fatty acid content present in the total lipid, and thereby make the larvae a more attractive component for inclusion in aquaculture feeds (St-Hilaire et al., 2007, p.).

It is evident that biology of the larvae of *H. illucens* is well studied, however, many aspects of the basic adult biology require further research attention to optimise them as a food source for poultry, aquaculture or livestock, in particular the morphology and reproductive behaviour of the adult fly.

9.3 REPRODUCTIVE BEHAVIOUR

Of interest to the black soldier fly industry is the reproductive behaviour of the adults as mated females are a prerequisite for breeding in an artificial setting.

Adult male *H. illucens*, like other members of Stratiomyidae (Alcock, 1990, 1993), have been reported to exhibit lekking behaviour, where a leaf is guarded territorially from other males and passing females are seized, with copulation beginning in the air as they fall to the ground (Tomberlin & Sheppard, 2001). As has been noted in the literature, the use of the term lek can be rather broad and vague (Bradbury, 1977). If we take a restricted definition of a lek, as per Bradbury (1977), then a lek requires the following conditions to be met: (a) an absence of male parental care, (b) existence of a mating arena, (c) male territories contain no resources, and (d) females have an opportunity to select a male for mating (Bradbury, 1977). Whether there is female mate choice occurring in the observed *H. illucens* mating interactions is debatable. The female flies towards a plant which is being used as a “lekking site” by several males and then a single male will fly up and snatch the female in mid-air (Tomberlin & Sheppard, 2001). It has not been demonstrated that there is female mate choice occurring here and I would suggest that they be classified as having lek-like mating behaviour (Bradbury & Davies, 1987). It has been proposed that the presence of vegetation for males to perch on was important for reproduction and without these sites there would be a reduction in the soldier fly population and associated benefits (Tomberlin & Sheppard, 2001). The circling of the female over the male would lead one to assume that the visual sensory system is important in finding a mate but there

could also be the possibility of mechano-sensory (e.g. female wing beat frequency) and chemo-sensory systems (pheromones) at play.

Further evidence of the importance of the visual sensory system is the importance of the presence of light in the mating behaviour of *H. illucens*. It has been noted that mating success in *H. illucens* adults increases in the presence of direct sunlight (Tomberlin & Sheppard, 2002) and artificial lighting with specific wavelengths of light can increase the rate of mating in indoor, controlled environments (Oonincx, Volk, Diehl, van Loon, & Belušič, 2016a; Zhang et al., 2010).

10 SPECIFIC AIMS OF THESIS

This thesis aims to investigate the following aspects of *H. illucens* biology.

Firstly, I will investigate and quantify the ultrastructural morphology of the antennae in *H. illucens* through the use of scanning electron microscopy.

Secondly, I will investigate the allometry of sensory traits and a dispersal trait (wing size) in the black soldier fly and compare the allometries of the sexes and between two separate populations. To do this, I will compare antennal length, antennal sensilla density, wing centroid size, eye size and ommatidia density against body size (measured as pronotum length). I will also investigate whether there are shape variations in wing morphologies between the sexes and populations.

Thirdly, I will investigate the effects larval density on developmental phenotypic plasticity to assess whether experimentally manipulated larval densities will give rise to different levels of investment in sensory traits in adults. Specifically, I predict that

high larval densities will result in adults that invest less in sensory traits when compared to adults raised from low larval densities.

Chapter Two: Methods

11 MORPHOLOGY, ALLOMETRY AND GEOMETRIC

MORPHOMETRICS

11.1 LARVAE

Larvae of *H. illucens* were raised to adult stage in the lab from two populations. The first population was reared from prepupae purchased from a Rotorua colony which were raised as larvae on table scraps in the compost heap of a resident. The second population were raised from eggs obtained from adults of the Rotorua colony raised at the University of Auckland and were fed a controlled diet of Formula 4-24 Drosophila feed (Carolina Biological Supply Company, North Carolina, USA). The diet was mixed with 2 parts water to 1 part diet with roughly 500mg of medium per larvae. New medium was added once a week. The prepupae were allowed to move out of their medium and into moist vermiculite for pupation. Upon emergence, the adults of both populations were killed by freezing in a -80°C freezer and then stored in 75% ethanol until ready for analysis.

11.2 PREPARATION OF THE LARVAE

The abdomen, head, wings and legs were removed from the pronotum. The wings were affixed to glass microscope slides for imaging and measurement. The antennae were removed from the head for analysis using scanning electron microscopy (SEM).

The head, pronotum, wings, abdomen and legs were stored in 75% ethanol for future use.

11.3 MEASUREMENTS

11.3.1 Antennae

Antennae were removed from ethanol and allowed to dry at room temperature for 24h prior to fixing to stainless steel stubs with adhesive carbon tape. The antennae were then sputter coated with a single coat of platinum using a Quorum Q150RS (Quorum Technologies, East Sussex, England). Where possible the lateral side of the antennae were used. In some cases, where the lateral side was damaged, the medial side was used. The samples were analysed using a FEI Quanta 200 field emission Environmental SEM (ESEM; FEI, Oregon, USA) conducted at 10.0 kV with water vapour at a pressure of 0.58 Torr and a spot size of 2.0. Images of the whole antennae were taken at a magnification of 60x whilst images of the eighth flagellomere were taken at a magnification of 100x.

11.3.2 Microtrichia densities

The SEM images were imported into FIJI ImageJ (Schindelin et al., 2012). The scale was set using the scale bar present in the original SEM image (1mm). A grid overlay of 10µm x 10µm quadrats was created on the digital image. For most images it was possible to fit eleven grid quadrats within the shallow depression of the eighth flagellomere and these were labelled one through eleven. Three quadrats from the

eleven available were then selected at random using the ‘sample’ function in R (v. 3.4.3) (Ihaka & Gentleman, 1996).

Sensilla were measured using the ImageJ tool “Multi-Point” by counting the tip of any sensilla that ended within the selected quadrat. In circumstances where a sensilla was broken but well within the quadrat, the base was counted.

All steps within ImageJ were recorded as a macro to assist in reproducibility although it should be noted that the Multi-Point tool in ImageJ does not record all of the points selected in the macro.

11.3.3 Pronotum

The pronotum length (PL) was measured using Jobmate Digital Callipers (Mitre 10 (New Zealand) Limited, Auckland, New Zealand) from the head socket to the angle of hind margin (Figure 11.1). This was used as a reference dimension due to its highly sclerotised nature making accurate measurements possible. Total body length was not measured due to the flexible, extendible nature of the abdomen.

Figure 11.1: Diagram of pronotum measurement. The prothorax was dissected from the head and abdomen and appendages were removed to aid in accurate measurement of the prothorax.

11.3.4 Eyes

The proximal end of the head, once dissected from the body, was affixed to a microscope slide with superglue. Images of the head were taken with a Leica Z16 APO Macroscope (Leica Microsystems, Wetzlar, Germany) fitted with a Lumenera Infinity 2-1 camera (Ontario, Canada) and Infinity Capture software. Where possible, the length of the left eye of each individual was measured dorsoventrally from the dorsal point of connection with the head to the ventral point of connection. In some cases, the

left eye was damaged and the right eye was used in its place. The measurements were taken using the FIJI ImageJ v.1.51s (Schindelin et al., 2012).

Eye length (EL) was reported as well as relative eye length ($\frac{EL}{PL}$).

Figure 11.2: Showing position of measurement for eye length (EL).

11.3.5 Ommatidia

A method adapted from Narendra et al. 2013 was used to analyse ommatidia densities (Narendra, Alkaladi, Raderschall, Robson, & Ribi, 2013). The front of the left eye was painted with clear nail varnish and allowed to dry (Figure 11.3). The dried varnish was then gently peeled off leaving a series of impressions in the varnish corresponding to individual ommatidia. The varnish was then mounted on a

slide and analysed using a Leica Macroscope fitted with an Infinity 2-1 camera and Infinity Capture software was used to take photos of each slide mounted ommatidia impression at a magnification of 57x. The image was then imported into FIJI ImageJ () and the density of ommatidia was counted within a 0.1 mm² quadrat using the multipoint tool.

Figure 11.3: Showing position of varnish for calculating ommatidia density (OD)

Figure 11.4: Showing grid overlaid on image of varnish. To aid in consistency across specimens, measurements were always taken from the grid segment closest to the proximal edge of the eye (seen here as the empty band of varnish to the left of the image).

11.3.6 Centroids

Wing centroids, a measure of the overall size of the wing, were used as a measure of wing size. A centroid is the square root of the sum of squared deviations of landmarks around their centroid (Bookstein, 1996; Klingenberg, McIntyre, & Zaklan, 1998). To collect these a Leica Z16 APO Macroscope (Leica Microsystems, Wetzlar, Germany) fitted with an Lumenera Infinity 2-1 camera (Ontario, Canada) and Infinity Capture software was used to take photos of each slide mounted wing at a magnification of 5.7x (n=84). The images were then digitised using tpsUtil32 (v.1.74) (Rohlf, 2015) to create a tps file. I then used tpsDigi2w32 (v.2.30) (Rohlf, 2015) to place a total of 16 landmarks on major wing vein margins and nodes (i.e. at the intersection of each major

vein and the wing margin, and where veins intersected with other veins) (Figure 11.5). To minimise bias, images were shuffled randomly and then reordered after all landmarks had been placed. All landmarks were placed by a single investigator (N.W.B) and in general the left wing was used, but in situations where this was not possible, the right wing was used and the image reversed to enable comparison. Frequently wings would become damaged due to flying into the enclosure during captivity, due to this I decided to avoid placing landmarks at the distal end of the wing, which was the area of the wing most frequently damaged, to maximise the number of individuals available for analysis. The two-dimensional, cartesian, landmark coordinates were imported into tpsRelw (v.1.69) (Rohlf, 2015) and from this wing centroids were extracted as a csv file.

Figure 11.5: Location of the 16 landmarks on the wing of an adult, female *H. illucens* from Rotorua

11.4 STATISTICAL ANALYSES

11.4.1 Sexual Dimorphism

Sexual dimorphism of traits was investigated in the Rotorua population only so as to remove variation between populations. The mean and standard error for males and females were calculated for relative eye size, length of eye, length of antennal segments, wing centroid size, eighth flagellomere microtrichia density and ommatidia density. A t-test was performed to assess statistical significance between sexes. Rather than performing Bonferroni procedures, which are conservative and cause a substantial reduction in the statistical power for rejecting an incorrect H_0 in each test (Nakagawa, 2004), I carefully selected traits that are likely to be involved in reproductive behaviour and reported the standardised effect size using Cohen's D (Cohen, 1992) and confidence intervals for effect size in addition to p values to allow an assessment of biological importance as well as statistical significance.

11.4.2 Allometry

I used the power function, $y = bX^a$, to investigate the relationship between body size (as approximated by pronotum length) and morphological features (static allometry). These variables were log transformed at base 10 to remove skewing and approximate a log-normal distribution. Log transformation also helps to increase homoscedasticity which minimises effects of extreme outliers whilst making for easier visualisation of the data (Campione & Evans, 2012). I decided upon using a Type II regression

technique as I was interested in investigating the line of best fit for my bivariate data set rather than predicting a value for Y when given X (Painting & Holwell, 2013; Taskinen & Warton, 2013). I used the statistical analysis program, R (Ihaka & Gentleman, 1996), and the package, SMATR (Warton, Duursma, Falster, & Taskinen, 2012), to perform the standardised major axis (SMA) regression on body size (approximated by pronotum length) against the following measurements: antennal length, eighth flagellomere microtrichia density, eye length, ommatidia density and wing centroids. The robust argument was used in SMATR to fit a SMA using Huber's M estimation to assist in minimising the effects of outliers which SMA regression is susceptible to (Taskinen & Warton, 2013; Warton et al., 2012). The SMA regression allowed us to test whether the slope deviated from isometry, i.e. is a greater or less than 1. The correlation of the variables, as an r^2 value, allowed me to estimate the strength of the linear relationship between Y and X .

11.4.3 Geometric Morphometrics

Geometric morphometrics was used to analyse shape differences between the wings of the Rotorua and lab-reared populations. The wing landmarks ($n=84$) for each individual were scaled using a Procrustes superimposition approach (Bookstein, 1996; Goodall, 1991). Effectively, this removes the dimension of size, position and orientation from the wings, leaving dimensionless geometric shapes which can then be compared to one another as single points in Kendall's shape space (Bookstein, 1996). Whilst this seems abstract, Klingenberg uses the helpful analogy of seeing a photo of a

friend, we recognise our friend based on their shape, despite the photo not being to scale, correct orientation, or in the same location (Klingenberg, 2016).

After Procrustes superimposition, I used MorphoJ (v. 1.8.0_161) (Klingenberg, 2011) to check landmarks for outliers by plotting the distribution of Mahalanobis distances (which provide an indication of how ‘unusual’ a landmark is in relation to other landmarks (Klingenberg, 2011)) in the dataset against a multivariate normal distribution (Klingenberg, Monteiro, & MacLeod, 2005). I then undertook a principle components analysis (PCA) which revealed that the first 5 principle components (PC) accounted for 80.4% of the variance between individuals. Using tpsRelw (v1.69) (Rohlf, 2015), I also conducted a relative warp analysis, which is analogous to a principal components analysis (PCA) but weights for bending energy (Bookstein, 1991; Rohlf, 1993, 2015). As I found that the first five relative warps accounted for the same amount of variance as the first five principle components (80.4%), I was able to continue using the principle components. By individually plotting PC2, PC3, PC4 and PC5 against PC1, I was able to visually compare the effects of sex and population. The principle component plots were shown with 90% confidence ellipses.

To investigate the effects of sex and population on wing shape, I performed a multivariate analysis of variance (MANOVA) on the first five principle components obtained for all specimens. All assumptions of the MANOVA were met. I also reported the partial eta-squared (η^2) as a measure for effect size.

12 INFLUENCE OF LARVAL DENSITY ON MORPHOLOGICAL TRAITS

An orthogonal experimental design, based upon a recent paper by Johnson et al. (Johnson et al., 2017), was used to investigate the effect of larval density on adult morphological traits. Eggs of *H. illucens* (provided by Plant and Food Research, Palmerston North) were hatched and the neonate larvae reared on wheat bran (1:3 ratio of wheat bran to water) in a Contherm Biosyn 6000cp growth chamber at 30°C with a 12:12 light:dark cycle. After five days, larvae were randomly allocated to four experimental treatments; (1) 125g of cow manure with twenty larvae, (2) 125g of cow manure with fifty larvae, (3) 250g of cow manure with twenty larvae and (4) 250g of cow manure with fifty larvae. Rather than varying the container size, as in Johnson et al. (2017), the containers remained the same size (500mL take away containers) with the volume of substrate changing between treatments. I made this change to reflect the habitat of the larvae of *H. illucens* which grow within a substrate as opposed to the lepidopteran larvae in Johnson et al.'s study which live upon a plant host.

When the larvae reached the prepupal stage, they were allowed to crawl out into moist vermiculite to pupate into adults. As adults emerged they were collected, frozen then placed into 75% ethanol until ready for dissection. The date of eclosion was recorded.

Measurements were taken in the same fashion as the morphology and allometry study.

12.1 STATISTICAL ANALYSIS

Generalised linear models (GLMs) were constructed using backwards elimination to predict antennal length, eye length and wing centroid size.

Chapter 3: Results

13 DESCRIPTION OF ANTENNAL MORPHOLOGY

Figure 12.1: An illustration of the antenna of *H. illucens* seen medially. SC = Scape, PE = Pedicel, F1 through F8 = Flagellomere one through eight.

The antennae of *H. illucens* are flagellar in form (D. Schneider, 1964) and made up of the scape, pedicel and eight flagellomeres (Figure 12.1). The male and female total antennal lengths and the length of flagellomeres one to eight show sexual dimorphism with the female being significantly larger than the male. This supports previous findings (Pezzi et al., 2017). The scape (b) is cylindrical in shape, the base of which is covered in two patches of microtrichia whilst the shaft is covered in chaetic sensilla pointing away from the head towards the pedicel. The pedicel (Figure 13.2c) is cylindrical with microtrichia in the joint connecting to the scape and chaetic sensilla of varying sizes pointing away from the head towards the flagellomeres. The first seven flagellomeres are largely covered in dense microtrichia which surround openings containing complex sensilla (Figure 13.3a). The first seven flagellomeres are fairly

uniform in appearance other than a bowl (Figure 13.3e) on the medial side of the antennae from flagellomeres four through six which contains densely packed trichoid sensilla (Figure 13.3f). The eighth flagellomere is flattened and lanceolate in form with a concave depression on the medial and lateral sides, the depression is filled with hair-like microtrichia whilst the outer rim contains longer, scimitar-like microtrichia (Figure 13.3d, e & f). At the proximal joint of the eighth and seventh flagellomere is a perforation measuring around 2.5µm in diameter.

Figure 13.2 Scanning electron micrographs of the antennae of *H. illucens*. (a) Overview of antennae of *H. illucens*. Sc, Scape; Pe, Pedicel; F1-F8, Flagellomeres one to eight. (b) Scape, showing microtrichia (Mt) at the base and two patches of chaetic sensilla (CS) of varying lengths. (c) Pedicel showing chaetic sensilla of varying lengths. (d) Overview of flagellomeres 1 – 7 with the shallow bowl (SB) outlined on flagellomeres four to six. (e) Overview of the shallow bowl with dense trichoid sensilla (TS) present.

Figure 13.3: (a) Trichoid sensilla in the shallow bowl, magnified. (b) A complex sensillum found amongst the dense microtrichia covering flagellomeres one to seven, in this case on the edge of the shallow bowl. (c) a perforation (star) at the proximal end of the eighth flagellomere (F8) depression. (d) a close-up of F8 with the depression outlined. (e) A magnified section of flagellomere eight showing hair-like microtrichia and scimitar-like microtrichia. (f) The tapered hair-like microtrichia found on flagellomere eight showing longitudinal striations.

14 DESCRIPTION OF EYE AND OMMATIDIA MORPHOLOGY

Figure 14.1: (a) the right eye of a female adult *H. illucens*. (b) the facets of the compound eye viewed at 97x magnification

Both the male and female adult *H. illucens* have large compound eyes that are located dorso-ventrally and strikingly coloured, with varying shades of blue, purple and green stripes (Figure 14.1a). The compound eyes are made up of numerous corneal lenses. These facets are arranged in tight rows traversing the entire compound eye (Figure 14.1b).

15 SEXUAL DIMORPHISM

Five of the ten measured traits analysed in *H. illucens* show sexual dimorphism (Table 15.1). Unpaired t-tests, which compared the mean size of each trait between males and females, showed a significant difference for total antennal length ($p < 0.001$), length of

flagellomeres 1-7 ($p < 0.001$), length of flagellomere 8 ($p < 0.001$), wing centroid ($P < 0.005$) and eye length ($p < 0.05$). For all traits that were sexually dimorphic, the males were the smaller of the pair. Full details for these analyses are presented in Table 3.1.

Table 15.1: Sensory traits of *H. illucens* were investigated for sexual dimorphism. Mean, maximum and minimum measurements presented with 95% confidence intervals, *t*-statistic, *p* value and Cohen's *D* (effect size).

Trait	Males				Females				95% CI	t	p	d	df
	n	Mean size $\pm$ SE	Max.	Min.	n	Mean size $\pm$ SE	Max.	Min.					
Pronotum Length	24	6.41 $\pm$ 0.05	6.87	6.1	27	6.42 $\pm$ 0.04	6.8	5.84	-0.132, 0.129	-0.0235	0.9813	-0.007	48.91
Antennae length	24	4.09 $\pm$ 0.05	5.03	3.578	27	4.74 $\pm$ 0.04	5.085	4.401	0.512, 0.782	8.4377	<0.001	2.34	48.74
Sensilla Density	7	49.52 $\pm$ 4.39	65.33	36.67	10	55.53 $\pm$ 1.47	61.33	47.33	-4.831, 16.850	1.2982	0.2335	0.735	7.351
Scape Length	24	0.64 $\pm$ 0.01	0.728	0.512	27	0.65 $\pm$ 0.01	0.769	0.55	-0.018, 0.039	0.70903	0.4817	0.198	48.6
Pedicel Length	24	0.22 $\pm$ 0.01	0.288	0.167	27	0.21 $\pm$ 0.01	0.284	0.142	-0.030, 0.011	-0.94294	0.3504	-0.264	48.49
Length Flagellomeres 1 -7	24	1.16 $\pm$ 0.03	1.724	0.981	27	1.60 $\pm$ 0.02	1.769	1.455	0.363, 0.512	11.858	<0.001	3.418	37.34
Length Flagellomere 8	24	2.06 $\pm$ 0.03	2.417	1.77	27	2.27 $\pm$ 0.02	2.496	2.054	0.133, 0.284	5.5739	<0.001	1.577	45.74
Wing Centroids	24	642.4 $\pm$ 11.37	794.1	527.6	26	705.8 $\pm$ 16.18	793	529.6	23.580, 103.313	3.2072	<0.005	0.894	44.1
Eye Length	24	256.6 $\pm$ 2.80	286.1	233.5	27	266.1 $\pm$ 2.92	296.1	234.5	1.326, 17.592	2.3373	0.0234	0.651	48.98
Ommatidia Density	23	142.1 $\pm$ 4.02	184	115	24	134.1 $\pm$ 3.43	172	111	-18.655, 2.644	-1.5153	0.1369	-0.443	43.6

Welch's unpaired independent t-tests were used to test for significant differences in mean size between the sexes for each trait.

The *t* statistic and *p* values from this are presented. Confidence intervals (CI), standard errors of the mean (SE) and effect size (Cohen's *d*) are also presented.

16 POPULATION DIFFERENCES

The interactions of population, sex and body size on measured traits were investigated using linear models and generalized linear models (poisson) Table 16.1).

The relationship between antennal length and population, sex and body size was explored using a linear model. The significance of body size ($t = 11.51$, $p < 0.0001$) indicates that antennal length is strongly influenced by body size. The slope of the relationship between antennal length and body size differs significantly between the sex of the individual ($t = -2.06$, $p < 0.05$). The final model had a p value < 0.0001 and an R^2 value of 0.83 suggesting a strong fit.

The relationship between sensilla density on the eighth flagellomere and population, sex and body size was explored using a generalised linear model (GLM) with a Poisson distribution. The significance of sex ($Z = -3.41$, $p < 0.001$) indicates that the sensilla density of males and females are different. Sensilla density is also significantly different between populations ($z = -2.841$, $p < 0.005$). There is no significant relationship between the sensilla density of individual flies and their body size ($z = -0.603$, $p = 0.5$). However, the slope of the relationship between sensilla density and the length of pronotum differs significantly between populations of flies ($t = 2.75$, $p < 0.01$).

The relationship between eye length and population, sex and body size was explored using a linear model. The significance of the difference between the sexes ($t = -5.12$, $p < 0.0001$) indicates that the eye length of males and females are different, with males having smaller eyes than females. Eye length is also significantly different between populations ($t = -3.25$, $p < 0.005$). There is a positive relationship between the eye length of individual flies and their body size ($t = 10.17$, $p < 0.0001$). In addition, the slope of the relationship between eye length and body size differs significantly between populations of flies ($t = 2.97$, $p = 0.003$). The final model had a p-value < 0.0001 and an R^2 value of 0.9.

The relationship between ommatidia density and population, sex and body size was explored using a GLM with a Poisson distribution. The significance of sex ($z = 3.094$, $p = 0.002$) indicates that the ommatidia density of males and females are different. Ommatidia density is also significantly different between populations ($z = 3.713$, $p < 0.001$). There is a weakly significant relationship between the ommatidia density of individual flies and their body size ($z = -1.655$, $p = 0.09$). However, the slope of the relationship between ommatidia density and body size differs significantly between populations of flies ($z = -3.891$, $p < 0.001$).

The relationship between wing centroid size and population, sex and body size was explored using a linear model. Wing centroid size is significantly different between populations ($t = 3.05$, $p < 0.005$). The slope of the relationship between wing centroid

size and body size differs significantly between populations of flies ($t = -2.992$, $p < 0.005$). The final model had a p -value < 0.001 and an R^2 value of 0.515.

Linear Model			
Antennal Length	Estimate	t-value	p-value
Intercept	1.41 ± 0.27	5.256	$p < 0.0001$
Sex	0.19 ± 0.36	0.549	0.5842
Body Size	0.52 ± 0.05	11.51	$p < 0.0001$
Sex*Body Size	-0.13 ± 0.06	-2.206	0.0303
Eye Length	Estimate	t-value	p-value
Intercept	69.04 ± 15.43	4.474	$p < 0.0001$
Sex	-9.93 ± 1.94	-5.118	$p < 0.0001$
Population	-120.4 ± 37.02	-3.252	$p < 0.005$
Body Size	31.3 ± 3.08	10.165	$p < 0.0001$
Population*Body Size	18.13 ± 6.10	2.973	$p < 0.005$
Wing Centroids	Estimate	t-value	p-value
Intercept	524.26 ± 137.24	3.82	$p < 0.0005$
Population	1009.08 ± 330.51	3.053	$p < 0.005$
Sex	-40.71 ± 17.36	-2.345	0.02
Body Size	32.15 ± 27.44	1.171	0.25
Population*Body Size	-162.75 ± 54.39	-2.992	$p < 0.005$
General linear model - Poisson Distribution			
Sensilla Density	Estimate	z-value	p-value
Intercept	4.36 ± 0.34	12.611	$p < 0.0001$
Population	-4.87 ± 1.71	-2.841	0.004491
Sex	-0.16 ± 0.05	-3.410	0.000651
Body Size	-0.04 ± 0.07	-0.603	0.546672
Population*Body Size	-0.72 ± 0.26	2.75	0.005967
Ommatidia Density	Estimate	z-value	p-value
Intercept	5.34 ± 0.14	36.231	$p < 0.0001$
Population	1.38 ± 0.37	3.713	$p < 0.0005$
Sex	0.06 ± 0.01	3.094	$p < 0.005$
Body Size	-0.05 ± 0.02	-1.655	$p < 0.09$
Population*Body Size	-0.24 ± 0.06	-3.891	$p < 0.0001$

t-values are presented for linear models and z-values for the poisson distribution (due to presence of count data). P values < 0.05 suggest that variable is a strong predictor for trait size

Table 16.1: Linear model and generalized linear model (poisson) statistics for the effect of population, sex and body size on trait size.

17 ALLOMETRY

Antennal length was positively allometric for males in both the Rotorua and laboratory populations, but isometric for females (Figure 17.1). This suggests that larger male flies are disproportionately investing in longer antennae. For females, I found that (a) there is a common slope between populations (Likelihood ratio (LR) = 0.26, $p = 0.6$), and (b) that the slope is isometric (LR = 0.301, $p = 0.86$), suggesting that females between the two populations show a similar proportional scaling relationship in antennal length against body size. For male antennal length, there is a common slope between populations (LR = 3.12, $p = 0.07$), however, this slope differed from one, i.e. was not isometric (LR = 7.495, $p = 0.02$).

Due to the expense and time involved in using the scanning electric microscope, a smaller sample size ($n=40$ which was then subset between sex and population) was available for analysing the sensilla density of sensilla on the eighth flagellomere. The sensilla density on the eighth flagellomere for both sexes in the Rotorua population showed positive allometric scaling suggesting a disproportionate investment in sensilla density by larger individuals. The lab population both show a negative gradient, with larger individuals having less sensilla density. The males show a negative allometry suggesting that they invest disproportionately less in sensilla as they get larger, whilst females show isometry indicating that sensilla density decreases proportionally with body size. Unsurprisingly, in the likelihood ratio test for common slope, there was no

evidence that populations shared a common slope for both males and females (male: LR = 9.93, $p = 0.002$; female: LR = 8.19, $p = 0.004$).

Eye length in the Rotorua population is positively allometric for both sexes. Due to the similar size of both slopes for male and female (Male: slope = 1.55; Female: slope = 1.51) a likelihood test was undertaken between the two sexes (Rotorua: LR = 0.01, $p = 0.91$), suggesting that there is a common slope with males and females both investing disproportionately in eye size as they get larger. The laboratory population both exhibited the same slope gradient (Male & Female: slope = 0.79). Females were exhibiting negative allometry ($p = 0.03$). Whilst for males alone I was unable to prove that the slope was different from one (suggesting isometry) ($p = 0.24$), when I performed a likelihood test on both males and females to test for a common slope, I found that (a) there was a common slope (Male & female: LR = 0.0001, $p = 0.99$) and (b) this slope was different from 1 indicating negative allometry (LR = 6.13, $p = 0.05$). Based on this, it is reasonable to say that there is a negative allometry exhibited by both males and females in the lab population. This suggests that larger individuals are investing disproportionately less in eye size.

For ommatidia density, both sexes in both groups exhibit a negative slope, showing that ommatidia density gets smaller as the individual gets larger. The Rotorua group showed strong negative allometry for ommatidia density (Male: slope = -3.79, $p < 0.001$; Female: slope = -3.12, $p < 0.001$). A likelihood test suggested that both groups shared a common slope (Rotorua: LR = 0.54, $p < 0.001$). The lab group showed a

slope of -1.72 and -0.84 for males and females respectively however, I was unable to show that these slopes were different from 1 for both these populations. However, a likelihood test between the sexes indicated that there was a common slope between the two sexes (Laboratory: $LR = 2.81$, $p = 0.09$) and that this slope was likely to be isometric ($LR = 7.94$, $p = 0.01$). There was no significant likelihood of a common slope between sexes in different populations.

For wing centroids, the Rotorua males and females and laboratory males show a negative slope with laboratory females exhibiting a positive slope. The likelihood test suggests that the Rotorua male and females are sharing a common slope which is positively allometric indicating that larger individuals are disproportionately investing in smaller wing size.

Table 17.1: The allometric scaling relationships between measured traits (antennal length, sensilla density, eye length, ommatidia density and wings) and body size (pronotum length) for *H. illucens*. A likelihood test to reject the null hypothesis that the slope is not different from 1 (isometric) was undertaken and *p* values are shown for this. A likelihood test is also presented to compare for common slope between males/females and, if there is a common slope, whether this is different from one.

Rotorua Population														
Trait	Sex	n	Intercept	Slope ($\pm$ SE)	95% CI	H0: slopes are isometric			H0: Common Slope			H0: Common slope isometric		
						r	p	df	LR	p	df	LR	p	df
Antennal Length	Male	24	-0.87	1.83	1.19, 2.80	0.54	0.01	22	0.26	0.61	1	0.3	0.9	2
	Female	27	-0.22	1.11	0.76, 1.62	0.11	0.59	25						
Sensilla Density	Male	7	-6.75	10.19	8.92, 11.64	1.00	<0.001	4	8.67	0.003	1	-	-	-
	Female	10	-1.41	3.84	2.13, 6.90	0.92	<0.001	8						
Eye Length	Male	24	1.16	1.55	1.14, 2.07	0.55	<0.001	22	0.01	0.9	1	19.23	<0.001	2
	Female	27	1.20	1.51	1.21, 1.89	0.61	<0.001	25						
Ommatidia Density	Male	23	5.20	-3.79	-5.54, -2.59	0.90	<0.001	21	0.55	0.4	1	62.68	<0.001	2
	Female	24	4.64	-3.12	-4.47, -2.18	0.86	<0.001	22						
Wings Centroids	Male	24	4.82	-3.66	-3.66, -1.70	0.76	<0.001	24	1.11	0.2	1	50.29	<0.001	2
	Female	26	5.52	-3.31	-4.78, -2.29	0.86	<0.001	22						

Lab Population														
Trait	Sex	n	Intercept	Slope ($\pm$ SE)	95% CI	H0: Slopes are isometric			H0: Common Slope			H0: Common slope isometric		
						r	p	df	LR	p	df	LR	p	df
Antennal Length	Male	15	-0.56	1.62	0.93, 2.83	0.46	0.09	13	0.0044	0.95	1	0.013	0.99	2
	Female	18	-0.09	0.99	0.76, 1.27	-0.03	0.91	16						
Sensilla Density	Male	13	-0.48	-3.37	1.86, 6.13	0.85	<0.001	11	6.4	0.01	1	-	-	-
	Female	9	2.41	-0.89	-1.97, -0.39	-0.12	0.76	7						
Eye Length	Male	15	1.78	0.79	0.54, 1.18	-0.33	0.24	13	0.0001	0.99	1	6.13	0.05	2
	Female	18	1.80	0.79	0.64, 0.97	-0.51	0.03	16						
Ommatidia Density	Male	11	3.41	-1.72	-3.46, -0.85	0.50	0.12	9	2.81	0.09	1	2.9	0.23	2
	Female	16	2.80	-0.84	-1.39, -0.50	0.13	0.17	14						
Wings Centroids	Male	15	4.21	-2.10	-3.60, -1.22	0.65	0.01	13	2.41	0.12	1	7.97	0.01	2
	Female	18	1.95	1.24	0.82, 1.88	0.34	0.01	16						

Table 17.2: Results from likelihood-ratio tests assessing whether the allometric slopes for all measured traits differed between each population, for both males and females. Where a significant p -value is present ($p < 0.05$) we can reject the null hypotheses that slopes are not different to one/isometric. A test for isometry was only performed if a common slope was found.

Sex	Trait	Between population likelihood-ratio tests					
		H0:Slopes are Equal			H0:Slopes are isometric		
		df	LR	p	df	LR	p
Male	Antennal Length	1	3.12	0.07	2	7.495	0.02
	Sensilla Density	1	9.938	0.002	-	-	-
	Eye Length	1	6.963	0.008	-	-	-
	Ommatidia Density	1	3.85	0.05	-	-	-
	Wings Centroids	1	0.27	0.6	2	25.67	$p < 0.001$
Female	Antennal Length	1	0.26	0.608	2	0.301	0.86
	Sensilla Density	1	8.19	0.004	-	-	-
	Eye Length	1	15.78	$p < 0.001$	-	-	-
	Ommatidia Density	1	15.79	$p < 0.001$	-	-	-
	Wings Centroids	1	11.61	$p < 0.001$	-	-	-

Figure 17.1: The allometric relationship of measured traits against body size (pronotum length). Panels A & F show antennal length for Rotorua and Laboratory populations, B & G show sensilla density, C & H show eye length, D & I show ommatidia density and E & J show wing centroid measurements. Blue = females and yellow = males.

18 GEOMETRIC MORPHOMETRICS OF WING SHAPE

The wing shape change derived from the principle components analysis (PCA) of the covariance matrix for the residuals of the 84 wings analysed are presented in Figure 18.1. The first three principle components (PC) accounted for 67.6% of the variation and were the only PCs that contained over 10% of the variation (Figure 18.1A).

In Figure 18.1(B) we can see male and females falling into two distinct groups with some overlap. Generally, males are ordinated along PC1 whilst females are ordinated along PC2. This is not the case with population (Figure 18.1E & F) which does not ordinate along PC1, PC2 or PC3, appearing to be highly variable and not explaining wing shape.

Using the first three principle components from the covariance matrix, a two-way multivariate analysis of variance was conducted to verify the visual observations from Figure 18.1. A statistically significant MANOVA effect (Table 18.1) was obtained for both sex (Pillais' trace = 0.69, $F_{(3, 76)} = 56.95$, $p < 0.001$) and population (Pillais' trace = 0.12, $F_{(3, 76)} = 3.49$, $p = 0.01$). There was no significant effect from the interaction between sex and population (Pillais' trace = 0.063, $F_{(3, 76)} = 1.702$, $p = 0.17$). The large F value for sex compared to the other variables indicates that this factor explains the groupings of plotted principle components. The multivariate effect size (η^2) for sex was estimated at 0.69, implying that 69% of the variance in the dependent variables

was accounted for by sex. The η^2 for education was estimated at 0.12, implying that 12% of the variance in the dependent variables was accounted for by sex.

The wireframe models (Figure 18.1D) show the shape change of the wing by the difference between the starting (black = reference i.e. overall mean shape) and target shapes (red = female, blue = male). With an increase in PC 1 we can see a slight proximal shift of landmark 1 and a large proximal shift in landmark 14 in females. In males, an increase in PC1 results in a large distal shift for landmark 14 and a slight posterior shift for landmarks 1, 12 and 13.

An increase in PC2 results in a proximal, posterior shift for landmarks 1, 12, 13, 15 and 16 and a distal shift of the remaining landmarks suggesting an elongation of the wing. For males, an increase in PC2 results in a distal shift of landmarks 1, 12, 13, 14, 15 and 16 and a proximal shift the remaining landmarks. When PC3 increases, a narrowing of the wing shape occurs for both males and females between the costal and proximal margins.

Table 18.1: MANOVA statistics are presented for the effect of sex, population and interaction between the two on H. illucens wing morphology.

2-way MAN OVA	MANOVA Statistics
Sex Effect	Pillai's Trace = 0.69; $F_{(3, 76)} = 56.95$; $p < 0.0001$
Population Effect	Pillai's Trace = 0.12; $F_{(3, 76)} = 3.49$; $p < 0.05$
Sex*Population	Pillai's Trace = 0.06; $F_{(3, 76)} = 56.95$; $p > 0.1$

A p-value < 0.05 represents a statistically significant effect of the independent variable on the dependent variables

Figure 18.1: (A) The eigenvalues for each principle component analysed are plotted in relation to how much of the variance was explained. (B, C, E & F) Principle component plots of the first three principle components which account for 69% of the variance in wing shape are plotted with sex (B & C) and population (E & F) with their 90% confidence ellipses. (D) Wireframes for the first three principle components are shown with the distortion of landmarks away from the mean shape due to the influence of sex. Black = mean landmark position for population, red = mean landmarks for females and blue = the mean landmark position for males.

19 PHENOTYPIC PLASTICITY

Using linear models, I investigated the effect of density and volume on the sensory and dispersal traits of *H. illucens* (Table 19.1). There were four treatments; (1) high density, low volume; (2) high density, high volume (no individuals emerged from this treatment); (3) low density, low volume; (4) low density, high volume.

The results show that the best predictor for antennal length was sex ($t = -5.39$, $p < 0.0001$) with males being on average, smaller than females. The low density, low volume treatment also showed an effect on antennal size with individuals in this treatment having larger antennae ($t = 2.03$, $p < 0.05$) than the other treatments.

The best predictor for eye length was body size ($t = 3.3$, $p < 0.005$). Interestingly, high density, low volume and low volume, high density treatments both have a significant effect with both showing larger eye sizes (Intercept estimate is 0.18 & 0.14mm respectively, $t = 2.34$ & 2.68 respectively, $p < 0.05$ for both treatments).

Wing centroids were predicted best by sex and body size with males exhibiting a smaller wing centroid size (-113.45 units compared to females). Treatment showed no significant effects on wing centroid size.

Table 19.1: Linear model statistics for the effect of density treatments, sex and body size on trait size.

Linear Model			
Antennal Length	Estimate	t-value	p-value
Intercept	3.3 ± 0.48	6.81	p<0.0001
High Density Low Volume	-0.04 ± 0.15	-0.318	p=0.75
Low Density High Volume	0.19 ± 0.10	1.844	p=0.07
Low Density Low Volume	0.20 ± 0.10	2.03	p<0.05
Sex - Male	-0.43 ± 0.08	-5.39	p<0.0001
Body Size	0.15 ± 0.09	1.64	p=0.10
Eye Length	Estimate	t-value	p-value
Intercept	1.32 ± 0.25	5.16	p < 0.0001
High Density Low Volume	0.18 ± 0.08	2.34	p<0.05
Low Density High Volume	0.14 ± 0.05	2.68	p<0.05
Low Density Low Volume	0.01 ± 0.05	0.24	p=0.8
Body Size	0.16 ± 0.05	3.3	p<0.005
Wing Centroid	Estimate	t-value	p-value
Intercept	312.73 ± 74.9	4.17	p < 0.0005
High Density Low Volume	29.85 ± 22.9	1.29	p=0.2
Low Density High Volume	-5.48 ± 15.84	-0.34	p=0.7
Low Density Low Volume	9.06 ± 15.34	0.59	p=0.5
Sex - Male	-113.45 ± 12.42	-9.13	P<0.0001
Body Size	142.70 ± 14.61	9.76	P<0.0001

t-values are presented for linear models. P values <0.05 suggest that variable is a strong predictor for trait size

Chapter 4: Discussion

20 DISCUSSION

This thesis is the first work to quantify and report on sensory traits, investment therein and developmental plasticity in the Stratiomyidae. There is a general lack of research into the allometry of sensory traits in insects. This is surprising, as sensory traits play an important role in insect behaviour and an understanding of relative investment in sensory morphology can be highly informative in understanding the selective pressures influencing them. The results of this research are interesting, both in a pure and applied sense. Differences in the sensory morphology of male and female *H. illucens* provide some insight into the unusual reproductive behaviour of the adults, whilst also providing insight into the reproductive behaviour of the species for the benefit of the insect protein industry.

20.1 ANTENNAL MORPHOLOGY

I found that the antennal morphology of *H. illucens* was similar to other members of the Hermetiinae (Jorgensen & James, 1968; Rozkosný, 1983), comprised of three parts, the scape, pedicel and an annulated flagellum made up of eight flagellomeres. A single shallow bowl was present across the medial faces of flagellomeres four to six which contained densely packed trichoid sensilla. Trichoid sensilla are also found on the flagellum of the Tephritidae (Hu, Zhang, Jia, Dou, & Wang, 2010) and other Stratiomyidae (Pezzi et al., 2017) however their sensory function has not been determined experimentally in the Stratiomyidae. It has been suggested that they have a

chemoreceptive function, mechanoreceptive function or a combination of both in other species of insects (Amornsak, Cribb, & Gordh, 1998; Hu et al., 2010; Pezzi et al., 2017). The trichoid sensilla of *H. illucens* closely resemble the appearance of those found on the antennae of *Bactrocera cucurbitae* (Tephritidae) (Hu et al., 2010).

Chaetic sensilla are found on the scape and pedicel of *H. illucens* and are variable in size. They are found on the scape and pedicel of other species of Stratiomyidae (Jorgensen & James, 1968; Rozkosný, 1983) and, in other species of Diptera, they have been attributed to a mechanoreceptive sensory function (de Freitas Fernandes, Linardi, & Chiarini-Garcia, 2002).

Flagellomeres one to six were covered with dense microtrichia that covered sensory pits containing complex sensilla, also found by Pezzi et al. (2017) who described them as having leaf like structures around the base of the sensilla which then branch out into digitiform structures (Pezzi et al., 2017). These are similar to sensilla found in *Chorisops tunisiae* (Mason, 2013), another member of the Stratiomyidae, which have the digitiform structures but not the leaf-like base.

The eighth flagellomere is surrounded by another type of microtrichia, termed scimitar microtrichia. These appear to be unique to *H. illucens*, not being found in other Stratiomyidae analysed using scanning electron microscopy (SEM) (Pezzi et al., 2017). However, light microscopy images of *Hermetia aurata* (Jorgensen & James, 1968) reveal a very similar eighth flagellomere shape. Unfortunately, fine detail of

ultrastructures was not able to be discerned but I hypothesise that an ultrastructural investigation comparing the two may reveal similarities in structures.

At the proximal end of the eighth flagellomere, just before it connects to the seventh flagellomere, is a perforation into the cuticle. It was not possible to increase the resolution of the perforation, although a small protrusion does appear to be located within this. This perforation has not been reported in *H. illucens* before and its structure and function would need to be further investigated to determine its function.

Within the shallow depression of the eighth flagellomere are numerous, hair-like microtrichia. The functional significance that these microtrichia serve is not immediately obvious. However, we do know that the eighth flagellomere accounts for around 50% of the length of the antennae with a shallow, lanceolate, concave bowl on either side increasing the surface area available. These bowls contain the hair-like microtrichia which, given the size of the region of antenna that is devoted to these structures, I would suggest play a sensory or sensory support function. A possible sensory candidate is mechanoreception which has been suggested in the Tephritidae (Hu et al., 2010) where it was speculated that the microtrichia could enhance the female's ability to sense mechanical stimuli during courtship displays either from direct contact or from air currents produced by the male's wing buzzing, one suggestion is that the microtrichia make the female less sensitive to sounds which could lead to sexual selection of males based on buzzing frequency (Hu et al., 2010; Lee, Chang, Hwang, & Lin, 1994; Miranda G., 2000; Webb, Calkins, Chambers,

Schwienbacher, & Russ, 1983). Vibrations do appear to illicit responses from *H. illucens*. Adult flies freeze in the presence of low frequency sounds and this is exploited by the insect protein industry to allow for easy entry to mating cages (Courtright, 2014). When *H. illucens* is in flight it produces an audible buzzing and so there could be potential for this to be used in male rival assessment and mate selection, with males territorially guarding leaves, fighting other males in the air and females circling plants that host a number of males guarding leaves (Tomberlin & Sheppard, 2001). Future studies could investigate several different areas around mechanoreception including whether there is innervation of the microtrichia through use of transmission electron microscopy, investigating if electrophysiological responses to vibrations change following coating of the eighth flagellomere microtrichia with nail varnish, and observing whether wing beat frequency can determine winners in male-male combat.

20.2 EYE MORPHOLOGY

Despite the often cited importance of light and vision to the mating behaviour of species within the genus, *Hermetia* (Alcock, 1990, 1993; Oonincx, Volk, Diehl, van Loon, & Belušič, 2016b; Zhang et al., 2010), there has only been one study investigating the neuroethology of visual sensory behaviour in *H. illucens* (Oonincx et al., 2016b). I found the eye of *H. illucens* was found to be large with densely packed ommatidia. The colouration of the eyes is a particularly striking feature of the species

and may be related to its perception of wavelengths of light. A previous study showed that specific wavelengths of light triggered males and females to copulate (Oonincx et al., 2016b) and only certain regions of the eye responded to certain wavelengths. Other studies of the compound eyes of insects have shown that the colouration of eye facets can narrow the spectral bandwidth of photoreceptors and may improve colour vision (Stavenga, Meglič, et al., 2017; Stavenga, Wehling, & Belušič, 2017). I hypothesise the colouration of the eye in *H. illucens* may correlate to the regions that respond to specific wavelengths of light. The compound eyes of *H. illucens* are likely to be an important sensory trait and an intriguing line of future research would be to investigate how the behavioural response to different wavelengths of light evolved and the selective pressures behind it.

20.3 SEXUAL DIMORPHISM AND INVESTMENT IN SENSORY TRAITS

I found that five of the ten measured traits in *H. illucens* were sexually dimorphic with females exhibiting larger antennae and antennal segments, wing centroids and eye length. The larger size of flagellomeres one through eight accounted for the increase in size of the female antennae when compared to the males' and supports the findings of Pezzi et al. (Pezzi et al., 2017). From the morphological study, we can see that flagellomeres one through seven contain numerous complex sensilla covered by dense microtrichia and a shallow bowl of trichoid sensilla, presumed to have an olfactory function and/or mechanosensory function (Amornsak et al., 1998; Hu et al., 2010;

Pezzi et al., 2017). The different life history traits of the male and female may explain why the females have larger antennae. After copulation, the females seek out suitable oviposition sites and previous studies suggest that olfaction plays an important role in this (Booth & Sheppard, 1984; Zheng et al., 2013), by increasing the size of the antennae, the surface area available to interact with chemical cues is increased.

Unfortunately, the densely packed microtrichia covering flagellomeres one to seven make it difficult to quantify the number of some important types of sensilla present. This prevents us from being able to determine how the number of these sensilla might scale with an increase in antennal size although this would be an interesting avenue to explore in the future if a way of removing the microtrichia was found.

Despite having larger antennae than males, females exhibit isometry with investment in the antennae increasing proportionately to body size. This indicates that a fitness benefit is not received by larger females investing disproportionately in increasing the size of their antennae, as would be expected if they were positively allometric. It may also be result from competing selective pressures, such as the metabolic costs (Niven & Laughlin, 2008), which would take resources away from other traits (e.g. egg mass). Metabolic costs are likely to be a serious constraint in this species due to adults not actively feeding and living off fat supplies from the larval stage (Tomberlin & Sheppard, 2002). It may be insightful to quantify reproductive traits, such as ovary size, in future studies to investigate whether there are trade-offs between investment in reproductive traits and sensory traits.

Males, on the other hand, exhibit a strong positive allometry for antennae size with antennal length increasing disproportionately to body size. This suggests that larger males receive a benefit from investment in disproportionately large antennae. I believe that there are two possible reasons for this based on the male life history. The first possible reason is that *H. illucens* exhibits male-male conflict for access to territory (Tomberlin & Sheppard, 2001). It is possible that the antennae play a role in this conflict, allowing males to assess the size of their opponent during their mid-air grappling. Larger males are more likely to be challenged and therefore being able to quickly assess the opponent helps to reduce energy loss, an important constraint in a short-lived species that does not actively feed. This would indicate that the trait is being influenced by sexual selection. Positive allometry has often been associated with sexual selection and whilst the universality of this association has been criticised (Bonduriansky, 2007), modelling predicts that sexually selected traits will show positive allometry, with strongest positive allometry usually occurring when males compete in large groups (Fromhage & Kokko, 2014) as is the case in *H. illucens*.

The second possible reason is that the mechanoreceptive or chemoreceptive function of the antennae may play a role in detecting females as they are in flight, with larger males investing disproportionately in antennal size to gain a competitive advantage in detecting passing females before smaller competitors do. This hypothesis is supported by the strong positive allometry shown in microtrichia density on the eighth flagellomere for the males from the wild population. The use of auditory cues from

wing beats for mate pairing is seen in many insect orders but is particularly evident in the Culicidae and Tephritidae (Cator, Arthur, Harrington, & Hoy, 2009; Miranda G., 2000; Sanford et al., 2011). Conversely, smaller males may not have sufficient resources to invest in larger antennae which could also explain the positive allometry observed.

Larger wings are associated with larger flight muscles which enable greater dispersal distances (Gage, 1995). There is sexual dimorphism in wing size and shape with females exhibiting larger, wider wings than males, which is intuitive when their life histories are compared as females need to search for oviposition sites, whilst males guard a leaf and wait for a female to approach (Axtell & Arends, 1990; Tomberlin & Sheppard, 2001).

Despite the sexual dimorphism shown in wing size, both males and females show a negative allometry with wings decreasing in size disproportionately to body size. Male wing size shows a negative allometry and negative gradient, suggesting that larger males do not invest in wing size and wing size in fact decreases with body size. This could be explained by smaller males investing in larger wings for the purposes of intercepting females in flight as an alternative mating strategy to competing with large males for territory. Males employing this strategy in other animal species have been described as ‘satellite’ males (Convey, 1989; Gross & Charnov, 1980; Humfeld, 2008; Leary, Fox, Shepard, & Garcia, 2005). Alternative reproductive tactics (ARTs) are not uncommon in species that exhibit male conflict, and allow individuals to maximise

mating success (Painting & Holwell, 2014) (a key assumption in the argument that sexually selected traits will exhibit positive allometries is that smaller males still have the opportunity to mate (Fromhage & Kokko, 2014). Further behavioural study of this species would be needed to assess whether smaller males are employing ARTs such as satellite tactics.

It is unclear why females exhibit a negative allometry for wing size. Perhaps larger females invest in other traits at the expense of wing size, or smaller individuals disperse further away from their eclosion site to reduce competition. Another option is that if the larval conditions are particularly poor, for example, having low nutritional value or high larval densities, then individual larvae are likely to mature at smaller sizes (as was seen in the laboratory population). In this scenario, it makes sense for the individuals to disperse further away from this poor larval site to search for better oviposition sites and so we would expect to see an investment in dispersal traits. The opposite of this situation, where larval conditions are optimal, producing larger adults, we would expect to see less investment in dispersal traits as there is little reason to disperse. Such plasticity in traits based on larval rearing conditions is seen in other orders of insects (Gage, 1995; Johnson et al., 2017) and it would be a beneficial strategy for a species that relies on a spatially, temporally variable oviposition resource such as decomposing organic matter. Again, there are many unexplored avenues where further study into the behaviour of the adult life history would assist in explaining these trends.

There has been little research investigating how *H. illucens* females locate mates and no mention in the literature of *H. illucens* or other Stratiomyidae utilising sex pheromones for mate attraction. The use of olfactory cues, which can assist mate pairing over distances or at low population densities, do not appear to be utilised by *H. illucens* (Axtell & Arends, 1990; Booth & Sheppard, 1984; Craig Sheppard, Larry Newton, Thompson, & Savage, 1994). As *H. illucens* larvae are usually found in high densities there is a reasonable probability of an adult fly finding a mate in the area surrounding the larval habitat upon pupation so the use of a pheromone may not be necessary, with visual cues being satisfactory. The larger eyes of the female may assist in determining optimal environmental conditions suitable for ovipositioning and, as the female is more mobile, navigation, orientation and obstacle avoidance (Fernald, 2004; Narendra et al., 2013; Nilsson, 1989). Both males and females show positive allometry, larger individuals investing in disproportionately large eyes. However, there appears to be a trade off as ommatidia density shows a negative allometry i.e. larger individuals have disproportionately lower investment in number of ommatidia. This may be due to metabolic constraints, the visual system being energetically expensive (Niven & Laughlin, 2008), so, rather than increasing the density of the ommatidia, larger ommatidia are utilised over the same area of eye.

20.4 POPULATION DIFFERENCES

By comparing males and females between populations using standardised major axis regression and linear modelling, I found that the laboratory-reared population were smaller than the wild Rotorua population and there were significant differences in the allometric relationship of wing centroid size, eye length, and sensilla density compared to body length. Specifically, laboratory females showed a positive allometry for wing size, (cf. strong negative allometry in Rotorua population), laboratory males and females both showed isometry for eye length (and a much weaker negative allometry for ommatidia density) and finally the sensilla density of males was strongly negatively allometric and isometric in females.

Environmental conditions experienced in the developmental stage of the insect can result in different phenotypic traits being expressed in the adult. For example, various traits have been shown to be affected by environmental conditions, with population density and chemical complexity of the diet affecting abundance and complexity of sensory traits in the Orthopterans, *Schistocerca americana* and *Locusta migratoria*, wing size, testes size and antennal length in Lepidopterans, *Uraba lugens* and *Plodia interpunctella* (Chapman, 2002; Gage, 1995; Greenwood & Chapman, 1984; Johnson et al., 2017; Rogers & Simpson, 1997). Given that both *H. illucens* populations were derived from the same genetic stock, the key difference between the two populations is that the laboratory population were reared in higher densities and fed a simpler standard diet compared to the uncontrolled conditions in which the wild, Rotorua

population were reared. Developmental plasticity may account for these observed differences in traits.

20.5 DEVELOPMENTAL PLASTICITY

H. illucens has to be able to tolerate a high degree of environmental stochasticity, so the ability to predict likely conditions in the future based on environmental stimuli experienced during larval development and then adapt adult traits to suit the predicted conditions would help to maximise individual fitness. Developmental plasticity allows for this and, from the density experiment I conducted, there is some evidence to suggest that *H. illucens* utilises developmental plasticity to determine antennal length.

Antennal length in *H. illucens* was found to be larger in low density treatments than it was in high density treatments. This may suggest that antennal length is used to find mates when adult densities are predicted to be low. This is consistent with previous research on the Lepidopteran, *U. lugens* (Johnson et al., 2017). An interesting follow up study would be to dissect out the testes and ovaries of the males and females to investigate if there are trade-offs between sensory traits and reproductive traits based on larval conditions.

Eye length was found to be significantly larger in high density, low volume and low density, high volume treatments which suggests that density does not determine the investment strategy of the individual for eye length. Wing centroid size was unaffected by density treatments, instead body size and sex were the significant predictors of

wing centroid size. This reflects what was found in the geometric morphometric study with sex being the primary driver in shape change.

The standard error in the estimates of the linear models was quite large, possibly reflecting a degree of variability in trait size within the treatments. This natural variation in the population may be similar to the bet-hedging strategy employed by some insects in variable environments which causes individuals to express life histories and traits that may appear to reduce their fitness but, overall, it offers the population a means of coping with environmental stochasticity and therefore ensures the survival of the trait (Menu & Desouhant, 2002; Menu, Roebuck, & Viala, 2000; Menu et al., 2000; Simons, 2011).

20.6 CONCLUSION

In this thesis, I investigated and quantified the sensory morphology of the antennae and compound eyes in *H. illucens*, investigated the allometry of sensory traits and made a comparison of the allometric relationships between sexes and populations, used geometric morphometrics to show that sex is the primary driver of wing shape, and investigated the relationship between investment in sensory traits and larval densities. The research undertaken in this thesis increases our knowledge of the adult biology of *H. illucens* and lays a foundation for the future study of the species. Future research directions include: (1) investigating the influence of larval diet on adult sensory investment, (2) investigate how diet and density influence adult investment in

reproductive traits, (3) examine whether microtrichia respond to mechanical stimulation, and (4) explore the evolution of the behavioural responses to specific wavelengths of light in this species.

Chapter 5: References

- Adams, D. C., Rohlf, F. J., & Slice, D. E. (2004). Geometric morphometrics: Ten years of progress following the 'revolution'. *Italian Journal of Zoology*, 71(1), 5–16. <https://doi.org/10.1080/11250000409356545>
- Alcock, J. (1990). A Large Male Competitive Advantage in a Lekking Fly, *Hermetia Comstocki* Williston (Diptera: Stratiomyidae). *Psyche: A Journal of Entomology*, 97(3–4), 267–279. <https://doi.org/10.1155/1990/72328>
- Alcock, J. (1993). The effects of male body size on territorial and mating success in the landmark-defending fly *Hermetia comstocki* (Stratiomyidae). *Ecological Entomology*, 18(1), 1–6. <https://doi.org/10.1111/j.1365-2311.1993.tb01073.x>
- Amornsak, W., Cribb, B., & Gordh, G. (1998). External morphology of antennal sensilla of *Trichogramma australicum* (Girault) (Hymenoptera: Trichogrammatidae). *International Journal of Insect Morphology and Embryology*, 27(2), 67–82. [https://doi.org/10.1016/S0020-7322\(98\)00003-8](https://doi.org/10.1016/S0020-7322(98)00003-8)
- Anand, H., Ganguly, A., & Halder, P. (2008). Potential value of acridids as high protein supplement for poultry feed. *International Journal of Poultry Science*. Retrieved from <http://agris.fao.org.ezproxy.auckland.ac.nz/agris-search/search.do?recordID=DJ2012052163>
- Andersson, M. B. (1994). *Sexual selection*. Princeton University Press.
- Axtell, R. C., & Arends, J. J. (1990). Ecology and management of arthropod pests of poultry. *Annual Review of Entomology*, 35(1), 101–126 Retrieved from

<https://www.annualreviews.org/doi/abs/10.1146/annurev.en.35.010190.000533?journalCode=ento>.

- Bartley, D. M., Carlberg, J. M., Olst, J. C., & Ford, R. F. (1980). Growth and conversion efficiency of juvenile American lobsters (*Homarus americanus*) in relation to temperature and feeding level. In *Proceedings of the World Mariculture Society* 11, 355–368. Retrieved from <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1749-7345.1980.tb00130.x>
- Berrigan, D. (1991). The Allometry of egg size and number in insects. *Oikos*, 60(3), 313–321. <https://doi.org/10.2307/3545073>
- Berrigan, D., & Pepin, D. J. (1995). How maggots move: Allometry and kinematics of crawling in larval Diptera. *Journal of Insect Physiology*, 41(4), 329–337. [https://doi.org/10.1016/0022-1910\(94\)00113-U](https://doi.org/10.1016/0022-1910(94)00113-U)
- Boland, M. J., Rae, A. N., Vereijken, J. M., Meuwissen, M. P., Fischer, A. R., Boekel, M. A. van, Hendriks, W. H. (2013). The future supply of animal-derived protein for human consumption. *Trends in Food Science & Technology*, 29(1), 62–73. Retrieved from <https://www.sciencedirect.com/science/article/pii/S0924224412001458>
- Bonduriansky, R. (2007). Sexual selection and allometry: A critical reappraisal of the evidence and ideas. *Evolution*, 61(4), 838–849. <https://doi.org/10.1111/j.1558-5646.2007.00081.x>

- Bookstein, F. L. (1991). *Morphometric tools for landmark data: geometry and biology*. Cambridge University Press.
- Bookstein, F. L. (1996). Biometrics, biomathematics and the morphometric synthesis. *Bulletin of Mathematical Biology*, 58(2), 313.
<https://doi.org/10.1007/BF02458311>
- Booth, D. C., & Sheppard, C. (1984). Oviposition of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): eggs, masses, timing, and site characteristics. *Environmental Entomology*, 13(2), 421–423.
<https://doi.org/10.1093/ee/13.2.421>
- Bradbury, J., & Davies, N. (1987). Relative roles of intra-and intersexual selection. *Sexual Selection: Testing the Alternatives*, 143–163.
- Bradbury, J. W. (1977). Lek mating behavior in the hammer-headed bat. *Zeitschrift Für Tierpsychologie*, 45(3), 225–255. <https://doi.org/10.1111/j.1439-0310.1977.tb02120.x>
- Briscoe, A. D., & Chittka, L. (2001). The evolution of color vision in insects. *Annual Review of Entomology*, 46(1), 471–510.
<https://doi.org/10.1146/annurev.ento.46.1.471>
- Byrd, J. H., & Castner, J. L. (2002). *Forensic Entomology: The utility of arthropods in legal investigations*. Boca Raton, FL: CRC Press.

- Campione, N. E., & Evans, D. C. (2012). A universal scaling relationship between body mass and proximal limb bone dimensions in quadrupedal terrestrial tetrapods. *BMC Biology*, 10(1), 60. <https://doi.org/10.1186/1741-7007-10-60>
- Caparros Megido, R., Gierts, C., Blecker, C., Brostaux, Y., Haubruge, É., Alabi, T., & Francis, F. (2016). Consumer acceptance of insect-based alternative meat products in Western countries. *Food Quality and Preference*, 52, 237–243. <https://doi.org/10.1016/j.foodqual.2016.05.004>
- Cardé, R. T. (2016). Moth navigation along pheromone plumes. *Pheromone Communication in Moths: Evolution, Behavior and Application* (173–189) Oakland, CA: University of California Press.
- Cator, L. J., Arthur, B. J., Harrington, L. C., & Hoy, R. R. (2009). Harmonic convergence in the love songs of the dengue vector mosquito. *Science*, 323(5917), 1077–1079. <https://doi.org/10.1126/science.1166541>
- Chapman, R. f. (2002). Development of phenotypic differences in sensillum populations on the antennae of a grasshopper, *Schistocerca americana*. *Journal of Morphology*, 254(2), 186–194. <https://doi.org/10.1002/jmor.10026>
- Chittka, L., & Menzel, R. (1992). The evolutionary adaptation of flower colours and the insect pollinators' colour vision. *Journal of Comparative Physiology A*, 171(2), 171–181. <https://doi.org/10.1007/BF00188925>
- Choi, Y.-C., Choi, J.-Y., Kim, J.-G., Kim, M.-S., Kim, W.-T., Park, K.-H., Jeong, G.-S. (2009). Potential usage of food waste as a natural fertilizer after digestion by

- Hermetia illucens* (Diptera: Stratiomyidae). *International Journal of Industrial Entomology*, 19(1), 171–174.
- Cohen, J. (1992). A power primer. *Psychological Bulletin*, 112(1), 155.
- Colgoni, A., & Vamosi, S. M. (2006). Sexual dimorphism and allometry in two seed beetles (Coleoptera: Bruchidae). *Entomological Science*, 9(2), 171–179.
<https://doi.org/10.1111/j.1479-8298.2006.00164.x>
- Convey, P. (1989). Influences on the choice between territorial and satellite behaviour in male *Libellula quadrimaculata* Linn. (Odonata: Libellulidae). *Behaviour*, 109(1), 125–141. <https://doi.org/10.1163/156853989X00187>
- Courtright, G. N. (2014, May 27). Method and apparatus for breeding flying insects. Yellow Springs, Ohio: United States Patent and Trademark Office. Retrieved from <http://patft.uspto.gov/netacgi/nph-Parser?Sect2=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/search-bool.html&r=1&f=G&l=50&d=PALL&RefSrch=yes&Query=PN/8733284>
- Craig Sheppard, D., Larry Newton, G., Thompson, S. A., & Savage, S. (1994). A value added manure management system using the black soldier fly. *Bioresource Technology*, 50(3), 275–279. [https://doi.org/10.1016/0960-8524\(94\)90102-3](https://doi.org/10.1016/0960-8524(94)90102-3)
- Dahanukar, A., Hallem, E. A., & Carlson, J. R. (2005). Insect chemoreception. *Current Opinion in Neurobiology*, 15(4), 423–430.
<https://doi.org/10.1016/j.conb.2005.06.001>

- Darwin, C. (1859). *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life* (6th ed.). London: W. Cowes & Sons.
- de Freitas Fernandes, F., Linardi, P. M., & Chiarini-Garcia, H. (2002). Morphology of the Antenna of *Dermatobia hominis* (Diptera: Cuterebridae) based on scanning electron microscopy. *Journal of Medical Entomology*, 39(1), 36–43.
<https://doi.org/10.1603/0022-2585-39.1.36>
- Deroy, O., Reade, B., & Spence, C. (2015). The insectivore's dilemma, and how to take the West out of it. *Food Quality and Preference*, 44, 44–55.
<https://doi.org/10.1016/j.foodqual.2015.02.007>
- DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, 13(2), 77–81.
[https://doi.org/10.1016/S0169-5347\(97\)01274-3](https://doi.org/10.1016/S0169-5347(97)01274-3)
- Diener, S., Zurbrugg, C., & Tockner, K. (2009). Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Management & Research: The Journal of the International Solid Wastes and Public Cleansing Association, ISWA*, 27(6), 603–610. Retrieved from journals.sagepub.com/doi/pdf/10.1177/0734242X09103838
<https://doi.org/10.1177/0734242X09103838> [doi]
- Eberhard, W. G. (2002a). Natural history and behavior of *Chymomyza mycopelates* and *C. exophthalma* (Diptera: Drosophilidae), and allometry of structures used

- as signals, weapons, and spore collectors. *The Canadian Entomologist*, 134(5), 667–687. <https://doi.org/10.4039/Ent134667-5>
- Eberhard, W. G. (2002b). The relation between aggressive and sexual behavior and allometry in *Palaeosepsis dentatiformis* flies (Diptera: Sepsidae). *Journal of the Kansas Entomological Society*, 75(4), 317–332.
- Eberhard, W. G., Huber, B. A., S., R. L. R., Briceño, R. D., Salas, I., & Rodriguez, V. (1998). One size fits all? Relationships between the size and degree of variation in genitalia and other body parts in twenty species of insects and spiders. *Evolution*, 52(2), 415–431. <https://doi.org/10.2307/2411078>
- Fernald, R. D. (2004). Eyes: variety, development and evolution. *Brain, Behavior and Evolution*, 64(3), 141–147. <https://doi.org/10.1159/000079743>
- Fisher, R. A. (1930). *The genetical theory of natural selection: a complete variorum edition* (2nd ed.). Oxford, UK: Oxford University Press.
- Fрати, F., Piersanti, S., Rebora, M., & Salerno, G. (2016). Volatile cues can drive the oviposition behavior in Odonata. *Journal of Insect Physiology*, 91–92, 34–38. <https://doi.org/10.1016/j.jinsphys.2016.06.007>
- Fromhage, L., & Kokko, H. (2014). Sexually selected traits evolve positive allometry when some matings occur irrespective of the trait. *Evolution*, 68(5), 1332–1338. <https://doi.org/10.1111/evo.12349>

- Gage, M. J. G. (1995). Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proc. R. Soc. Lond. B*, 261(1360), 25–30. <https://doi.org/10.1098/rspb.1995.0112>
- Glazier, D. S., & Deptola, T. J. (2011). The amphipod *Gammarus minus* has larger eyes in freshwater springs with numerous fish predators. *Invertebrate Biology*, 130(1), 60–67. <https://doi.org/10.1111/j.1744-7410.2010.00220.x>
- Gnatzy, W., Grünert, U., & Bender, M. (1987). Campaniform sensilla of *Calliphora vicina* (Insecta, Diptera). *Zoomorphology*, 106(5), 312–319. <https://doi.org/10.1007/BF00312005>
- Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Toulmin, C. (2010). Food Security: The challenge of feeding 9 billion people. *Science*, 327(5967), 812–818. <https://doi.org/10.1126/science.1185383>
- Goldsmith, T. H., & Philpott, D. E. (1957). The microstructure of the compound eyes of insects. *The Journal of Cell Biology*, 3(3), 429–440. <https://doi.org/10.1083/jcb.3.3.429>
- Goodall, C. (1991). Procrustes methods in the statistical analysis of shape. *Journal of the Royal Statistical Society. Series B (Methodological)*, 53(2), 285–339.
- Gould, S. J. (1966). Allometry and size in ontogeny and phylogeny. *Biological Reviews*, 41(4), 587–638. <https://doi.org/10.1111/j.1469-185X.1966.tb01624.x>
- Greenwood, M., & Chapman, R. F. (1984). Differences in numbers of sensilla on the antennae of solitary and gregarious *Locusta migratoria* L. (Orthoptera:

- Acrididae). *International Journal of Insect Morphology and Embryology*, 13(4), 295–301. [https://doi.org/10.1016/0020-7322\(84\)90004-7](https://doi.org/10.1016/0020-7322(84)90004-7)
- Gross, M. R., & Charnov, E. L. (1980). Alternative male life histories in bluegill sunfish. *Proceedings of the National Academy of Sciences*, 77(11), 6937–6940.
- Hallberg, E., & Hansson, B. S. (1999). Arthropod sensilla: Morphology and phylogenetic considerations. *Microscopy Research and Technique*, 47(6), 428–439. [https://doi.org/10.1002/\(SICI\)1097-0029\(19991215\)47:6](https://doi.org/10.1002/(SICI)1097-0029(19991215)47:6)
- Hallem, E. A., Dahanukar, A., & Carlson, J. R. (2006). Insect odor and taste receptors. *Annual Review of Entomology*, 51(1), 113–135. <https://doi.org/10.1146/annurev.ento.51.051705.113646>
- Hamerman, E. J. (2016). Cooking and disgust sensitivity influence preference for attending insect-based food events. *Appetite*, 96, 319–326. <http://dx.doi.org/10.1016/j.appet.2015.09.029>
- Hansson, B. S., & Stensmyr, M. C. (2011). Evolution of insect olfaction. *Neuron*, 72(5), 698–711. <https://doi.org/10.1016/j.neuron.2011.11.003>
- Hosken, D. J., Minder, A. M., & Ward, P. I. (2005). Male genital allometry in Scathophagidae (Diptera). *Evolutionary Ecology*, 19(5), 501–515. <https://doi.org/10.1007/s10682-005-1023-z>
- Howard, J., Blakeslee, B., & Laughlin, S. B. (1987). The intracellular pupil mechanism and photoreceptor signal: noise ratios in the fly *Lucilia cuprina*.

- Proc. R. Soc. Lond. B*, 231(1265), 415–435.
<https://doi.org/10.1098/rspb.1987.0053>
- Hu, F., Zhang, G.-N., Jia, F.-X., Dou, W., & Wang, J.-J. (2010). Morphological characterization and distribution of antennal sensilla of six fruit flies (Diptera: Tephritidae). *Annals of the Entomological Society of America*, 103(4), 661–670. <https://doi.org/10.1603/AN09170>
- Humfeld, S. C. (2008). Intersexual dynamics mediate the expression of satellite mating tactics: unattractive males and parallel preferences. *Animal Behaviour*, 75(1), 205–215. <https://doi.org/10.1016/j.anbehav.2007.05.015>
- Huxley, J. (1932). *Problems of relative growth*. Baltimore, MA: Johns Hopkins University Press.
- Huxley, J. S., & Teissier, G. (1936). Terminology of relative growth. *Nature*, 137(3471), 780. <https://doi.org/10.1038/137780b0>
- Huxley, Julian S. (1924). Constant differential growth-ratios and their significance. *Nature*, 114(2877), 895. <https://doi.org/10.1038/114895a0>
- Huxley, Julian S., Needham, J., & Lerner, I. M. (1941). Terminology of relative growth-rates. *Nature*, 148(3747), 225. <https://doi.org/10.1038/148225a0>
- Ihaka, R., & Gentleman, R. (1996). R: A language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, 5(3), 299–314.
<https://doi.org/10.1080/10618600.1996.10474713>

- Jeram, S., & Pabst, M. A. (1996). Johnston's organ and central organ in *Nezara viridula* (L.) (Heteroptera, Pentatomidae). *Tissue and Cell*, 28(2), 227–235.
[https://doi.org/10.1016/S0040-8166\(96\)80011-5](https://doi.org/10.1016/S0040-8166(96)80011-5)
- Johnson, T. L., Symonds, M. R. E., & Elgar, M. A. (2017). Anticipatory flexibility: larval population density in moths determines male investment in antennae, wings and testes. *Proc. R. Soc. B*, 284(1866), 20172087.
<https://doi.org/10.1098/rspb.2017.2087>
- Jorgensen, N. M., & James, M. T. (1968). Evolution of the antenna in the Stratiomyidae (Diptera). *Annals of the Entomological Society of America*, 61(4), 870–878. <https://doi.org/10.1093/aesa/61.4.870>
- Jr, C. B., Newton, G. L., Hale, O. M., & Barker, R. W. (1977). Manure residue as a substrate for protein production via *Hermetia illucens* larvae [for swine rations]. In 9. *Cornell Agricultural Waste Management Conference. Ann Arbor, Mich.(USA). 1977*.
- Keil, T. A. (1997). Functional morphology of insect mechanoreceptors. *Microscopy Research and Technique*, 39(6), 506–531. [https://doi.org/10.1002/\(SICI\)1097-0029\(19971215\)39:6<506::AID-JEMT5>3.0.CO;2-B](https://doi.org/10.1002/(SICI)1097-0029(19971215)39:6<506::AID-JEMT5>3.0.CO;2-B)
- Kelly, C. D. (2005). Allometry and sexual selection of male weaponry in Wellington tree weta, *Hemideina crassidens*. *Behavioral Ecology*, 16(1), 145–152.
<https://doi.org/10.1093/beheco/arh141>

- Klingenberg, C. P. (1996). Multivariate allometry. In *Advances in Morphometrics* (pp. 23–49). Springer, Boston, MA. https://doi.org/10.1007/978-1-4757-9083-2_3
- Klingenberg, C. P. (2011). MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, 11(2), 353–357. <https://doi.org/10.1111/j.1755-0998.2010.02924.x>
- Klingenberg, C. P. (2016). Size, shape, and form: concepts of allometry in geometric morphometrics. *Development Genes and Evolution*, 226(3), 113–137. <https://doi.org/10.1007/s00427-016-0539-2>
- Klingenberg, C. P., McIntyre, G. S., & Zaklan, S. D. (1998). Left–right asymmetry of fly wings and the evolution of body axes. *Proceedings of the Royal Society of London B: Biological Sciences*, 265(1402), 1255–1259. <https://doi.org/10.1098/rspb.1998.0427>
- Klingenberg, C. P., Monteiro, L. R., & MacLeod, N. (2005). Distances and directions in multidimensional shape spaces: implications for morphometric applications. *Systematic Biology*, 54(4), 678–688. <https://doi.org/10.1080/10635150590947258>
- Knight, K. (2017). Male mosquitoes sing from the same song sheet. *Journal of Experimental Biology*, 220(3), 230–230. <https://doi.org/10.1242/jeb.155135>
- Lakes-Harlan, R., Stölting, H., & Stumpner, A. (1999). Convergent evolution of insect hearing organs from a preadaptive structure. *Proceedings of the Royal Society*

- of London B: Biological Sciences*, 266(1424), 1161–1167.
<https://doi.org/10.1098/rspb.1999.0758>
- Land, M. F. (1989). Variations in the structure and design of compound eyes. In *Facets of Vision* (pp. 90–111). Berlin, Heidelberg: Springer
https://doi.org/10.1007/978-3-642-74082-4_5
- Land, M. F. (1997). Visual acuity in insects. *Annual Review of Entomology*, 42(1), 147–177. <https://doi.org/10.1146/annurev.ento.42.1.147>
- Lane, R. P. (1981). Allometry of size in three species of biting midges (Diptera, Ceratopogonidae, Culicoides). *Journal of Natural History*, 15(5), 775–788.
<https://doi.org/10.1080/00222938100770571>
- Leary, C. J., Fox, D. J., Shepard, D. B., & Garcia, A. M. (2005). Body size, age, growth and alternative mating tactics in toads: satellite males are smaller but not younger than calling males. *Animal Behaviour*, 70(3), 663–671.
<https://doi.org/10.1016/j.anbehav.2004.12.013>
- Lee, W.-Y., Chang, J.-C., Hwang, Y.-B., & Lin, T.-L. (1994). Morphology of the antennal sensilla of the oriental fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae). *Zoological Studies*, 33(1), 65–71.
- Legay, J.-M. (1977). Allometry and systematics Insect egg form. *Journal of Natural History*, 11(5), 493–499. <https://doi.org/10.1080/00222937700770421>

- Leong, S. Y., Kutty, S. R. M., Malakahmad, A., & Tan, C. K. (2016). Feasibility study of biodiesel production using lipids of *Hermetia illucens* larva fed with organic waste. *Waste Management*, 47, 84–90.
- Li, Q., Zheng, L., Cai, H., Garza, E., Yu, Z., & Zhou, S. (2011). From organic waste to biodiesel: Black soldier fly, *Hermetia illucens*, makes it feasible. *Fuel*, 90(4), 1545–1548.
- Mason, F. (2013). Updated Italian checklist of soldier flies (Diptera, Stratiomyidae). *ZooKeys*, (336), 61–78. <https://doi.org/10.3897/zookeys.336.6016>
- May, B. M. (1961). The occurrence in New Zealand and the life-history of the soldier fly *Hermetia illucens* (L.)(Diptera: Stratiomyidae). *NZJ Sci*, 4(5).
- McCallan, E. (1974). *Hermetia illucens* (L.) (Diptera, Stratiomyidae), a cosmopolitan American species long established in Australia and New Zealand. *Entomologist's Monthly Magazine*, 232–233.
- Menu, F., & Desouhant, E. (2002). Bet-hedging for variability in life cycle duration: bigger and later-emerging chestnut weevils have increased probability of a prolonged diapause. *Oecologia*, 132(2), 167–174.
<https://doi.org/10.1007/s00442-002-0969-6>
- Menu, F., Roebuck, J., & Viala, M. (2000). Bet-Hedging diapause strategies in stochastic environments. *The American Naturalist*, 155(6), 724–734.
<https://doi.org/10.1086/303355>

- Miranda G., X. (2000). Sexual dimorphism in the aristae of *Ceratitis capitata* (Diptera, Tephritidae) and its possible importance in courtship. *Journal of the New York Entomological Society*, 108(3 & 4), 339–348. [https://doi.org/10.1664/0028-7199\(2000\)108\[0339:SDITAO\]2.0.CO;2](https://doi.org/10.1664/0028-7199(2000)108[0339:SDITAO]2.0.CO;2)
- Moczek, A. P., & Emlen, D. J. (2000). Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do alternative reproductive tactics favour alternative phenotypes? *Animal Behaviour*, 59(2), 459–466. <https://doi.org/10.1006/anbe.1999.1342>
- Myers, H. M., Tomberlin, J. K., Lambert, B. D., & Kattes, D. (2008). Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. *Environmental Entomology*, 37(1), 11–15.
- Nakagaki, B. J., & Defoliart, G. R. (1991). Comparison of diets for mass-rearing *Acheta domesticus* (Orthoptera: Gryllidae) as a novelty food, and comparison of food conversion efficiency with values reported for livestock. *Journal of Economic Entomology*, 84(3), 891–896. <https://doi.org/10.1093/jee/84.3.891>
- Nakagawa, S. (2004). A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behavioral Ecology*, 15(6), 1044–1045. <https://doi.org/10.1093/beheco/arh107>
- Nakamura, S., Ichiki, R. T., Shimoda, M., & Morioka, S. (2015). Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the

- laboratory: low-cost and year-round rearing. *Applied Entomology and Zoology*, 51(1), 161–166. <https://doi.org/10.1007/s13355-015-0376-1>
- Narendra, A., Alkaladi, A., Raderschall, C. A., Robson, S. K. A., & Ribi, W. A. (2013). Compound eye adaptations for diurnal and nocturnal lifestyle in the intertidal ant, *Polyrhachis sokolova*. *PLOS ONE*, 8(10), e76015. <https://doi.org/10.1371/journal.pone.0076015>
- Needham, J., & Lerner, I. M. (1940). Terminology of relative growth-rates. *Nature*, 146(3706), 618. <https://doi.org/10.1038/146618a0>
- Newton, G. L., Sheppard, D. C., Watson, D. W., Burtle, G. J., Dove, C. R., Tomberlin, J. K., & Thelen, E. E. (2005). The black soldier fly, *Hermetia illucens*, as a manure management/resource recovery tool. In *Symposium on the state of the science of Animal Manure and Waste Management* (pp. 5–7).
- Newton, L., Sheppard, C., Watson, D. W., Burtle, G., & Dove, R. (2005). Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. *Animal and Poultry Waste Management Center*, Raleigh, NC: North Carolina State University
- Nilsson, D.-E. (1989). Optics and evolution of the compound eye. In *Facets of Vision* (pp. 30–73). Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-74082-4_3

- Niven, J. E., Anderson, J. C., & Laughlin, S. B. (2007). Fly photoreceptors demonstrate energy-information trade-offs in neural coding. *PLOS Biology*, 5(4), e116. <https://doi.org/10.1371/journal.pbio.0050116>
- Niven, J. E., & Laughlin, S. B. (2008). Energy limitation as a selective pressure on the evolution of sensory systems. *Journal of Experimental Biology*, 211(11), 1792–1804. <https://doi.org/10.1242/jeb.017574>
- O'Hanlon, J. C., Holwell, G. I., & Herberstein, M. E. (2014). Pollinator deception in the orchid mantis. *The American Naturalist*, 183(1), 126–132. <https://doi.org/10.1086/673858>
- Okada, J., & Toh, Y. (2006). Active tactile sensing for localization of objects by the cockroach antenna. *Journal of Comparative Physiology A*, 192(7), 715–726. <https://doi.org/10.1007/s00359-006-0106-9>
- Olberg, R. M. (2012). Visual control of prey-capture flight in dragonflies. *Current Opinion in Neurobiology*, 22(2), 267–271. <https://doi.org/10.1016/j.conb.2011.11.015>
- Ooninx, D. G. A. B., Volk, N., Diehl, J. J. E., van Loon, J. J. A., & Belušič, G. (2016a). Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (*Hermetia illucens*) informing the design of LED-based illumination to enhance indoor reproduction. *Journal of Insect Physiology*, 95(Supplement C), 133–139. <https://doi.org/10.1016/j.jinsphys.2016.10.006>

- Oonincx, D. G. A. B., Volk, N., Diehl, J. J. E., van Loon, J. J. A., & Belušič, G. (2016b). Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (*Hermetia illucens*) informing the design of LED-based illumination to enhance indoor reproduction. *Journal of Insect Physiology*, 95(Supplement C), 133–139. <https://doi.org/10.1016/j.jinsphys.2016.10.006>
- Orr, H. A. (2009). Fitness and its role in evolutionary genetics. *Nature Reviews Genetics*, 10(8), 531–539. <https://doi.org/10.1038/nrg2603>
- Painting, C. J., & Holwell, G. I. (2013). Exaggerated trait allometry, compensation and trade-offs in the New Zealand giraffe weevil (*Lasiornychus barbicornis*). *PLOS ONE*, 8(11), e82467. <https://doi.org/10.1371/journal.pone.0082467>
- Painting, C. J., & Holwell, G. I. (2014). Flexible alternative mating tactics by New Zealand giraffe weevils. *Behavioral Ecology*, 25(6), 1409–1416. <https://doi.org/10.1093/beheco/aru140>
- Pezzi, M., Leis, M., Chicca, M., Falabella, P., Salvia, R., Scala, A., & Whitmore, D. (2017). Morphology of the antenna of *Hermetia illucens* (Diptera: Stratiomyidae): an ultrastructural investigation. *Journal of Medical Entomology*, 54(4), 925–933. <https://doi.org/10.1093/jme/tjx055>
- Pujol-Luz, J. R., Francez, P. A. da C., Ururahy-Rodrigues, A., & Constantino, R. (2008). The black soldier-fly, *Hermetia illucens* (Diptera, Stratiomyidae), used to estimate the postmortem interval in a case in Amapá State, Brazil. *Journal of Forensic Sciences*, 53(2), 476–478.

- Ramos-Elorduy, J., González, E. A., Hernández, A. R., & Pino, J. M. (2002). Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. *Journal of Economic Entomology*, 95(1), 214–220. <https://doi.org/10.1603/0022-0493-95.1.214>
- Rogers, S., & Simpson, S. (1997). Experience-dependent changes in the number of chemosensory sensilla on the mouthparts and antennae of. *Journal of Experimental Biology*, 200(17), 2313–2321.
- Rohlf, F. J. (1993). Relative warp analysis and an example of its application to mosquito. *Contributions to Morphometrics*, 8, 131.
- Rohlf, F. J. (2015). The tps series of software. *Hystrix, the Italian Journal of Mammalogy*, 26(1), 9–12.
- Rohlf, F. J., & Marcus, L. F. (1993). A revolution in morphometrics. *Trends in Ecology & Evolution*, 8(4), 129–132. [https://doi.org/10.1016/0169-5347\(93\)90024-J](https://doi.org/10.1016/0169-5347(93)90024-J)
- Rozkosný, R. (1983). *A Biosystematic Study of the European Stratiomyidae (Diptera): Volume 2 - Clitellariinae, Hermediinae, Pachygasterinae and Bibliography*. Springer Science & Business Media.
- Rumpold, B. A., & Schlüter, O. K. (2013). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science & Emerging Technologies*, 17, 1–11. <https://doi.org/10.1016/j.ifset.2012.11.005>

- Sanford, M. R., Demirci, B., Marsden, C. D., Lee, Y., Cornel, A. J., & Lanzaro, G. C. (2011). Morphological differentiation may mediate mate-choice between incipient species of *Anopheles gambiae* s.s. *PLOS ONE*, 6(11), e27920. <https://doi.org/10.1371/journal.pone.0027920>
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676. <https://doi.org/10.1038/nmeth.2019>
- Schneider, D. (1964). Insect antennae. *Annual Review of Entomology*, 9(1), 103–122. <https://doi.org/10.1146/annurev.en.09.010164.000535>
- Schneider, E. S., & Schmitz, H. (2014). Thermomechanical properties of the stimulus transducing cuticle in the infrared organ of *Merimna atrata* (Coleoptera, Buprestidae). *Journal of Morphology*, 275(9), 991–1003. <https://doi.org/10.1002/jmor.20276>
- Schoener, T. W. (2011). The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science*, 331(6016), 426–429. <https://doi.org/10.1126/science.1193954>
- Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology*, 39(4), 695–698.

- Sheppard, D. Craig, Newton, G. L., Thompson, S. A., & Savage, S. (1994). A value added manure management system using the black soldier fly. *Bioresource Technology*, 50(3), 275–279.
- Shingleton, A. (2010). Allometry: the study of biological scaling. *Nature Education Knowledge*, 3(10), 2.
- Simons, A. M. (2011). Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20110176.
<https://doi.org/10.1098/rspb.2011.0176>
- Skals, N., Anderson, P., Kanneworff, M., Löfstedt, C., & Surlykke, A. (2005). Her odours make him deaf: crossmodal modulation of olfaction and hearing in a male moth. *Journal of Experimental Biology*, 208(4), 595–601.
<https://doi.org/10.1242/jeb.01400>
- Smallegange, R. C., Kelling, F. J., & Otter, C. J. D. (2008). Types and numbers of sensilla on antennae and maxillary palps of small and large houseflies, *Musca domestica* (Diptera, Muscidae). *Microscopy Research and Technique*, 71(12), 880–886. <https://doi.org/10.1002/jemt.20636>
- Städler, E. (1984). Contact Chemoreception. In *Chemical Ecology of Insects* (pp. 3–35). Boston, MA: Springer. https://doi.org/10.1007/978-1-4899-3368-3_1
- Stavenga, D. G., Meglič, A., Pirih, P., Koshitaka, H., Arikawa, K., Wehling, M. F., & Belušič, G. (2017). Photoreceptor spectral tuning by colorful, multilayered

- facet lenses in long-legged fly eyes (Dolichopodidae). *Journal of Comparative Physiology A*, 203(1), 23–33. <https://doi.org/10.1007/s00359-016-1131-y>
- Stavenga, D. G., Wehling, M. F., & Belušič, G. (2017). Functional interplay of visual, sensitizing and screening pigments in the eyes of *Drosophila* and other red-eyed dipteran flies. *The Journal of Physiology*, 595(16), 5481–5494. <https://doi.org/10.1113/JP273674>
- Steinbrecht, R. A. (1987). 11 - Functional morphology of pheromone-sensitive sensilla. In G. D. Prestwich & G. J. Blomquist (Eds.), *Pheromone Biochemistry* (pp. 353–384). New York, NY: Academic Press. <https://doi.org/10.1016/B978-0-12-564485-3.50016-6>
- St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., Irving, S. (2007). Fish offal recycling by the black soldier fly produces a foodstuff high in Omega-3 Fatty acids. *Journal of the World Aquaculture Society*, 38(2), 309–313.
- Stork, N. E. (1988). Insect diversity: facts, fiction and speculation. *Biological Journal of the Linnean Society*, 35(4), 321–337. <https://doi.org/10.1111/j.1095-8312.1988.tb00474.x>
- Stork, N. E. (2018). How many species of insects and other terrestrial arthropods are there on earth? *Annual Review of Entomology*, 63(1), 31–45. <https://doi.org/10.1146/annurev-ento-020117-043348>

- Stork, N. E., McBroom, J., Gely, C., & Hamilton, A. J. (2015). New approaches narrow global species estimates for beetles, insects, and terrestrial arthropods. *Proceedings of the National Academy of Sciences*, 112(24), 7519–7523.
<https://doi.org/10.1073/pnas.1502408112>
- Stumpner, A., & Helversen, D. von. (2001). Evolution and function of auditory systems in insects. *Naturwissenschaften*, 88(4), 159–170.
<https://doi.org/10.1007/s001140100223>
- Tabassum-Abbasi, Abbasi, T., & Abbasi, S. A. (2016). Reducing the global environmental impact of livestock production: the minilivestock option. *Journal of Cleaner Production*, 112, Part 2, 1754–1766.
<https://doi.org/10.1016/j.jclepro.2015.02.094>
- Taskinen, S., & Warton, D. I. (2013). Robust tests for one or more allometric lines. *Journal of Theoretical Biology*, 333, 38–46.
<https://doi.org/10.1016/j.jtbi.2013.05.010>
- Teissier, G. (1960). Relative Growth. In *The physiology of Crustacea*. (pp. 537–560). New York, NY: Academic Press 1960-61.
- Thompson, D. W. (1917). *On growth and form*. Cambridge, UK: University Press.
Retrieved from
<http://tinyurl.galegroup.com.ezproxy.auckland.ac.nz/tinyurl/5MTk7X>
- Tomberlin, J. K., & Sheppard, D. C. (2001). Lekking behavior of the black soldier fly (Diptera: Stratiomyidae). *Florida Entomologist*, 84(4), 729–730.

- Tomberlin, J. K., & Sheppard, D. C. (2002). Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. *Journal of Entomological Science*, 37(4), 345–352. <https://doi.org/10.18474/0749-8004-37.4.345>
- Tomberlin, J. K., Sheppard, D. C., & Joyce, J. A. (2002). Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. *Annals of the Entomological Society of America*, 95(3), 379–386.
- Tomberlin, J., Sheppard, D., & Joyce, J. (2005). Black soldier fly (Diptera: Stratiomyidae) colonization of pig carrion in south Georgia. *Journal of Forensic Sciences*, 50(1), 152–153.
- Tschirner, M., & Simon, A. (2015). Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. *Journal of Insects as Food and Feed*, 1(4), 249–259.
- United Nations, Department of Economic and Social Affairs. (2015). *World Population Prospects: The 2015 Revision, Key Findings and Advance Tables* (Working Paper No. ESA/P/WP.241).
- Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). Edible insects: future prospects for food and feed security. *FAO Forestry Paper (FAO)*. Retrieved from <http://agris.fao.org.ezproxy.auckland.ac.nz/agris-search/search.do?recordID=XF2013000989>

- Warrant, E. J. (2017). Visual tracking: hot pursuit with tiny eyes. *Current Biology*, 27(6), R234–R237. <https://doi.org/10.1016/j.cub.2017.02.032>
- Warton, D. I., Duursma, R. A., Falster, D. S., & Taskinen, S. (2012). smatr 3– an R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution*, 3(2), 257–259. <https://doi.org/10.1111/j.2041-210X.2011.00153.x>
- Webb, J. C., Calkins, C. O., Chambers, D. L., Schwienbacher, W., & Russ, K. (1983). Acoustical aspects of behavior of mediterranean fruit fly, *Ceratitis Capitata*: Analysis and identification of courtship sounds. *Entomologia Experimentalis et Applicata*, 33(1), 1–8. <https://doi.org/10.1111/j.1570-7458.1983.tb03224.x>
- West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, 20(1), 249–278. <https://doi.org/10.1146/annurev.es.20.110189.001341>
- Wilkinson, G. S. (1993). Artificial sexual selection alters allometry in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). *Genetics Research*, 62(3), 213–222. <https://doi.org/10.1017/S001667230003192X>
- Woods Jr., W. A., Hendrickson, H., Mason, J., & Lewis, S. M. (2007). Energy and predation costs of firefly courtship signals. *The American Naturalist*, 170(5), 702–708. <https://doi.org/10.1086/521964>

- Zacharuk, R. Y. (1980). Ultrastructure and function of insect chemosensilla. *Annual Review of Entomology*, 25(1), 27–47.
<https://doi.org/10.1146/annurev.en.25.010180.000331>
- Zhang, J., Huang, L., He, J., Tomberlin, J. K., Li, J., Lei, C., Yu, Z. (2010). An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. *Journal of Insect Science*, 10(1), 202.
<https://doi.org/10.1673/031.010.20201>
- Zheng, L., Crippen, T. L., Holmes, L., Singh, B., Pimsler, M. L., Benbow, M. E., Tomberlin, J. K. (2013). Bacteria mediate oviposition by the black soldier fly, *Hermetia illucens* (L.), (Diptera: Stratiomyidae). *Scientific Reports*, 3.
<https://doi.org/10.1038/srep02563>
- Zuidhof, M. J., Molnar, C. L., Morley, F. M., Wray, T. L., Robinson, F. E., Khan, B. A., Goonewardene, L. A. (2003). Nutritive value of house fly (*Musca domestica*) larvae as a feed supplement for turkey poults. *Animal Feed Science and Technology*, 105(1–4), 225–230. [https://doi.org/10.1016/S0377-8401\(03\)00004-X](https://doi.org/10.1016/S0377-8401(03)00004-X)