



A University of Sussex PhD thesis

Available online via Sussex Research Online:

<http://sro.sussex.ac.uk/>

This thesis is protected by copyright which belongs to the author.

This thesis cannot be reproduced or quoted extensively from without first obtaining permission in writing from the Author

The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the Author

When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given

Please visit Sussex Research Online for more information and further details

NOVEL MORPHOLOGICAL AND PHYSIOLOGICAL SCALING RELATIONSHIPS IN THE SOUTHERN RED WOOD ANT

Craig Darren Perl

University of Sussex

This thesis is submitted to the University of Sussex in application for the degree of
Doctor of Philosophy

December 2017

Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration with others except where specifically indicated in the text. No part of this dissertation has been submitted to any other university in application for a higher degree.

Signature:

Craig Perl

1st December, 2017

Summary

Red wood ants (*Formica rufa*) are visual navigators whose colonies contain workers that differ substantially in size. By investigating the allometry of the ants' compound eyes, and the regions within them, I showed that facets in particular regions scaled differently: both grade and slope shifts occurred. Facets in some eye regions were absolutely larger than others, while other facet regions scaled at different rates with body size.

I next compared eye scaling between nests from the same population. Nevertheless, the method by which ants increased their eye size differed between nests. I found that ants from some nests primarily increased eye size through facet number and others through facet diameter. This showed that scaling rules at the cellular levels can differ even within a single population.

Comparisons among *Formica* species revealed that differential eye scaling was not restricted to just *F. rufa*. Differential scaling was found in *F. sanguinea* but not *F. lugubris* or *F. fusca*. Surprisingly, scaling between facet diameter and number was conserved across all four species, demonstrating that whole-organ scaling among species can be conservative whilst differing vastly between organ-regions.

Moving beyond morphology, I next investigated whether physiological scaling was equally as variable among nests. Metabolic rate scaling was negatively allometric and the same among four nests. Respiratory water loss was found to be determined solely by metabolic rate. Metabolic rate co-varies with different ventilation types, however, switches in ventilation type are driven by movement. This demonstrates that increases in metabolic rate are not sufficient to explain changes in ventilation type but are sufficient to explain respiratory water loss.

Acknowledgements

First and foremost, I would like to acknowledge the unfathomable amount of support I have received from my supervisor, Jeremy Niven. His unquenchable pursuit of scientific rigour (and cake) is an inspiration to all of us. It would behove everyone to emulate Jeremy's unparalleled dedication to his students. My deepest gratitude to my co-supervisor Paul Graham, for not only facilitating access to wood ants but for the best welcome I could have hoped for on my first day at Sussex – being called a pleb and told to move some chairs around.

To all of the CCNR dwellers, mostly I'm sorry. Marta, Sofia, Cornelia, Laura, James, Sergio, Adrian and Paul, I couldn't have asked for better office mates; willing to tolerate the most unsavoury comments being shouted across the room, your acceptance has been truly appreciated. I believe we even got some work done sometimes. Special mention to Sian; you're a terrible human and I wouldn't be shouting about murders across the office if you weren't there to normalise that sort of behaviour so this is clearly all your fault.

Other PhD students living outside of the third-floor basement deserve huge thanks for their role in allowing me to fitfully grasp hold of a few scraps of sanity. Joanne has fed me more mugs of tea than I can possibly count, each one an invaluable corridor of escape into literature, sentient spiders and science fiction. And sometimes Sambuca. To Rosaline, who I think just about tolerates me now, your forthright advice has been as insightful as it is illuminating.

David Treanor has served dutifully as my companion through the world of ant research, whiskey consumption and Brighton housing. David Sullivan, another survivor of Ewhurst Road, showed me that you can achieve a surprising amount with little supervision and a lot of computer games. Scott Clay cannot receive enough recognition;

procrastinator-in-chief, uncompromising drinking companion and consumer of more chicken than I thought humanly possible. You are an inspiration to all of us. Jenny James, I never thought anyone would be able to hate everything with such a fervency, thank you for helping me to reach my true apathetic potential. Also, thanks for occupying my sofa constantly, though the flat does feel a bit empty now. The writhing tentacles of madness that live in my head would be larger, scarier and crawling from my ears if not for Chris and Tanya Accleton-Pennell, our joint passion for rock climbing is something I hope we pursue long into the future.

Special thanks go to Declan Patrick MacManus, your words and music have filled my mind and soul, turning me into a very strange person indeed. Even bigger thanks, appreciation and love go to Corinne who has supported me more than I deserve. I am truly grateful for her undying comfort and encouragement, no matter how pouty I'm being. Or how much I'm blathering about statistics. Or insects. Or Elvis Costello. She is wonderful and I could not ask for a better companion.

This work is dedicating to my parents, Gary and Lorraine. You have both been unwaveringly accommodating throughout all my academic pursuits despite never really understanding what I was doing, why on earth I would want to do it and when it will stop. Thank you.

Publications

To date, the following publications have arisen in whole or part from this thesis:

Perl, Craig D. and Niven, Jeremy E. (2016) Differential scaling within an insect compound eye. *Biology Letters*, **12** (3).

Perl, Craig D. and Niven, Jeremy E. (2016) Colony-level differences in the scaling rules governing wood ant compound eye structure. *Scientific Reports*, **6**, p. 24204.

Perl, Craig D., Rossoni, Sergio, and Niven, Jeremy E. (2017) Conservative whole-organ scaling contrasts with highly labile suborgan scaling differences among compound eyes of closely related *Formica* ants. *Ecology and Evolution*, **7**, 1663–1673.

Perl, Craig D. and Niven, Jeremy, E. (2017) Metabolic rate scaling, ventilation patterns and respiratory water loss in red wood ants: Activity drives ventilation changes, metabolic rate drives water loss. *In review*.

Contents

Declaration.....	ii
Summary	iii
Acknowledgements	iv
Publications	vi
Contents.....	vii
Chapter 1: General introduction.....	I
1.1 Morphological scaling	I
1.2 Types of allometry	2
1.3 Ecological implications in insects	4
1.4 Developmental scaling mechanisms in insects	7
1.5 Insect compound eyes and scaling	9
1.6 Evolvability of scaling.....	12
1.7 Physiological scaling	14
1.8 Metabolic rate.....	15
1.9 Gas exchange in insects.....	19
1.10 Ants.....	24
1.11 Summary of contributions	26
Chapter 2: Differential scaling within an insect compound eye	29
2.1 Abstract.....	29
2.2 Introduction.....	29
2.3 Material and methods	31
2.3.1 Animals	31
2.3.2 Specimen preparation	31
2.3.3 Measurements.....	32
2.3.4 Statistics.....	33
2.4 Results	33
2.5 Discussion	34
2.5.1 Proximate mechanisms	35
2.5.2 Functional implications.....	35
Chapter 3: Colony-level differences in wood ant eye scaling.....	37
3.1 Abstract.....	37
3.2 Introduction.....	37
3.3 Methods.....	41
3.3.1 Animals	41
3.3.2 Specimen preparation	42

3.3.3 Measurements.....	42
3.3.4 Statistics.....	43
3.4 Results	44
3.4.1 Size variation in wood ants.....	44
3.4.2 Eye morphology.....	44
3.5 Discussion.....	48
3.6 Supplementary materials	Error! Bookmark not defined.
Chapter 4: Conservative whole-organ scaling contrasts with highly labile sub-organ scaling differences among compound eyes of closely-related <i>Formica</i> ants.....	59
4.1 Abstract.....	59
4.2 Introduction.....	59
4.3 Materials and Methods.....	62
4.3.1 Animals	62
4.3.2 Specimen preparation	64
4.3.3 Statistics.....	65
4.4 Results	67
4.4.1 Allometric scaling of compound eyes and facets of <i>Formica</i> species	67
4.4.2 Scaling of facet number with diameter.....	69
4.4.3 Intra-eye scaling.....	70
4.5 Discussion.....	77
4.6 Supplementary materials	82
Chapter 5: Metabolic rate scaling, ventilation patterns and respiratory water loss in red wood ants.....	90
5.1 Abstract.....	90
5.2 Introduction.....	90
5.3 Materials and methods.....	93
5.3.1 Animals	93
5.3.2 Metabolic rate and water loss measurement	94
5.3.3 Ventilation type classification and exclusions.....	95
5.3.4 Dry mass	96
5.3.5 Analysis	96
5.4 Results	98
5.4.1 Experiment 1	98
5.4.2 Experiment 2.....	105
5.5 Discussion.....	108
5.5.1 Ventilation pattern is independent of metabolic rate.....	109
5.5.2 The absence of discontinuous gas exchange	111
5.5.3 Metabolic rate drives respiratory water loss independent of ventilation pattern....	112

5.5.4 Homogeneous physiological scaling among colonies.....	113
5.5.5 Intraspecific metabolic scaling in wood ant foragers	114
5.6 Supplementary material	Error! Bookmark not defined.
Chapter 6: General discussion	118
6.1 The contributions of this thesis.....	118
6.2 Importance of differential scaling	120
6.3 Variability in morphological and physiological scaling	122
6.4 Insect ventilation patterns and their causes	123
6.5 Ecological implications of homogenous metabolic rate among nests.....	124
6.6 Open questions	125

List of tables

Table 2.1 Linear model co-efficients corresponding to data presented in Fig. 2.2.

Table 3.1 Scaling co-efficients from gross morphological eye measurements.

Table 3.2 Loadings from principle component analysis of nest, eye area and femur length, mean facet diameter and facet number.

Table 4.1 Number of ants and number of nests used per species for whole-eye scaling and for intra-eye scaling.

Table 4.2 Structure of linear mixed effect models for all analyses.

Table 4.3 Scaling exponents and standard error for each species for each scaling relationship.

Table 4.4 Correlations, eigenvalues and relative contributions of all three factors for all three principle components.

Table 4.5 Slope and intercept estimates derived from linear mixed effect models analysing differences between *Formica* species in terms of whole organ eye scaling.

Table 4.6 Slope and intercept estimates derived from linear mixed effect models analysing differences in facet diameter scaling between different regions of the eye within different *Formica* species.

Table 4.7 Slope and intercept estimates derived from linear mixed effect models analysing differences in facet diameter scaling within homologous regions of the eye between different *Formica* species.

Table 5.1 Structure of linear mixed effects models used for assessing metabolic and water loss rate scaling of *Formica rufa*.

Table 5.2 Different models used to assess variability of metabolic rate scaling exponents.

Table 5.3 Mean metabolic rate of different ant workers under different ventilation regimes.

Table 5.4 Summary of scaling exponents derived from different ant workers.

List of figures

Figure 1.1 Types of allometric change.

Figure 1.2 Cross section of different types of insect compound eye showing individual ommatidia.

Figure 2.1 Regional differences exist in the diameters of facets from compound eyes of wood ant workers.

Figure 2.2 The diameters of facets from different eye regions scale differentially.

Figure 3.1 Size variation in wood ant (*Formica rufa*) workers.

Figure 3.2 Electron micrograph of *Formica rufa* head and compound eye surface.

Figure 3.3 Differential scaling of compound eyes and facets among colonies.

Figure 3.4 The size range and distribution of workers from three nests used in the investigation assessed by femur length.

Figure 3.5 Differences in the head size of large and small workers.

Figure 3.6 Cluster analysis of femur length, eye area and nest after dimension reduction using Principle Component Analysis (PCA).

Figure 3.7 Cluster analysis of facet count, mean facet diameter and nest after dimension reduction using PCA.

Figure 3.8 Eye area is well approximated by an oval.

Figure 4.1 Phylogeny of *Formica* species

Figure 4.2 Scaling relationships in the four species of *Formica* as derived from linear mixed effect models.

Figure 4.3 Cluster analysis of facet number, mean facet diameter and species after dimension reduction using principle component analysis (PCA).

Figure 4.4 Intra-eye facet diameter scaling within species as derived from linear mixed effect models.

Figure 4.5 Intra-eye facet diameter scaling among species as derived from linear mixed effect models.

Figure 4.6 Eye casts from the four species used in this study.

Figure 4.7 Allometric scaling relationships in the four species of *Formica* as derived from linear models.

Figure 4.8 Intra-eye facet diameter allometric scaling within species as derived from linear models.

Figure 4.9 Intra-eye facet diameter allometric scaling among species as derived from linear models.

Figure 5.1 *Formica rufa* workers used two different ventilation types for gas exchange.

Figure 5.2 *Formica rufa* worker metabolic rate is higher when ventilating continuously than cyclically and scales with negative allometry.

Figure 5.3 *Formica rufa* metabolic rate does not differ between nests. Mean metabolic rate (\pm standard error).

Figure 5.4 *Formica rufa* workers used two different ventilation types for gas exchange, which is reflected in the patterns of respiratory water loss.

Figure 5.5 Respiratory water loss of *Formica rufa* workers scales with negative allometry and is higher when ventilating continuously compared with cyclically.

Figure 5.6 Respiratory water loss is driven entirely by increases in metabolic rate. *Formica rufa* worker water lost through ventilation as a function of metabolic rate.

Figure 5.7 *Formica rufa* workers do not change their metabolic rate when restrained or unrestrained.

Figure 5.8 *Formica rufa* metabolic rate differed between restrained or unrestrained cyclically ventilating ants.

Figure 5.9 *Formica rufa* worker size did not differ among nests.

Chapter I: General introduction

I.1 Morphological scaling

It is a truth universally acknowledged, that a species exhibiting some variation in body size must be in want of organs that modulate their dimensions appropriately. The relative change in organ size in response to a change in body size is known as *allometric scaling* or *allometry*. Organs can maintain a fixed size relative to body size, a condition called *isometry*. The term scaling will refer to all instances of changes in organ size with body size, given that the term allometry is often employed in the strict sense of *positive allometry*. Positive allometry occurs when an organ becomes proportionally larger as body size increases. An example of this can be found in stag beetles, where larger adult males have disproportionately large mandibles compared with smaller males (Kawano, 2000). Though widely documented (Bonduriansky, 2007; Gould, 1973; McCullough et al., 2015) and truly eye-catching, positive allometry is unrepresentative of most organ scaling. The vast majority of organs (Voje, 2016) become relatively smaller with increasing body size – *negative allometry* (Eberhard, 2009; Eberhard et al., 1998). This means that smaller individuals have relatively larger organs than their larger conspecifics. Quantitative investigations into relative organ size have existed since at least 1897 (reported in Gayon, 2000) and the early 20th century yielded further significant contributions, most notably from D'arcy Thompson, who expounding the important role of physical laws on biological growth (Thompson, 1992). However, Julian Huxley and Georges Teissier are credited with providing a standardised nomenclature and the power function most typically used to quantify allometric changes:

$$Y = bx^{\alpha}$$

where x is body size, Y is organ size, α is the scaling exponent and b is the initial growth index (Huxley and Teissier, 1936). This power function shows that modifications to two

parameters will influence how an organ responds to changes in body size; these are slope (α) and grade (b) shifts. A grade shift occurs when the mean size of an organ changes but the rate with which organ size increases with each unit of body size remains the same (Fig. 1.1). Thus, the intercept of the regression line changes and is modelled as an increase or decrease in b . Alternatively, there can be a change in the slope of the line, reflecting a change in the magnitude of the response by an organ to changing body size. This is modelled as changes in the value of α . If $\alpha > 1$, the organ scales with positive allometry, if $\alpha < 1$, the organ scales with negative allometry and if $\alpha = 1$, the organ scales isometrically.

1.2 Types of allometry

There are three ways in which an organism can experience organ scaling (Cock, 1966; Gould, 1966). The first is called *static* allometry, which captures how the size of an organ relates to body size among conspecifics at a given developmental stage (McCullough et al., 2015), most frequently the adult stage. Static allometry has been investigated extensively across many vertebrate (Brooke et al., 1999; Campione and Evans, 2012; Christiansen, 1999; Nudds, 2007; Prange et al., 1979) and invertebrate taxa (Cariveau et al., 2016; Emlen and Nijhout, 2000; Huxley, 1924; Tokeshi et al., 2000), particularly in reference to exaggerated secondary sexual characters (Alatalo et al., 1988; Baker and Wilkinson, 2001; Bonduriansky, 2007; Bonduriansky and Day, 2003; Cuervo and Møller, 2009; Emlen, 2008; Gould, 1973; Petrie, 1992).

Animals also experience relative organ size changes throughout development, typically organs enlarge as animals mature. The scaling of organs during development is

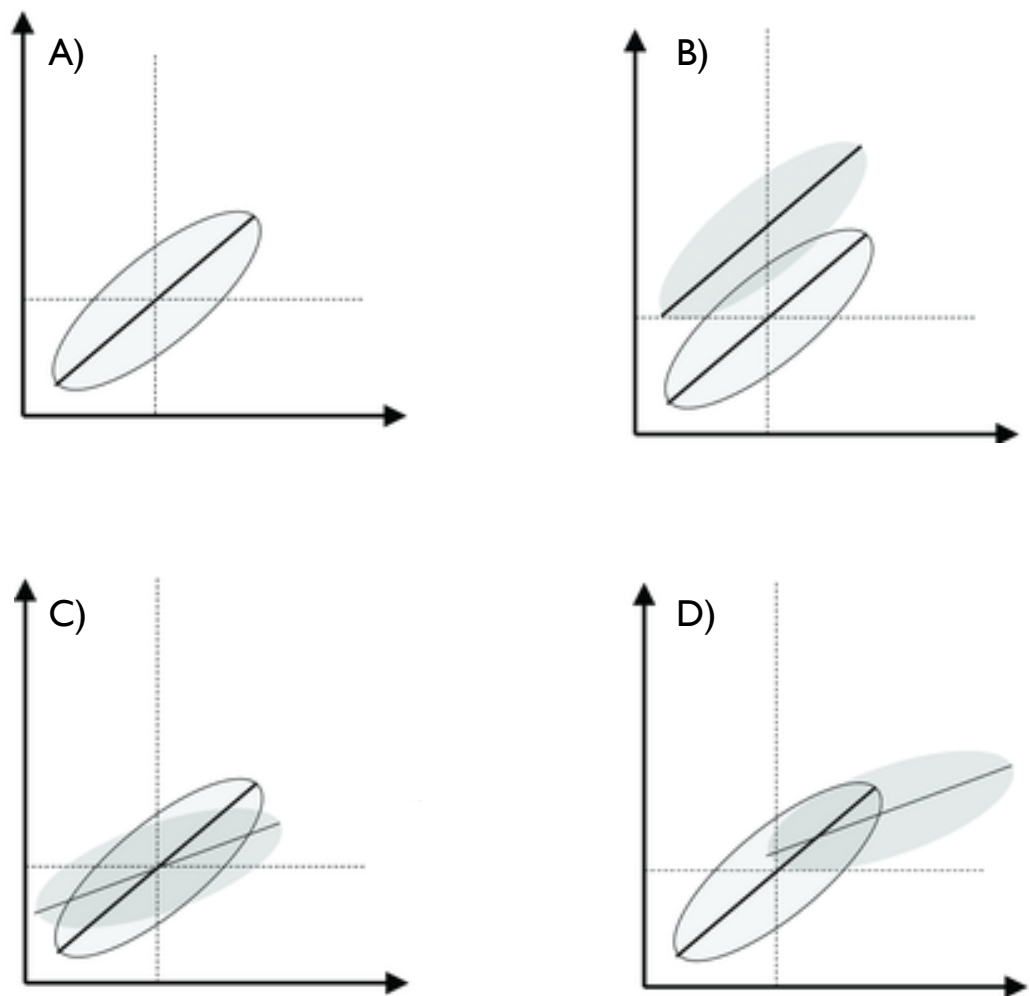


Figure 1.1 Types of allometric change. X-axis is body size or proxy thereof, y-axis is organ size. A) Original ancestral state; B) Grade shift; C) Slope shift; D) Both grade and slope shift. Adapted from Pélabon et al., 2014.

called *ontogenetic* allometry (Pélabon et al., 2013). Relative organ size can change vastly over developmental time for both vertebrates (McLellan et al., 2002; Pélabon et al., 2013; Tavares et al., 2016) and invertebrates (Gould, 1966; Moreira et al., 2017; Whitman, 2008). Within the insects, there are two distinct maturation processes; complete and incomplete metamorphosis, which are used in holometabolous and hemimetabolous orders, respectively (Gullan and Cranston, 2005). Within the hemimetabola, body size increases rapidly in a step-wise fashion through a series of moults. Immature stages (nymphs) are miniature wingless versions of the adults (imagoes) and most of their

organs get larger with each moult, increasing with body size (Gullan and Cranston, 2005). The exceptions are characters, such as wings, that grow at later nymphal stages, typically just before maturation. By contrast, the holometabola have strongly demarcated stages within their life history with an egg, larval, pupal and imago stage; each with different ecological and morphological adaptations (Gullan and Cranston, 2005). As such, *ontogenetic* allometry is far less obvious in these insects compared with incompletely metamorphosing insects. However, holometabolous insects often have several larval instars, where ontogenetic allometry may be apparent (Tammaru and Esperk, 2007).

The final framework through which animals can express organ scaling is *evolutionary* allometry (Voje et al., 2014). This captures how homologous organs among species (Rosenberg, 2002) or between different populations of the same species (Toju and Sota, 2006) change in relation to body size. There has been significant disagreement surrounding the exact role of *evolutionary* allometry (Egset et al., 2012; Mirth et al., 2016); how evolvable scaling relationships are or whether they are constrained developmentally or physiologically (Pélabon et al., 2014; Voje et al., 2014). This topic is assessed in section 1.6. Allometry at all three organisational levels (*static*, *ontogenetic*, *evolutionary*) can be compared both intra- and interspecifically.

1.3 Ecological implications in insects

Scaling is ubiquitous throughout animal species because growth is unavoidable (*ontogenetic* allometry) and individuals rarely have equal access to food and are genetically variable (*static* allometry). When *static* and *ontogenetic* allometries are expressed differentially between populations and species, *evolutionary* allometry is the natural consequence (Cheverud, 1982; Pélabon et al., 2013). Body size and scaling impact upon many life history traits (Chown and Gaston, 2010; Dial et al., 2008) making them a crucial

part of our understanding of animal ecology and behaviour (Kalinkat et al., 2015). Body size (and the relative scaling of organs) inherently affects interactions with both the biotic (predation, parasitism, intra-specific competition) and abiotic (temperature regulation, environmental rugosity) environment, and therefore it can be broadly posited that organ scaling is a crucial determinant of fitness.

One of the clearest examples of the importance of scaling is in the direct fitness benefits yielded by sexually selected, positively allometric traits. This has been well characterised in *Onthophagus* dung beetles, in which the males' horns show positive allometry. Larger males have horns that are disproportionately big and are referred to as majors, the smaller males have small or non-existent horns (Emlen, 1997). There is a threshold body size, above which males are majors and below which they are minors (Moczek and Nijhout, 2002). Different morphs have different methods of sexual competition, majors guard females and partake in gladiatorial combat (Moczek and Emlen, 2000) whereas minors "sneak" access by digging directly into female tunnels (Emlen, 1997). Major males achieve a higher level of fitness than minors (Hunt and Simmons, 2001), albeit only moderately (Simmons et al., 2004). Body size and correct organ scaling determine fitness in conjunction with behaviour. The change in behaviour coincides with the size at which horns grow (Hunt and Simmons, 2000). Mismatches in size, organ and behaviour (e.g. a minor male guarding a burrow) would have disastrous fitness consequences. In contrast to positively allometric sexually selected traits, male insect genitals are negatively allometric (Eberhard, 2009; Eberhard et al., 1998). This is likely due to stabilising selection; males do not attract or compete with females directly through large genitals and it is more beneficial to have an aedeagus that is compatible with the majority of females (Eberhard, 2009). Hence, stabilising selection occurs, producing low scaling slopes.

As well as influencing intraspecific competition, scaling is vital for modulating interspecific interactions. The scaling of proboscis length in camellia weevils is essential for laying eggs on seeds of their host plant (Toju and Sota, 2006). Proboscis length is matched to the relative thickness of the seed pericarp. Moreover, there are significant differences in the scaling of proboscis length between different weevil populations, indicating that allometric shifts have been selected for and have a critical role in determining fitness (Toju and Sota, 2006).

Scaling is also important for mutualistic interactions; pollinator proboscis length is important for determining degree of floral specialisation (Cariveau et al., 2016). Across taxa, proboscis length matches the corolla length of the flowers that pollinators visit (Stang et al., 2009), and therefore, it is essential for pollinators to scale their proboscis' accurately. Furthermore, scaling of proboscis length is key to interpreting plant-pollinator networks, body size and relative proboscis length being important mediators of floral association. Allometric measurements can be used to make predictions about species where direct morphometrics are difficult to acquire. Using only taxonomy to make these predictions explains 61% of the variation in proboscis length among five families of bees, however, including allometric measures raises this to 91% (Cariveau et al., 2016).

Sensory organs are vitally important for fitness because these structures enable accurate perception and interaction with the world, permitting food and mate location as well as navigation (Graham and Collett, 2002; Srinivasan, 2010; Zeil, 1983), thus the scaling of these structures is also critical. Olfactory sensitivity in bumblebees and honeybees scales with body size; larger foragers are more sensitive than smaller nestmates (Riveros and Gronenberg, 2010; Spaethe et al., 2007) and the same is true of vision (Jander and Jander, 2002; Kapustjanskij et al., 2007; Kelber et al., 2006; Streinzer

et al., 2016; Warrant, 2008). Higher visual sensitivity enables the detection of signal, and therefore vision, with fewer available photons, such as at dawn and dusk. This puts smaller-bodied species at a disadvantage; being active in lower ambient light allows access to floral resources earlier in the day, before they are depleted by successive visitors (Kapustjanskij et al., 2007; Kelber et al., 2006). Smaller body sizes in bees can be advantageous though, providing a greater range of potential nesting sites (Michener, 2001) and an increased resilience to periods of scarcity (Streinzer et al., 2016). To compensate for their small body sizes, the eyes of stingless bees scale with positive allometry. This maintains the advantages of small body sizes, relatively to larger bees, but compensates somewhat for the lower sensitivity induced by smaller eye sizes (Streinzer et al., 2016).

Having organs correctly matched to body size, behaviour and environment is essential for maintaining fitness. Therefore, it is not surprising that the coordination of organ and body size scaling is carefully regulated.

1.4 Developmental scaling mechanisms in insects

Adult organ growth in hemimetabolous insects occurs in step-wise increases through a series of larval moults, each moult typically having larger organs than the previous (Nijhout and Callier, 2015). In holometabolous insects, adult organ growth occurs *via* the development of larval imaginal discs; undifferentiated ectodermally-derived cellular monolayers (Currie et al., 1988; Morata and Lawrence, 1979). Imaginal discs grow larger throughout larval development and differentiate into adult tissue during pupation (Andersen et al., 2013). Though genetics (Shingleton et al., 2009; Stevenson et al., 1995) and temperature (Nijhout and Callier, 2015; Shingleton et al., 2009) can affect imaginal

disc growth, changes in nutritional status are probably the most important determinants of adult organ size.

Both somatic and imaginal disc growth is mediated by insulin-like peptides (ILPs) and their receptors (Shingleton et al., 2005; Wu and Brown, 2006). Secretion of ILPs is proportional to nutritional status (Nijhout et al., 2014; Wu and Brown, 2006) and along with ecdysone, stimulate somatic growth during the larval stage (Nijhout and Callier, 2015). Release of ILPs is stimulated by the fat body on ingestion of amino acids (Andersen et al., 2013). The fat body accumulates resources during larval development and then acts as a sentinel organ, modulating global growth in response to nutrition (Nijhout and Callier, 2015). Because ILPs stimulate growth in the larval soma and imaginal discs (Emlen et al., 2012; Nijhout and Callier, 2015), the fat body is ultimately responsible for coordinating adult organ scaling.

The exact mechanism that scales organs with adult body size is unknown (Andersen et al., 2013; Nijhout and Callier, 2015). However, ILPs are likely important; both pre-pupation larval body size and imaginal disc growth respond to insulin receptor activity (Shingleton et al., 2005). Furthermore, larval body size determines adult body size and the larval stage sequesters resources used to grow imaginal discs (Andersen et al., 2013). Therefore, it has been speculated that there must be a link between pre-pupation body size and adult organ size (Andersen et al., 2013). In *Manduca sexta*, ecdysone is responsible for scaling wing size with adult body size and in insects in general, ecdysone determines final adult weight (Nijhout and Callier, 2015). Thus, scaling of adult organ size is likely mediated by both ILPs and ecdysone.

Organs can differentially respond to changes in nutrition, and hence the same global levels of ILPs, by expressing different amounts of insulin receptors on the surface of the imaginal disc cells (Emlen et al., 2012). A greater number of insulin receptors

increases sensitivity to ILPs. Hence, two different organs can respond differently to the same amount of ILP, providing a mechanism for differential growth and organ scaling (Emlen et al., 2012; Nijhout et al., 2014).

1.5 Insect compound eyes and scaling

Insect compound eyes are no less susceptible to scaling than other organs, though due to their unique structure, body size imposes strict limits on visual acuity. Compound eyes can be classified as either apposition, superposition or neural superposition (Fig. 1.2). Though they share much in the way of morphology and anatomy (Kunze, 1979; Land, 1997), superposition eyes pool photon capture over several ommatidia to increase sensitivity and are typically found in nocturnal or crepuscular species (Land, 1997). Though much of what is discussed applies equally to superposition and apposition eyes, we will focus primarily on apposition eyes, the type found in ants.

Insect compound eyes are composed of repeating visual subunits, known as ommatidia (Land, 1997). Each ommatidium is effectively a tube, with a hexagonal lens (or facet) at one end and photoreceptors at the other (Fig. 1.2). The photoreceptors are aligned to form a light-collecting structure along the centre of the ommatidium called a rhabdom (Land, 1997). Each ommatidium samples a single point in space, contributing a single pixel to the complete image. Fewer ommatidia translate to fewer pixels, creating a lower resolution (Land, 1997). The angle between two rhabdoms of adjacent ommatidia (the interommatidial angle) also determines resolution. A small interommatidial angle means that neighbouring ommatidia sample from points close together. The larger the interommatidial angle, the greater the region that two adjacent ommatidia are sampling. This creates a blurrier (less resolved) image. Facet diameter also affects resolution. Whenever a wave front passes through an aperture, diffraction

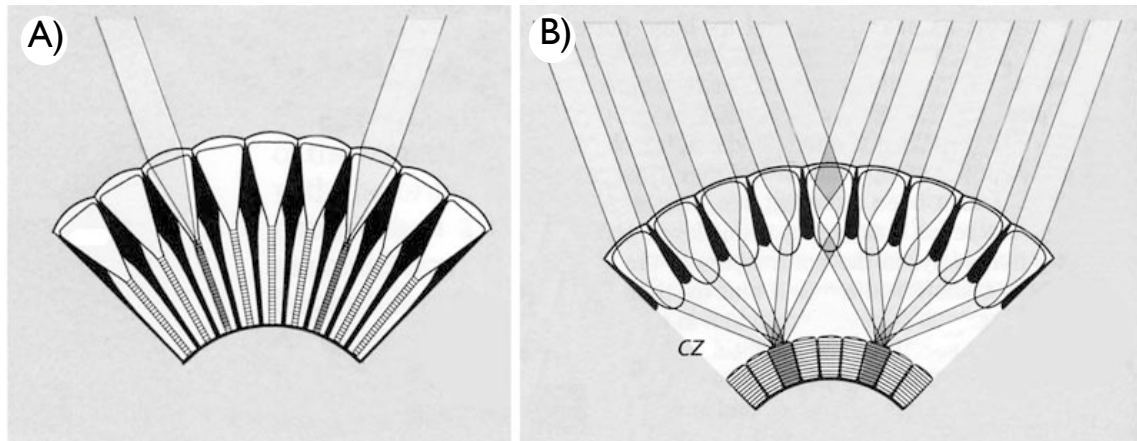


Figure 1.2 Cross section of different types of insect compound eye showing individual ommatidia. A) Apposition compound eye. Commonly found in diurnal insects. Each ommatidium collects light independently, with a single lens focussing light on a single set of photoreceptors (rhabdom). B) Superposition compound eye. Light collected by multiple ommatidia are focussed onto a single rhabdom to increase sensitivity. cz: clear zone. Modified from Warrant, 2004.

takes place, creating an interference pattern. Interference becomes worse with smaller apertures, with maximum interference when the aperture is equal to the wavelength of the wave passing through. Hence, the smaller the facet diameter, the worse the interference pattern on the rhabdom and the poorer the image formed (Land, 1997). Facet diameter also affects sensitivity; larger facets enable more photons to be captured per second, allowing vision at lower ambient light levels (Fischer et al., 2011).

Insect eyes function best when they are as large as possible, allowing for large facet diameters and small interommatidial angles, which provide high resolution and sensitivity (Land, 1997; Land and Nilsson, 2002; Warrant and McIntyre, 1993). However, eye size is restricted by the availability of space and resources (Nilsson, 1990; Spaethe and Chittka, 2003), both of which are governed by body size (Jander and Jander, 2002; Kapustjanskij et al., 2007; Rutowski, 2001; Spaethe and Chittka, 2003; Streinzer et al., 2013). Increasing body size provides more space for greater numbers of ommatidia and for larger facets (Fischer et al., 2011). However, body size is limited by physiological and evolutionary constraints, as well as resource availability. Many adult insects fly, and a compound eye with human equivalent acuity would be too large to facilitate flight (Land,

1997). Furthermore, body sizes in insects are thought to be limited due to relatively low ambient oxygen partial pressures combined with their tracheal system (see 1.8) (Harrison et al., 2010; Klok and Harrison, 2009). To combat the limitations imposed by the nature of compound eyes and a small body size, compound eyes are typically heterogeneous (Collett and Land, 1975; Homberg and Paech, 2002; Land, 1997). This heterogeneity extends to morphology (facet diameter), anatomy (photoreceptor width) and physiology (photoreceptor response) (Burton et al., 2001). Thus, some sections of an eye have greater sensitivity and/or resolution. Highly acute regions often have specific functions, such as mate detection (Burton and Laughlin, 2003) or polarisation sensitivity (Labhart and Meyer, 1999).

Given the limits imposed on vision by body size, it is unsurprising that the response of compound eyes to increases in body size has been investigated in many insect taxa, including; damselflies (Scales and Butler, 2016), crickets (Zagorski and Merry, 2014), Hemiptera (Dander and Jander, 1994; Döring and Spaethe, 2009), flies (Stevenson et al., 1995), butterflies (Merry et al., 2006; Merry et al., 2011; Rutowski, 2001; Rutowski et al., 2009; Ziemba and Rutowski, 2000), bees (Jander and Jander, 2002; Kapustjanskij et al., 2007; Spaethe and Chittka, 2003; Streinzer et al., 2013, 2016) and ants (Baker and Ma, 2006; Bernstein and Finn, 1971; Klotz et al., 1992; Moser et al., 2004; Schwarz et al., 2011; Zollikofer et al., 1995).

In the investigations listed above eye size is estimated in a variety of ways; height, length, area, diameter and ommatidia number. Regardless of the estimation method, compound eyes increase in size with increasing body size, both intra- (Bernstein and Finn, 1971; Stevenson et al., 1995) and interspecifically (Jander and Jander, 2002; Scales and Butler, 2016). Many studies that report results of eye scaling are only reporting monotonic increases in size, rather than actual scaling relationships (Döring and Spaethe,

2009; Kapustjanskij et al., 2007; Moser et al., 2004; Streinzer et al., 2013; Zagorski and Merry, 2014; Ziemba and Rutowski, 2000). Of those that do report scaling relationships, the overwhelming majority show eye scaling with negative allometry, the exceptions being stingless bee eyes scaling with positive allometry (Streinzer et al., 2016) and nymphalid butterfly eyes scaling at near isometry (Rutowski et al., 2009). Increases in eye size can be mediated through increases in facet number (Schwarz et al., 2011; Zollikofer et al., 1995) or by increasing both facet number and facet diameter (Kapustjanskij et al., 2007; Schwarz et al., 2011; Spaethe and Chittka, 2003). The way in which compound eyes get bigger will be selected for by species specific visual ecology (Bauer and Kredler, 1993; Jander and Jander, 2002), which determines the necessity for increases in sensitivity or resolution. Furthermore, changes in eye size may also respond to mechanisms of body size increase, reflecting relative increases in cell size or number (Chown et al., 2007; Stevenson et al., 1995).

1.6 Evolvability of scaling

Though scaling is essential to morphological evolution (Shingleton et al., 2009), there is debate surrounding the ultimate ability of allometry to be the subject of selection. Low evolvability of allometry implies that there is an evolutionary constraint: "...processes that preclude a trait from reaching a phenotypic optimum or slow down its evolution toward this optimum" (Pélabon et al., 2014).

Two processes may constrain the evolution of allometry; development and function (Bolstad et al., 2015; Pélabon et al., 2014; Tobler and Nijhout, 2010). Developmental constraints are hypothesised to influence evolutionary allometry because the three distinct levels of scaling (*evolutionary, static and ontogenetic*) are interlinked (Bolstad et al., 2015; Cheverud, 1982; Gould, 1966; Pélabon et al., 2013). Allometric

variation subject to selection is limited by the scope of variation provided by *ontogenetic* and *static* allometric parameters (Frankino et al., 2005; Voje et al., 2014). However, these allometries may be constrained in their variation because organs must function as part of a whole and, therefore, do not have an infinite morphospace in which to move. This potentially explains the evolutionary stability of allometric scaling, especially between closely related lineages (Gould, 1966; Pélabon et al., 2014). Functional constraints reflect the consequences of stabilising selection. The *static* allometry of an organ may be constrained because to deviate from certain boundaries would result in severe fitness deficits (Frankino et al., 2005; Mirth et al., 2016). Therefore, within a species, allometries do not vary much because to do so is heavily selected against (Weber, 1990). Further constraints may be caused by pleiotropy, where changes in scaling parameters have a negative effect upon another system (Bolstad et al., 2015).

If allometry were constrained to the extent that evolvability was null, we might predict that closely related species would not differ in their slopes and intercepts, nor would we expect slopes and intercepts to respond to selection. However, this is not so. Comparisons of allometry between closely related species have revealed differences in scaling relationships (Baker and Wilkinson, 2001; Simmons and Tomkins, 1996). There is evidence that scaling does differ between populations (Emlen and Nijhout, 2000; Toju and Sota, 2006) and artificial selection experiments have manipulated both slopes (Bolstad et al., 2015; Stillwell et al., 2016; Voje et al., 2014) and intercepts (Bolstad et al., 2015; Egset et al., 2012; Frankino et al., 2005; Frankino et al., 2007; Stillwell et al., 2016). Nevertheless, some experiments have been criticised on methodological grounds (Mirth et al., 2016; Stillwell et al., 2016). Principally, that scaling cannot be investigated as simply a monotonic increase in organ size with body size, but must be analysed specifically in the sense of a power relationship (Huxley and Teissier, 1936; Pélabon et al., 2014).

The current consensus is that both slopes and intercepts are evolvable between lineages, however, changes in intercept (grade shifts) are much easier to select for than slope shifts (Egset et al., 2012; Pélabon et al., 2014; Tobler and Nijhout, 2010; Toju and Sota, 2006; Voje et al., 2014). Thus, changes in the mean value of a character are easier to select for than changes in the rate at which that character scales with increasing body size. However, simply because something is possible under strong artificial selection, does not necessarily mean that such conditions exist in nature. If the evolvability of allometry is limited due to stabilising selection and functional constraints, the capacity to artificially induce changes in allometry is irrelevant because the outcome is the same – allometries may not be as free to evolve as theoretically possible.

1.7 Physiological scaling

Analogous to morphological scaling, physiological traits also scale with body size. These physiological traits can be analysed using the same linear allometric equation used to establish how morphological traits respond to increasing body size, both intra- or interspecifically.

Locomotion is one such size-dependent physiological feature. The speed at which an animal travels is linked with body size and has been examined across many taxa, including mammals (Heglund and Taylor, 1988; Winter, 1999), birds (Alerstam et al., 2007), amphibians (Wilson et al., 2000) and insects (Berrigan and Pepin, 1995; Dudley and Srygley, 1994; Hurlbert et al., 2008). Speed mostly scales with a negative allometry (Alerstam et al., 2007; Bejan et al., 2006; Dudley and Srygley, 1994; Heglund and Taylor, 1988; Hurlbert et al., 2008), independent of the mode of locomotion (Bejan et al., 2006). However, some species of lizard (Garland, 1984) and amphibians during some developmental stages (Wilson et al., 2000) have mass-independent speeds. Across

terrestrial animals, walking is a combination of physiology and morphology, with longer legs reducing locomotive costs (Pontzer, 2007). For ants specifically, running speed is determined not just by body size, but also leg length (Hurlbert et al., 2008) and life history traits, such as being thermophilic (Sommerer and Wehner, 2012).

Closely associated with speed is wing beat or stride frequency, which also scales with mass. Stride frequency across mammals declines with increasing mass (Heglund and Taylor, 1988; Heglund et al., 1974), a pattern reflected in crabs (Whittemore et al., 2015) and lizards (Huey, 1982b; Whittemore et al., 2015). Wing beat frequencies in birds and bats scale with negative interspecific allometry (Norberg and Norberg, 2012) but with positive allometry across insects species (Byrne et al., 1998).

Alongside locomotion, there are many other physiological traits that scale with body size across many species. Egg size in insects and reptiles scales with body size (Ford and Seigel, 1989; García-Barros, 2000; King, 2000; Sturm, 2016) as does clutch size in reptiles (Ford and Seigel, 1989; King, 2000). Even urination speed (Yang et al., 2014) and more holistic life history traits, such as lifespan (Atanasov, 2007; Holm et al., 2016) scale with body size across taxa separated by vast swathes of time and huge differences in body size. Overall, physiological processes are intimately correlated with body size, and metabolic rate is no exception.

1.8 Metabolic rate

Metabolism is the sum of all the catabolic and anabolic processes within an animal, the net energy of which is usable for all cellular and physiological processes (Alexander, 1999; Chown and Nicolson, 2004). Metabolic rate is important because it is viewed as a modulator of other physiological processes; often referred to as a “pacemaker” (Glazier, 2015; Hoppeler and Weibel, 2005). Metabolic rate has wide ranging impacts over

behaviour and ecology (Biro and Stamps, 2010; Burton et al., 2011; Gillooly et al., 2001; Glazier, 2015; West et al., 1997) as well as life history traits such as; reproductive capacity (Blueweiss et al., 1978; Hammond and Diamond, 1997; McNab, 1980), life span (Hulbert et al., 2007; Speakman, 2005) and growth (Brown et al., 2004; Sears et al., 2012). However, the exact direction of causality is not as obvious as might be assumed. Increases in these life history traits might drive increases in metabolic rate rather than the converse. There are instances where metabolic rate and reproductive capacity do not correlate (Earle and Lavigne, 1990; Schimpf et al., 2012) or even have a negative relationship (Blackmer et al., 2005). Likewise, though there is evidence of growth being driven by metabolic rate (Derting, 1989; Glazier, 2015; Sears et al., 2012), changes in ontogenetic metabolic rate scaling in cockroaches implies the opposite (Woodland et al., 1968). It is likely that increases in growth instigate higher metabolic rates; growth incurs a metabolic cost, through the production of new tissue, and therefore we should expect metabolic rate to compensate accordingly (Glazier, 2015). Locomotion is another trait that is linked with metabolic rate (Niven and Scharlemann, 2005; Snelling et al., 2011; Weibel and Hoppeler, 2005). Movement probably drives metabolic rates because animals choose when and how to move; there is no *a priori* reason to suppose that spontaneous increases in metabolic rate would cause increases in locomotion (Glazier, 2015). Furthermore, a higher maximum speed requires a greater capacity to generate energy, and therefore may promote a higher metabolic rate (Biro and Stamps, 2010; Glazier, 2015; Reinhold, 1999; Speakman, 2005). Relationships between life history traits and metabolic rate are further confounded because many physiological traits (see above), including metabolic rate, co-vary with body size (Griebeler and Werner, 2016).

Not all determinants of metabolic rate are ambiguous, many environmental factors have definitive effects on metabolism (Burton et al., 2011; Glazier, 2005). Factors

such as low oxygen availability cause metabolic rate depression in vertebrates (Bickler and Buck, 2007; Mortola, 1993) and invertebrates (Gorr et al., 2010; Hoback and Stanley, 2001). The two most prominent determinants of metabolic rate across animals are temperature and body size (Chown and Nicolson, 2004; Clarke and Fraser, 2004; Gillooly et al., 2001; Kleiber, 1934; Rubner, 1883; White et al., 2006). External temperatures largely determine ectotherm body temperature and are consequently important for determining ectotherm metabolic rate (Clarke and Johnston, 1999; Huey, 1982a; Killen et al., 2010; Whitford, 1973). Contrary to expectations, endotherm metabolic rate is also affected by environmental temperature (Clarke et al., 2010; White et al., 2007b). Furthermore, birds and mammals have a significantly elevated metabolic rate independent of body mass and temperature in comparison to ectotherms of equivalent size (White et al., 2006). Among insects, the effect of temperature was already “the most over-confirmed fact in insect physiology” over 50 years ago (Keister and Buck, 1964). It is well established that insect metabolic rates increase with increasing ambient temperatures (Basson and Terblanche, 2010; Chown and Nicolson, 2004; Käfer et al., 2015; Lighton and Bartholomew, 1988; Tribe and Bowler, 1968; Vogt and Appel, 1999).

Size is also an important determinant of metabolic rate (Clarke and Johnston, 1999; White et al., 2005). This is because larger animals have a greater mass of active tissue and this translates into higher metabolic rates. However, metabolic rate most commonly scales with negative allometry, therefore larger animals are relatively less metabolically active than smaller ones. This observation has created considerable debate, firstly about what causes this and secondly, whether there is a general rule that can explain metabolic rate scaling (Brown et al., 2004; Chown et al., 2007; Glazier, 2005; Glazier, 2010; Griebeler and Werner, 2016; West et al., 1997; West et al., 1999).

There has been substantial investigation into whether there is a universal metabolic scaling exponent (Agutter and Wheatley, 2004), beginning with Kleiber's report on metabolic scaling across mammals (Kleiber, 1932). The most recent permutation of this idea is the metabolic theory of ecology (MTE). The MTE claims to explain the $\frac{3}{4}$ power metabolic scaling law observed across all of life, proposing that this is caused by the fractal nature of nutrient supply and uptake networks (Brown et al., 2004; West et al., 1997; West et al., 1999). However, not all authors agree that interspecific metabolic rate allometry scales to the power of $\frac{3}{4}$ (Chown et al., 2007; Glazier, 2005; Glazier et al., 2015; Griebeler and Werner, 2016; White et al., 2006). Some investigators propose an exponent of $\frac{2}{3}$ (Dodds et al., 2001; Heusner, 1982; White et al., 2006), purportedly caused by relative increases in surface area to volume ratios with body size (Agutter and Wheatley, 2004). Other authors propose no universal exponent; that it likely varies depending on taxonomy, physiology and environment (Clarke et al., 2010; Glazier, 2005; Glazier, 2009; Griebeler and Werner, 2016; Hayssen and Lacy, 1985; Terblanche et al., 2007; White et al., 2006). The values $\frac{2}{3}$ and one have been proposed to provide boundaries within which the value of the exponent fluctuates. The extremes of $\frac{2}{3}$ and one depend respectively on whether surface-related processes (e.g. nutrient uptake, heat loss) (Glazier et al., 2015; Hirst et al., 2014) or mass-related energy usage dominates (Glazier, 2005; Glazier, 2008). The shape of an organism influences surface area related processes and therefore the metabolic rate scaling exponent. Surface area related processes dominate in some taxa, especially marine invertebrates. This is demonstrated in marine invertebrates, which frequently change shape during ontogeny. Elongation or flattening result in isometric metabolic rate scaling, rather than an exponent <1 as predicted by resource transport theory (Glazier et al., 2015; Hirst et al., 2014). Both surface area and resource transport processes are

frequently present in an organism, hence the prevalence of intermediate values (Glazier, 2005).

There has been no definitive conclusion concerning the universality of the quarter-power scaling law. However, mounting evidence suggests that even if the MTE provides an adequate theoretical fit, there are enough empirical instances where it does not apply (Chown et al., 2007; Hirst et al., 2014; White et al., 2006). Thus, it becomes difficult to justify the application of a single all-encompassing scaling law (Agutter and Wheatley, 2004; Dodds et al., 2001).

1.9 Gas exchange in insects

Alongside the general environmental and physiological determinants of metabolic rate, arthropods (Chown and Nicolson, 2004) have an additional factor that is linked with variation in metabolism, namely ventilation patterns (Contreras and Bradley, 2009, 2010; Gibbs and Johnson, 2004). Insects breathe through a series of pores aligned laterally along the abdomen and thorax called spiracles. Each of these spiracles can be opened or closed via a muscular valve. When an insect is placed inside a respirometer, the pattern of spiracle opening and closing translates into different patterns of breathing, represented as volume of carbon dioxide excreted. There are three principle types of gas exchange; continuous, cyclic and discontinuous (Chown and Nicolson, 2004). These ventilation patterns are well documented in insects (Basson and Terblanche, 2011; Contreras and Bradley, 2010; Contreras and Bradley, 2011; Duncan et al., 2002; Marais et al., 2005), and especially ants (Gibbs and Johnson, 2004; Lighton and Berrigan, 1995; Lighton and Garrigan, 1995; Quinlan and Lighton, 1999; Schilman et al., 2005). Discontinuous gas exchange cycles (DGC) piqued the interest of biologists when they were hypothesised to have a role in water regulation in *Agapema galbina* (Lepidoptera)

pupae (Buck and Kesiter, 1955). There are many hypotheses explaining the evolution and proximate causes of DGC, though currently no consensus (Chown and Holter, 2000; Chown et al., 2006; Hetz and Bradley, 2005; Lighton, 1998; Lighton and Berrigan, 1995; Matthews and White, 2010). In comparison, functional explanations for the other forms of gas exchange, cyclic and continuous, are lacking.

Discontinuous gas exchange cycles are typified by short bursts of carbon dioxide release followed by a longer period of no gas exchange at all and are commonly associated with quiescent animals (Lighton, 1998). The DGC has been demarcated into three distinct portions (Chown and Nicolson, 2004; Lighton, 1994): (1) The closed phase in which spiracles are entirely shut and there is no gas exchange; (2) The flutter phase in which spiracles open and close rapidly, allowing some gas exchange to occur; and (3) The open phase in which the spiracles are fully open and carbon dioxide leaves the trachea and oxygen enters.

There are at least six hypotheses seeking to explain DGC. The oldest explanation for DGC is the hygric hypothesis, which posits that the adaptive function is to preserve water (Buck and Kesiter, 1955; Lighton, 1996; Quinlan and Gibbs, 2006). The hygric hypothesis states that the opening of spiracles to allow gas exchange also allows the loss of large amounts of water vapour (Schimpf et al., 2009). By using DGC, insects keep their spiracles mostly closed and therefore reduce water stress. This hypothesis has been treated with scepticism for several reasons. There is evidence that insects under water stress do not use DGC, precisely when DGC would be most advantageous (Quinlan and Hadley, 1993). Similarly, there are xeric species that might benefit from a ventilation pattern that reduces water loss and do not use DGC (Lighton and Garrigan, 1995). Furthermore, there is evidence that respiratory water loss is minimal compared

with cuticular water loss and therefore preventing water loss *via* closing spiracles would have minimal adaptive value (Hadley, 1994; Quinlan and Hadley, 1993).

The hygric hypothesis has been displaced by a series of other adaptive hypotheses. The chthonic hypothesis argues that DGC are an adaptive function to living in hypercapnic or hypoxic environments, principally in subterranean habitats (Gibbs and Johnson, 2004; Lighton, 1996; Lighton, 1998; Lighton and Berrigan, 1995). In claustral habitats, there is minimal diffusion of carbon dioxide away from a ventilating insect due to severely reduced movement of air. By ventilating discontinuously, an insect allows the maximum amount of time for excreted carbon dioxide to diffuse away before excreting more into the local environment. If carbon dioxide were continuously excreted, the build-up would create a hypercapnic environment, potentially leading to lethal exclusion of oxygen. The DGC prevents the build-up of dangerous levels of carbon dioxide and maintains preferential gas concentrations (Lighton, 1996; Lighton, 1998; Lighton and Berrigan, 1995). This hypothesis has been directly tested in the ant, *Pogonomyrmex barbatus*, the queens of which are subterranean post-mating. It was found that the ratio of water to carbon dioxide excretion did not differ among ventilation types (Gibbs and Johnson, 2004) indicating that the chthonic hypothesis cannot explain all instances of DGC.

The oxidative damage hypothesis states that DGC prevent over-exposure to oxygen (Hetz and Bradley, 2005). Over exposure to oxygen causes cellular oxidative damage, which has been shown to increase mortality (Boardman et al., 2012; Orr and Sohal, 1994). Keeping spiracles closed is hypothesised to exclude oxygen from the tracheae and prevent excessive exposure. When insects are quiescent, metabolic demand is too low to use the amount of oxygen being delivered, hence the extended periods of spiracular closure (Hetz and Bradley, 2005). The oxidative damage hypothesis

specifically rejects the chthonic and hygric hypotheses; both would predict high oxygen concentrations within tracheae under hyperoxic conditions. Instead, there is modulation of tracheal oxygen partial pressures irrespective of external oxygen concentrations (Hetz and Bradley, 2005). Experimental support for the oxidative damage hypothesis is mixed; direct observations indicated no link between hyperoxia and increases in reactive oxygen species within *Samia cynthia* pupae (Lepidoptera) (Boardman et al., 2012). However, it has been shown that in response to decreasing environmental oxygen, ants (Lighton and Garrigan, 1995) and dung beetles (Chown and Holter, 2000) will decrease the length of their closed phase.

Other investigators maintain that different ventilation patterns allow the exchange of greater or lesser amounts of oxygen and carbon dioxide relative to metabolic demand (Contreras and Bradley, 2010), but DGC may not be an adaptive trait at all. Discontinuous ventilation could be the result of spiracles responding to setpoints of carbon dioxide and oxygen concentration (Chown and Holter, 2000; Förster and Hetz, 2010). There is also a non-adaptive neural hypothesis that reconciles contradictory experimental findings and numerous adaptive hypotheses. It has been suggested that DGC are the result of reductions in brain activity (Matthews and White, 2010). In quiescent states, there is devolution of spiracular control to decentralised ganglia (Niven et al., 2008), where the default ventilatory control is DGC (Matthews and White, 2010). Decapitated cockroaches (Edwards and Miller, 1986) and ants (Lighton and Garrigan, 1995) engage in DGC, implying that DGC are mediated by ganglia rather than the brain. Cockroaches have also been shown to switch to DGC when their brains were sufficiently cooled, a way of inducing reduced brain activity (Matthews and White, 2013). Furthermore, DGC are prevalent in ant queens, which frequently have atrophied brains after mating, living the rest of their lives underground (Julian and Gronenberg, 2002).

Neural tissue is expensive (Niven and Laughlin, 2008) and therefore when inactive, reducing brain activity would be energetically beneficial.

The benefit of the neural control hypothesis is that it is compatible with other DGC hypotheses. For instance, if inactive with a minimally active brain, metabolic rates will be lower and therefore, it follows that DGC could be the default state because there is less requirement for gas exchange (Contreras and Bradley, 2010). The neural hypothesis is also compatible with the setpoint hypothesis; the proximate mechanism by which the ganglia regulate gas exchange could be spiracular responses to local gas concentrations (Chown and Holter, 2000; Förster and Hetz, 2010). Recent opinion has moved towards the idea of multiple adaptive explanations of DGC (Chown, 2002; Chown and Holter, 2000; Matthews and White, 2010). This paradigm is built on the conflicting multiple adaptive hypotheses (White et al., 2007a) and several independent evolutions of DGC within the insects (Marais et al., 2005).

The amount of attention given to DGC over the past 60 years is disproportionate to the prevalence of DGC among insects; it has been observed in only five orders: Coleoptera, Hymenoptera, Blattodea, Lepidoptera and Orthoptera (Marais et al., 2005). Thus, it is imperative to establish the adaptive value, or lack thereof, of the other forms of gas exchange which are prevalent throughout the other insect orders (Marais et al., 2005). Compared with DGC, cyclic and continuous ventilation patterns are largely unexplored. The term cyclic can also be applied to DGC, in that it is a ventilation pattern that releases carbon dioxide in short bursts at regular periods. Here it shall be used in the strict sense of pronounced, regular peaks and troughs of carbon dioxide release, where carbon dioxide release never quite reaches zero, unlike in DGC. Continuous ventilation has no obvious regularity regarding carbon dioxide release, spiracles are kept open and gas exchange occurs constantly (Gray and Bradley, 2006; Lighton, 1994). Cyclic

gas exchange is normally associated with a higher metabolic rate than DGC, and continuous gas exchange occurs at the highest metabolic rates (Chown and Nicolson, 2004; Gibbs and Johnson, 2004). We are unaware of any adaptive hypotheses about cyclic gas exchange, however it has been shown that some instances of cyclic gas exchange are in fact DGC. Under low flow rates in a respirometer, the closed and flutter phases can be lost due to temporal averaging of carbon dioxide release (Gray and Bradley, 2006). Continuous gas exchange is used by insects at high temperatures or during activity (Basson and Terblanche, 2011; Contreras and Bradley, 2010; Klok and Chown, 2005). The stimulus to change between different ventilation patterns is currently thought to be induced by higher metabolic rates creating increasingly higher demand for gas exchange, resulting in longer spiracular opening times (Contreras and Bradley, 2010; Gibbs and Johnson, 2004).

1.10 Ants

The southern red wood ant, *Formica rufa*, is a locally abundant ant found in conifer and mixed forests of southern England. Its distribution extends across central Europe into Fennoscandia (Collingwood, 1979). The ants form nests composed of large mounds of twigs, pine needles and leaves rather than the subterranean nests of other ants. In the UK, and certainly in Sussex (pers. obs.), these nests are polygynous. In other populations across Europe, nests are known to be monogynous (Collingwood, 1979). Virgin queens mate on the nest surface, shed their wings and return to the same nest from which they hatched. New nests are formed from budding, where a mated queen will depart her home nest with a contingent of workers to found a new nest nearby (Collingwood, 1979; Keller, 1991)

The workers actively forage from early spring, their diet is honeydew; the sugar-laden excretions of arboreal aphids (Skinner, 1980). Larvae are fed on invertebrates encountered by the foragers (Collingwood, 1979; Skinner, 1980). Foraging occurs along well-demarcated paths (Rosengren, 1977), with foragers following the pheromone trails left by homeward bound nestmates that have successfully found food. Trail fidelity is very high, workers return to the same trails after over-wintering (Rosengren and Fortelius, 1986).

Formica rufa have a variety of navigation tools that allow them to reach food sources and return to the nest (Knaden and Graham, 2016). One such tool is path integration. On an outward journey, an ant merges information about total distance and direction travelled into a single vector. This vector allows the ant to know its position relative to the nest and to directly return to the nest having followed a tortuous route. Memories of the vector when feeding allow an ant to return directly to that food source (Cheng et al., 2006; Collett et al., 1999; Fernandes et al., 2015).

Unlike many ants, *F. rufa* also exhibits visually driven navigation behaviours. Though foragers will mark trails with scent, visual landmarks are crucial for navigating back to the nest after foraging for food (Graham and Collett, 2002; Graham et al., 2003; Nicholson et al., 1999). Conspicuous objects and landmark recognition are important for creating robust navigational memories and learning new routes (Graham et al., 2003). Memories of landmarks are stored from particular vantage points (Harris et al., 2007) and therefore to return to a familiar location the insect moves until the current view matches the view from the stored memory (Harris et al., 2007; Judd and Collett, 1998).

There are three other species of *Formica* studied in this thesis: *F. fusca*, *F. lugubris* and *F. sanguinea*. The most closely related species to *F. rufa* is *F. lugubris*, which is found across Europe, but is restricted to northern England (Collingwood, 1979). Just like *F.*

rufa, they feed mostly on honeydew and will hunt other invertebrates with which to feed larvae. Unlike *F. rufa*, *F. lugubris* are polydomous, a condition where workers will move between nests, providing food and resources to multiple nests. This creates a vast network of densely-packed, interconnected nests (Ellis and Robinson, 2014; Ellis et al., 2014).

Formica sanguinea is a facultatively dulotic Eurasian species; they will raid nests of *Raptiformica* subgroup (D'Ettorre and Heinze, 2001; Goropashnaya et al., 2012; Mori et al., 2000) stealing pupae and larvae which are then raised in the *F. sanguinea* nests (Mori et al., 2000). On maturation, these stolen juveniles then perform brood and nest care in place of *F. sanguinea* workers (Hölldobler and Wilson, 1990). *Formica fusca* differ from the previous three species in that their nests are rather small, approximately 500 freely foraging workers (Collingwood, 1979; Wallis, 1964); significantly smaller than the several thousand in *F. rufa* and *F. lugubris* colonies. *Formica fusca* have single or multi-gyne subterranean nests and forage for honeydew and invertebrate prey (Collingwood, 1979).

1.1.1 Summary of contributions

This thesis makes several novel contributions to the field of allometry. In terms of morphological scaling, we show that different regions of the *Formica rufa* compound eye scale differentially. We assessed this by measuring how facet diameters in different regions of the eye vary with increasing body size. Different regions of the eye respond to increases in size with different magnitudes. To our knowledge, this is the first demonstration of differential scaling within a single organ. This demonstrates that homologous cells within an organ can respond with high resolution to their developmental environment. This process of internal differential scaling provides a hitherto unconsidered mechanism by which to evolve novel organ morphology.

We next considered other levels of underexplored morphological scaling, and this led us to comparisons of eye scaling among *F. rufa* nests within a population. We show that though eye area scaling was similar among nests, the increase in eye size with body size was mediated differently between some nests. Workers from some nests increase their eye size primarily through increases in facet number, while other workers from different nests increase eye size by increasing facet diameter. This shows that whole-organ scaling rules can vary within a population level.

We were also curious about how widespread differential scaling within an organ was across the genus, and, if it were restricted to certain species if this could shed some light on either a phylogenetic or ecological component to the phenomenon. Differential slope shifts were only present in two of four species. There was no obvious ecological, life history or phylogenetic correlate. However, the scaling between facet diameter and number was conserved across all four species, indicating that whole-organ scaling rules are conservative across the genus. This contrasts with the differences in facet diameter scaling between homologous regions among species. This shows that individual species can have specific sensory adaptations whilst still conforming to genus-wide scaling rules.

Having established that substantial differences existed in the morphological scaling between *F. rufa* nests from a population, we investigated if a similar phenomenon existed at a physiological level. Metabolic rate scaling proved invariant between nests, indicating that physiological scaling is likely not as developmentally susceptible to environmental changes as morphological scaling. We determined that in *F. rufa* changes in ventilation pattern are caused by changes in relative activity independent of metabolic rate. Previous studies concluded that metabolic rate drives ventilation patterns, however, our data indicates that increases in metabolic rate and changes in ventilation pattern are likely co-occurring with the onset of movement. We also show that

estimates of intraspecific metabolic scaling exponents in insects are dependent on model structure and the inclusion of ventilation type. This has important implications for exponent estimates across the fields of respiratory metabolism and physiological ecology.

Chapter 2: Differential scaling within an insect compound eye

2.1 Abstract

Environmental and genetic influences cause individuals of a species to differ in size. As they do so, organ size and shape are scaled to available resources whilst maintaining function. The scaling of entire organs has been investigated extensively but scaling within organs remains poorly understood. By making use of the structure of the insect compound eye, we show that different regions of an organ can respond differentially to changes in body size. Wood ant (*Formica rufa*) compound eyes contain facets of different diameters in different regions. When the animal body size changes, lens diameters from different regions can absolutely increase or decrease in size either at the same rate (a 'grade' shift) or at different rates (a 'slope' shift). These options are not mutually exclusive, and we demonstrate that both types of scaling apply to different regions of the same eye. This demonstrates that different regions within a single organ can use different rules to govern their scaling, responding differently to their developmental environment. Thus, the control of scaling is more nuanced than previously appreciated, diverse responses occurring even among homologous cells within a single organ. Such fine control provides a rich substrate for the diversification of organ morphology.

2.2 Introduction

In natural environments, adults from a single species can vary enormously in body size owing to a combination of genetic and environmental factors. Organ size changes to accompany changes in body size, a process known as allometric scaling (Huxley and Teissier, 1936; Shingleton et al., 2009). Theories of organ scaling (Emlen et al., 2012; Shingleton et al., 2009) have focused on entire organs and how their relative proportions

change with whole body size, largely ignoring changes in the size and number of cells within organs (Stevenson et al., 1995). Here, we investigate scaling within an organ, the compound eye of an insect.

Organ scaling has been studied in numerous taxa but particularly in holometabolous insects (Berrigan, 1991; Emlen, 2008; Miyatake, 1993; Nijhout and Grunert, 2010; Tomkins, 1999) because the organs of adults of these insects develop during pupation from cellular monolayers, called imaginal discs (Currie et al., 1988; Morata and Lawrence, 1979). Insect compound eyes provide an opportunity to explore scaling within an organ because the facet array provides a read-out at cellular-level resolution of relative investment in individual facets (Shingleton et al., 2005; Stevenson et al., 1995). During development individual retinal cells arise from an ommatidial progenitor (Egelhaaf et al., 1988; Friedrich et al., 1996) and do not contribute to adjacent ommatidia as they differentiate (Wolff and Ready, 1991). Therefore, facet scaling provides some information about resource allocation at the cellular level within an imaginal disc during development.

We studied the scaling of wood ant (*Formica rufa* L.) worker compound eyes. The area of their compound eyes as well as the numbers of facets and their diameters increase with body size, though they do so with negative allometry. We found substantial heterogeneity in scaling of facet diameter between different regions of the compound eye, demonstrating hitherto unknown control of scaling of structures within organs.

2.3 Materials and methods

2.3.1 Animals

Formica rufa (Hymenoptera: Formicidae) colonies were collected from Broadstone Warren, Sussex, UK (51°04'40.8"N, 0°01'48.0"E) between June 2013 and August 2014, and maintained indoors at 21°C under a 12 L : 12 D cycle.

2.3.2 Specimen preparation

Individual ants were restrained and transparent nail varnish (Rimmel London, UK) was applied to both compound eyes to create a cast. Once dried, the casts were removed, flattened and mounted onto 12.5 mm specimen stubs (Agar Scientific, UK). The rear left femur of each ant was used as a proxy for the size of the ant (Espadaler and Gómez,

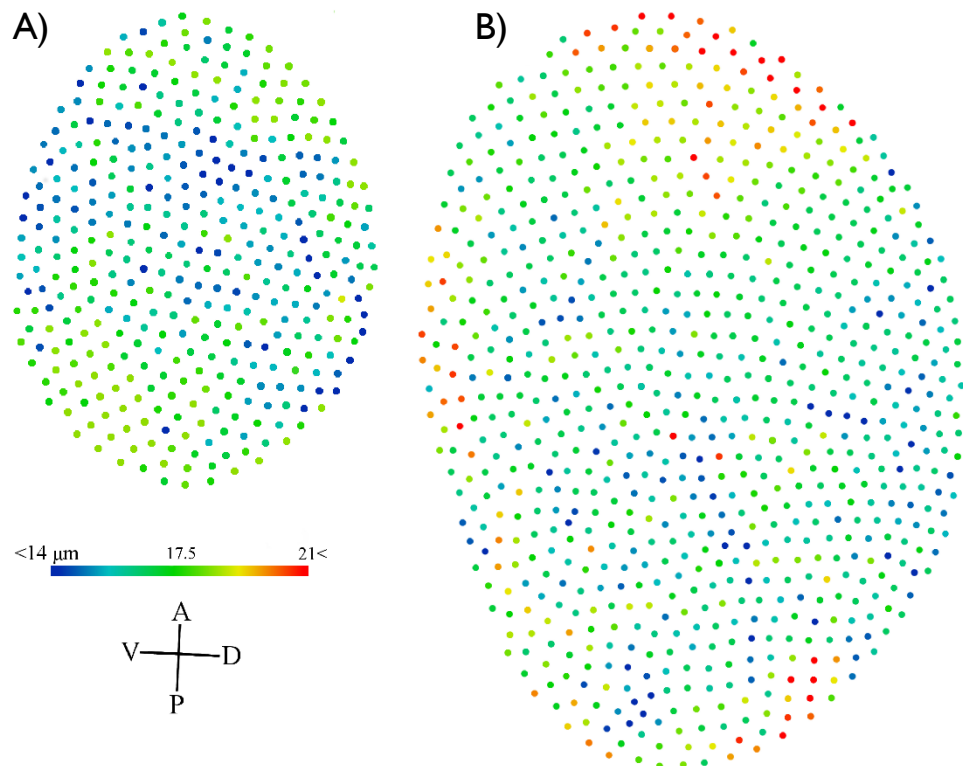


Figure 2.1 Regional differences exist in the diameters of facets from compound eyes of wood ant workers. A) A heat-map of the diameter of each facet from a single wood ant worker compound eye, 382 facets in total. B) As in A) but for an eye from a larger worker. A – anterior, D – dorsal, P – posterior, V – ventral.

2001). Specimens were imaged using a scanning electron microscope (S420 Stereoscan, LEO Electron Microscopy Ltd., Germany).

2.3.3 Measurements

Nine facet diameters from four separate eye regions were selected at random and measured from 66 ants (2376 facets in total) from three colonies. The diameters of every facet from a representative small and large ant were measured to produce eye 'heatmaps'. Diameters were measured from scanning electron micrographs using ImageJ v. 1.48 (Schneider et al., 2012). Facets were sampled from near the margins of the eye

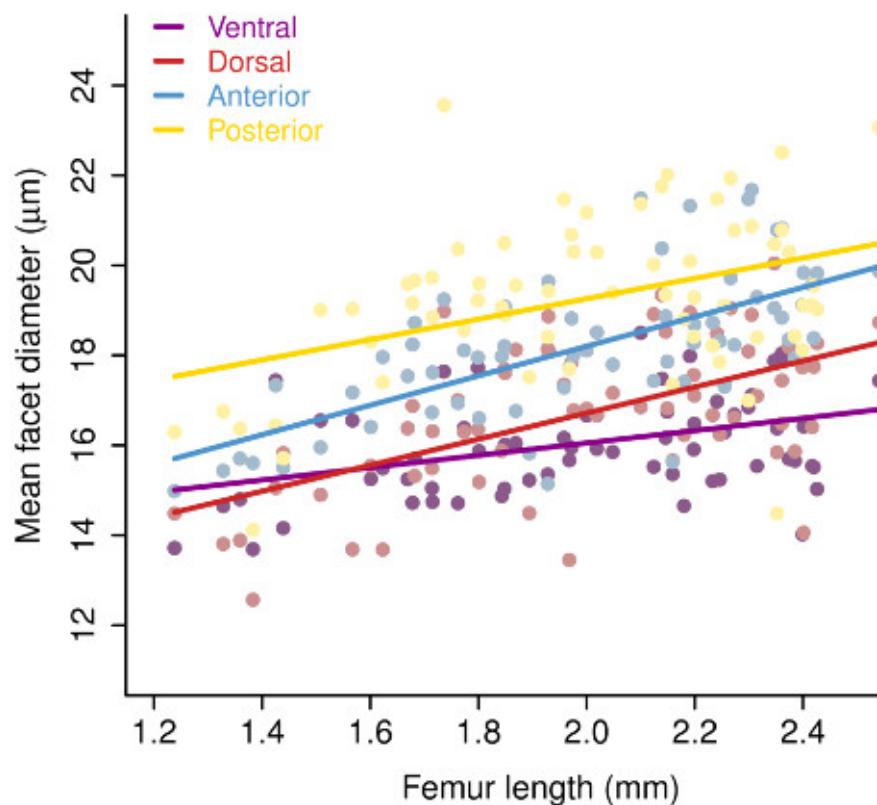


Figure 2.2 The diameters of facets from different eye regions scale differentially. The graphs show the scaling of facets from the anterior, posterior, dorsal or ventral regions of the compound eye for workers from three wood ant nests.

in each of the regions respectively. This prevented facets from being sampled from the centre of the eye or from ambiguous regions. Facets were not sampled from the outer two rows because these were often deformed to accommodate the eye meeting the cuticle of the head.

2.3.4 Statistics

Statistics were calculated using R v. 3.1.2. (R Core Team, 2016). Facet diameter scaling was investigated with linear mixed effect models by using the lme function from the 'nlme' package (Pinheiro et al., 2016). Custom contrast matrices were used to make post hoc multiple pair-wise comparisons (t-tests) of linear mixed effect models with the estimable function from the 'gmodels' package (Warnes et al., 2015).

2.4 Results

We measured the facet diameters of the eyes of small and large workers (Fig. 2.1), creating maps of facet diameters (Schwarz et al., 2011). These maps revealed differences in facet diameters between the large and the small workers, as well as regions of the eye in which facets differed systematically in diameter. In the eye of the larger worker, the largest facets are found mainly in the anterior–dorsal region, whereas in the smaller worker eye, the largest facets are restricted to the anterior–dorsal and ventral–posterior regions (Fig. 2.1). To quantify differences in facet diameter between regions and across a range of worker body sizes, we measured facet diameters from four regions (anterior, posterior, ventral and dorsal) of the compound eye.

Comparisons among eye regions showed that, for a given body size, facets differed in absolute diameter between regions within an individual ant. Facet diameters were, however, larger across all regions of the larger worker eyes than in those of

smaller workers. The intercept of the posterior region was significantly higher than that of the anterior region ($t_{65,188} = 2.69$, $p = 0.008$). The dorsal region had a significantly lower intercept than either the posterior ($t_{65,188} = 3.28$, $p = 0.001$) or ventral eye regions ($t_{65,188} = 2.05$, $p = 0.04$). There were no differences among the comparisons of the remaining regions ($t_{65,188} < 1.47$, $p > 0.1$).

Thus, as workers increase in size, facets in some regions are absolutely larger than others but increase in diameter proportionally producing grade shifts (Fig. 2.2). Comparison among eye regions also revealed significant differences in the slope of the scaling relationship, indicating that facet diameters in some regions became relatively larger than others with increasing body size. The facet diameters in the anterior ($t_{65,188} = 3.36$, $p = 0.001$) and dorsal regions of the eye ($t_{65,188} = 2.65$, $p = 0.009$) increased with body size with a greater amount than those from the ventral eye region. There were no differences among the comparisons of the remaining regions ($t_{65,188} < 1.81$, $p > 0.07$). Thus, as workers increase in size, facets in the anterior–dorsal region increase in diameter at a greater rate than the rest of the eye.

Table 2.1 Linear model co-efficients corresponding to data presented in Fig. 2.2.

Eye region	Slope \pm s.e.	Intercept \pm s.e.	p-value	r^2
Anterior	3.27 ± 0.47	11.65 ± 0.93	>0.0001	0.44
Dorsal	2.90 ± 0.52	10.92 ± 1.04	>0.0001	0.32
Posterior	2.27 ± 0.65	14.72 ± 1.32	0.001	0.15
Ventral	1.37 ± 0.42	13.31 ± 0.85	0.002	0.13

2.5 Discussion

Comparison of facet diameters among different regions of the wood ant compound eye shows that they scale heterogeneously. In some regions, large facets increase in diameter proportionally with absolutely smaller facets in other regions, indicative of grade shifts. This implies that facets in these regions are of equal importance, and that additional

resources associated with increased body size are allocated proportionately. Some regions differ in slope of their scaling relationships, showing that available resources are disproportionately allocated within the developing eye imaginal disc, larger individuals investing more in the anterior and dorsal regions of the compound eye than the ventral region.

2.5.1 Proximate mechanisms

We propose that individual cells within an imaginal disc use nutrients to different extents. Growth and nutrition are linked by insulin production (Nijhout et al., 2014; Wu and Brown, 2006). Cells may show regional differences in their expression of insulin receptors, so that when exposed to the same increased levels of insulin-like peptides those that express more insulin receptors will grow at a faster rate. This mechanism is analogous to that proposed to account for the differential growth of imaginal discs underlying exaggerated traits and could provide the basis for the evolution of organ shape changes (Emlen et al., 2012). One putative mechanism is that adjusting the number of insulin and ecdysone receptors in different parts of an imaginal disc could alter the shape of an entire organ. Such changes could, for example, contribute to the evolution of the horns of adult males from different species of *Onthophagus*, which differ in the number of prongs and their shape (Emlen et al., 2005).

2.5.2 Functional implications

Increases in facet diameter improve sensitivity by improving photon capture (Land, 1997). Thus, differences in facet diameter within the wood ant compound eye are presumably a consequence of needing regions of high sensitivity and resolution with limited resources and space available. Such specialized regions are common in compound

eyes and are typically associated with specific aspects of behaviour where high performance is required, such as mate or prey detection (Land, 1997). However, previous studies have not considered that particular regions of the compound eye may differ from one another in terms of their scaling (Everett et al., 2012).

Slope shifts indicate differential investment in particular regions depending on body size. Such differential investment may be related to task differentiation; despite lacking distinct morphological castes, larger ants forage further from the nest than smaller ants (Wright et al., 2000), which may necessitate greater investment in vision. However, rhabdom structure and interommatidial angles are needed to determine the impact of these differences in scaling upon wood ant vision.

More generally, increased investment in specific regions of the compound eye or other sensory structures may confer an advantage on larger individuals of a particular species in specific tasks, especially when such regions are linked to the detection of mates or prey. This raises the possibility that eye regions such as the love spot of male houseflies (Burton and Laughlin, 2003) may also show scaling indicative of greater investment in larger individuals. This would produce exaggerated sensory structures analogous to the exaggerated morphological traits more typically associated with sexual selection, such as *Onthophagus* beetle horns (Emlen et al., 2005). However, the lattice structure of the compound eye may constrain investment in such regions, preventing them from showing the extreme positive allometry of beetle horns.

Chapter 3: Colony-level differences in wood ant eye scaling

3.1 Abstract

Differential organ growth during development is essential for adults to maintain the correct proportions and achieve their characteristic shape. Organs scale with body size, a process known as allometry that has been studied extensively in a range of organisms. Such scaling rules, typically studied from a limited sample, are assumed to apply to all members of a population and/or species. Here we study scaling in the compound eyes of workers of the wood ant, *Formica rufa*, from different colonies within a single population. Workers' eye area increased with body size in all the colonies showing a negative allometry. However, both the slope and intercept of some allometric scaling relationships differed significantly among colonies. Moreover, though mean facet diameter and facet number increased with body size, some colonies primarily increased facet number whereas others increased facet diameter, showing that the cellular level processes underlying organ scaling differed among colonies. Thus, the rules that govern scaling at the organ and cellular levels can differ even within a single population.

3.2 Introduction

Understanding how organ size and shape is controlled during development is a major challenge in biology. The control of organ morphology is particularly problematic for organisms that need to develop organs to meet specific requirements under fluctuating conditions and resources. In natural environments, adults from a single species can vary enormously in body size due to a combination of genetic and environmental factors. The changes in organ size that accompany changes in body size can be characterised by allometric scaling relationships. The scaling of any feature with size can be described by:

$$Y = bx^{\alpha}$$

Where x is a measure of body size, Y is the size of the organ in question, α is the scaling exponent and b is the initial growth index (Huxley and Teissier, 1936).

When no change occurs in the relative size of an organ with body size ($\alpha = 1$) the relationship is described as isometric (Lease and Wolf, 2010). More typically, however, organs show negative allometries ($\alpha < 1$) becoming smaller relative to larger body sizes (Eberhard, 2009; Eberhard et al., 1998). Even with negative allometries organs can be absolutely larger in animals with a greater body size, proportionally smaller when compared to the same organ in smaller conspecifics (Gayon, 2000; Huxley and Teissier, 1936; Klotz et al., 1992). In rare cases, organ size may show positive allometry increasing in size greater than body size ($\alpha > 1$) (Emlen, 1997; Gould, 1973). Such positive allometry is often associated with organs under sexual selection (Emlen, 1997; Gould, 1973).

The scaling of different organs within a body is the product of differential growth; as an organism grows larger, certain organs grow at a faster rate than others (Eberhard et al., 1998; Emlen et al., 2012). This is thought to occur through differential resource allocation (Bonduriansky and Day, 2003; Emlen et al., 2012; Kodric-Brown et al., 2006), whereby resources are distributed to different organs at different rates. Scaling has been studied in many taxa including mammals and birds (Burton, 2006; Emlen, 2008; Gatesy, 1991; Prange et al., 1979) and especially insects (Berrigan, 1991; Emlen, 2008; Miyatake, 1993; Nijhout and Grunert, 2010; Tomkins, 1999). In part, this is due to the power of genetic tools available in the fruit fly *Drosophila melanogaster* (Shingleton et al., 2009) but also because of the mode of development of holometabolous insects: the organs of these adult insects develop at the end of a period of larval growth from ectodermally-derived

cellular monolayers called imaginal discs (Currie et al., 1988; Morata and Lawrence, 1979).

Differential resource allocation to imaginal discs during pupation is mediated through insulin-like peptides (ILPs) and their receptors (Shingleton et al., 2005). During the larval (or feeding) stage ILPs are produced in response to changes in nutrition (Nijhout et al., 2014; Wu and Brown, 2006) and, along with ecdysone, are responsible for inducing somatic growth (Nijhout and Callier, 2015). During the pupal (or non-feeding) stage imaginal disc cell growth is also mediated by ILPs, but ILP release is controlled via ecdysone levels instead of responding to nutrition (Nijhout and Callier, 2015). Insulin receptors are expressed by imaginal disc cells (Shingleton et al., 2008) the greater the number of receptors, the more sensitive the disc is to increases in ILPs (Emlen et al., 2012). Hence, greater nutrition leads to increases in organ size, but the scaling of different organs varies depending on the relative sensitivities to ILPs (Emlen and Allen, 2003; Lavine et al., 2015; Shingleton et al., 2007). Additional factors, including genetics (Bargum et al., 2004; Shingleton et al., 2009; Stevenson et al., 1995) and temperature (Nijhout et al., 2014; Shingleton et al., 2009), will also have an impact upon scaling and size changes in response to feeding.

Organs such as the compound eyes and wings of insects provide an opportunity to explore scaling at the cellular level because external structures visible in adult organs provide a read-out at a cellular-level resolution (Bonduriansky and Day, 2003; Emlen et al., 2012). In compound eyes this means that the size of the facets are representative of the level of cellular growth and division that occurs during development (Oliver and Gruss, 1997). The scaling of compound eyes with body size has been investigated in numerous insect species (Baker and Ma, 2006; Bernstein and Finn, 1971; Jander and Jander, 2002; Kapustjanskij et al., 2007; Klotz et al., 1992; Schwarz et al., 2011; Spaethe

and Chittka, 2003; Streinzer et al., 2013; Zollikofer et al., 1995). In all these investigations insect compound eyes increase in size (measured as either eye length or area) with increasing body size but show negative allometry. Some species, such as *Cataglyphis albicans*, *C. bicolor*, *C. fortis* (Zollikofer et al., 1995), *Camponotus pennsylvanicus* (Klotz et al., 1992) and *Melophorus bagoti* (Schwarz et al., 2011), primarily increase facet number as they get larger whereas others, such as the bee *Bombus terrestris* (Kapustjanskij et al., 2007; Spaethe and Chittka, 2003) and the ants *Solenopsis* sp. (Baker and Ma, 2006), and *Formica integroides* (Bernstein and Finn, 1971) increase facet diameter and facet number.

Wood ants, *Formica rufa* (L.), form nest mounds (Fig. 3.1a) containing up to 100 queens and 100,000-400,000 workers without distinct castes (Collingwood, 1979). Workers form large trails within woodlands (Fig. 3.1b) and use visually-guided navigation whilst foraging (Graham and Collett, 2002), ensuring that resource allocation to the compound eye is important for their ecology, and suggesting that scaling relationships within the visual system have a functional consequence. We studied the scaling of wood ant compound eyes, exploring organ-level morphological changes in eye area, facet number and size and how this differed between nests. We found substantial heterogeneity in eye scaling between different nests within the same population. This heterogeneity calls into question many inherent assumptions made by studies examining differential organ scaling.

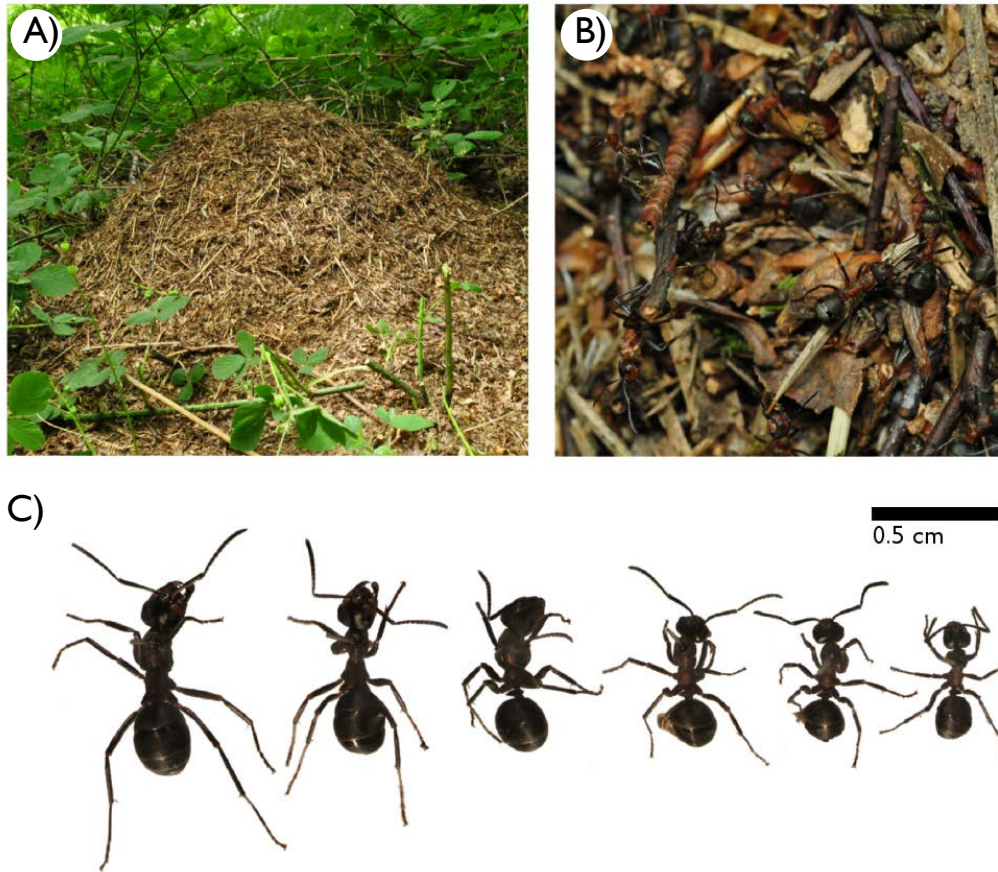


Figure 3.1 Size variation in wood ant (*Formica rufa*) workers. A) A wood ant nest, and B) workers on a foraging trail. C) Workers from a single nest are morphologically undifferentiated but span a wide range of body sizes.

3.3 Methods

3.3.1 Animals

Whole colonies of *Formica rufa* (L.) (Hymenoptera: Formicidae) were collected from Ashdown Forest, Sussex (N 51 4.680, E 0 1.800) between June 2013 and August 2014, and maintained under a 12:12 hour light:dark cycle indoors at 21°C. Foraging workers from nest #1 and #2 were sampled simultaneously during the end of 2013, ants from nest #3 were sampled from August 2014.

3.3.2 Specimen preparation

Individual ants were selected from a colony at random and restrained with Plasticine (Early Learning Centre, UK). Transparent nail varnish (Rimmel London, UK) was applied to both compound eyes using a cocktail stick to create a cast. Ants were then stored at 4°C for a minimum of 48 hours to ensure the casts completely dried. The nail varnish casts were removed, flattened with incisions and mounted on to 12.5 mm specimen stubs (Agar Scientific, UK). Casts were made as in Ribi *et al.* (1989). The rear left femur of each ant was mounted along with the eye cast as a proxy for the size of the ant. Nail-varnish eye casts and femurs were gold-coated and imaged using a scanning electron microscope (S420 Stereoscan, LEO Electron Microscopy Ltd., Germany).

3.3.3 Measurements

Sixty six ants from three separate colonies were measured; 17 from nest #1, 30 from nest #2 and 19 from nest #3. Femur length, facet diameters, facet counts, eye areas and eye dimensions were measured from scanning electron micrographs using ImageJ v.1.48 (Schneider *et al.*, 2012). Facet diameters are known to be non-uniform across compound eyes of insects. To account for this variation we split the eye into four regions (anterior, posterior, dorsal and ventral). Facet diameters were measured from three sets of three facets from these eye regions (*i.e.* 3 measurements from each region, 12 measurements from each cast). In two ants, the diameters of all the facets were measured. Eye area was calculated by approximating the shape of the eye as an oval. To validate this approximation we measured the real eye area from 15 ants (five ants per nest) and compared the real measurements with the approximations using a linear regression. The results of this analysis (Fig. 3.8) indicated that approximating eyes as ovals provided an accurate measure of eye area (intercept = 4.39 ± 5.62 , $p = 0.448$; slope = 0.96 ± 0.03 , p

< 0.0001 ; $r^2 = 0.98$). Facets were counted by hand from one of the eyes of each ant from scanning electron micrographs. Right eye was primarily used but the left eye was employed on occasions where the right eye was rendered unusable. Left eyes were employed too infrequently to establish if a statistical difference existed between them. Femur length was selected as a proxy for mass, though it scales positively with mass in Formicines (Espadaler and Gómez, 2001).

3.3.4 Statistics

Eye area, mean facet diameter and facet number were analysed using analysis of covariance (ANCOVA) constructed with R base-package (R Core Team, 2016). Non-significant ANCOVA terms were eliminated step-wise until only significant terms remained in the model. For cases in which data violated the assumptions of ANCOVA, we compared the output to robust linear models constructed using the `lmRob` function from the ‘robust’ package (Wang et al., 2017). There were no differences between analyses performed with robust linear models and ANCOVAs.

Principle component analysis (PCA) was conducted using R base-package (R core team, 2014) and cluster analysis was conducted using the `Mclust` function from the “mclust” library (Fraley et al., 2012), which uses Bayesian Information Criterion (BIC) scores from model-based inferences to calculate the optimum number of clusters. Data were normalised prior to PCA to ensure equal variance amongst groups.

Custom contrast matrices were used to make post-hoc multiple pair-wise comparisons of ANCOVAs with the `estimable` function from the ‘gmodels’ package (Warnes et al., 2015). All statistics were calculated using R v.3.1.2.

3.4 Results

3.4.1 Size variation in wood ants

Wood ant workers lack distinct morphological castes but they span a wide range of body sizes (Fig. 3.1c). Even with a single colony, the smallest workers can be less than half the size of the largest (Fig. 3.1c: 3.4). Irrespective of size, workers possess small, flat compound eyes located laterally on their head (Fig. 3.2) but larger workers possess larger eyes than their smaller counterparts (Fig. 3.5).

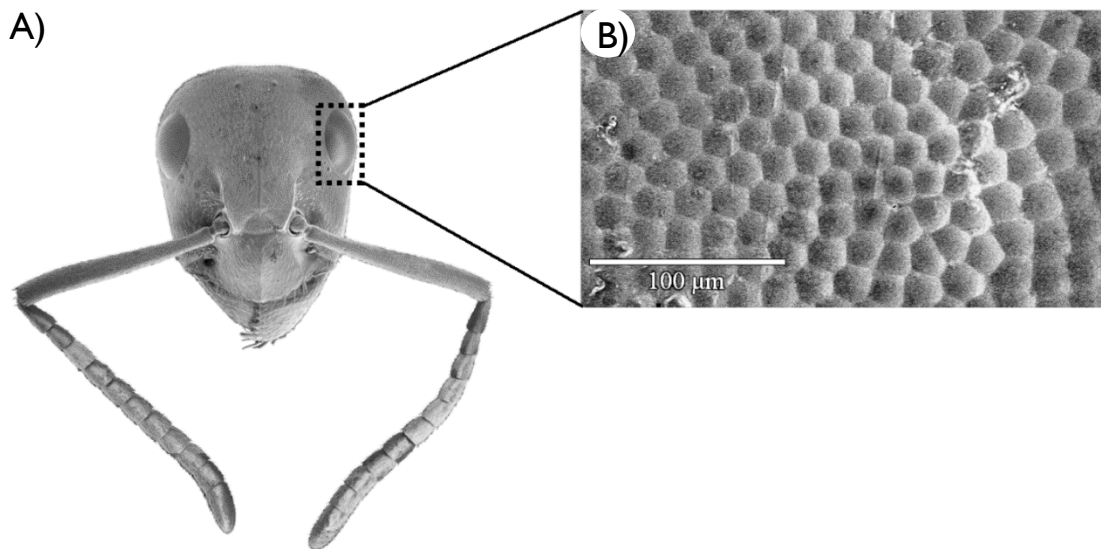


Figure 3.2 A) A frontal view of the head of a large worker viewed under a scanning electron microscope. B) Close-up of surface of worker eye showing details of the facet array.

3.4.2 Eye morphology

We quantified the differences in the area of compound eyes of small and large workers from three separate nests, using the square-root of eye area to preserve dimensionality (Fig. 3.3). As expected, in all three nests the compound eye area increased with increasing ant size ($F_{63,59} = 297.16$, $p < 0.001$; subscript denotes sample size, degrees of freedom). The compound eyes of smaller workers were absolutely smaller but larger relative to their mass than those of their larger counterparts (Fig. 3.3a). Consequently,

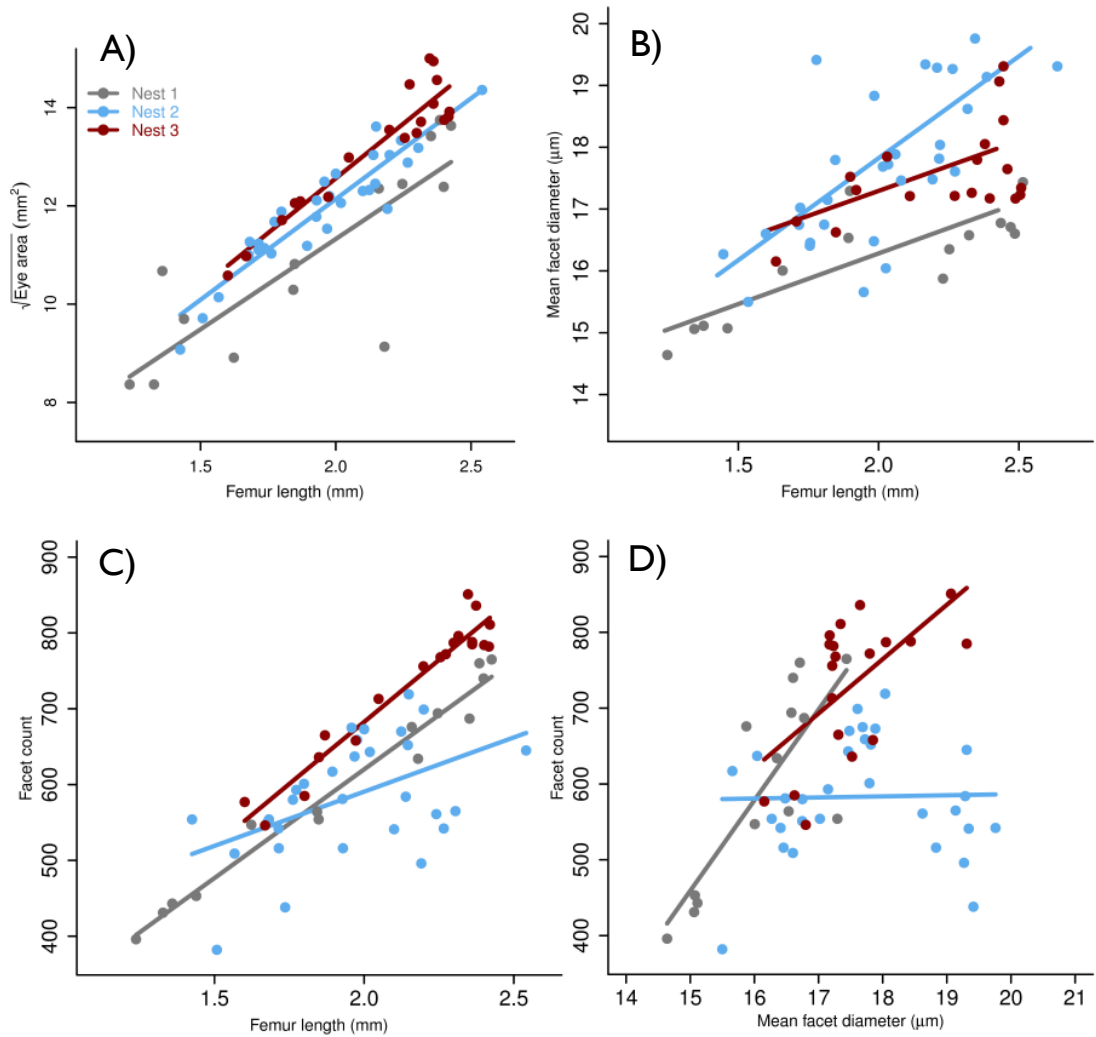


Figure 3.3 Differential scaling of compound eyes and facets among colonies. Allometric scaling of A) eye $\sqrt{\text{area}}$ (mm) (r^2 values: Nest #1: 0.66; Nest #2: 0.86; Nest #3: 0.87), B) mean facet diameter (μm) (r^2 values: Nest #1: 0.66; Nest #2: 0.46; Nest #3: 0.28) and C) facet number with a proxy of body size (femur length) (r^2 values: Nest #1: 0.97; Nest #2: 0.21; Nest #3: 0.93). D) Scaling of facet number with mean facet diameter (r^2 values: Nest #1: 0.63; Nest #2: -0.04; Nest #3: 0.32). Sample sizes: Nest #1: 17; Nest #2: 29; Nest #3: 19.

for each of the three nests eye area had a negative allometric scaling relationship scaling relationship with hind femur length (Espadaler and Gómez, 2001), which we used as a proxy for body size (Table 3.1). Comparisons among the three nests showed significant differences in mean eye area ($F_{63,59} = 12.25$, $p < 0.001$) but failed to reveal a significant interaction between eye area and body size ($F_{63,57} = 1.54$, $p = 0.22$), indicating that the rate of increase in eye area with body size is the same between nests. Pairwise comparisons between nests revealed that the mean eye area of ants from nest #1

differed from that of nest #2 ($t_{63,59} = 2.95$, $p < 0.01$) and #3 ($t_{63,59} = 4.91$, $p < 0.001$). The mean eye area of ants from nest #2 also differed from that of nest #3 ($t_{63,59} = 2.74$, $p < 0.01$).

We assessed the differences in eye area scaling between nests using principle component analysis (PCA) followed by cluster analysis. PCA was used to reduce the three variables of interest (femur length, eye area and nest) to two principle components. The first two principle components explained 97% of the variation in the data. Principle component 1 (PC1) was negatively correlated with all three variables, though primarily femur length and eye area, whereas PC2 was strongly positively correlated with nest affiliation (Table 3.1, Fig. 3.6). Subsequent cluster analysis revealed that there were four clusters; one corresponding to nest #1, another for nest #2, and a further two clusters for points belonging to nest #3 (Table 3.1, Fig. 3.6).

Changes in both the diameter and number of facets could account for the scaling of eye area with body size, and for the differential scaling of eye area among nests. We measured the diameter of facets from the compound eyes of small and large workers from all three nests. The diameter of every facet from a representative small and large ant were measured, yielding facet ranges of 12.45-21.51 μm and 15.33-23.22 μm , respectively. Our measurements showed that mean facet diameters scale with negative allometry ($F_{63,59} = 50.91$, $p < 0.001$) (Fig. 3.3b). However, mean facet diameter was relatively larger in small ants compared with their larger counterparts. Consequently, for each of the three nests facet diameter had a negative allometric scaling relationship (Table 3.1). Comparison among the three nests showed significant differences in their mean facet diameter ($F_{63,59} = 15.89$, $p < 0.001$). Ants from nest #1 had significantly smaller mean facet diameters than those from nest #2 ($t_{63,59} = 5.57$, $p < 0.001$) or nest #3 ($t_{63,59} = 3.40$, $p < 0.01$). Ants from nest #3 had significantly larger facet diameters than those

from nest #2 ($t_{63,59} = 2.31$, $p = 0.02$). Yet despite the differences in mean facet diameter, the rate of increase in facet diameter with body size did not differ between nests ($F_{63,57} = 3.13$, $p = 0.05$).

We also counted all the facets from the compound eyes of each worker from which we had previously measured the area and facet diameters. As expected, the number of facets per eye increased with body size ($F_{63,57} = 206.27$, $p < 0.001$) (Fig. 3.3c). Our counts revealed that smaller ants had relatively more facets than their larger counterparts and, akin to area and facet diameter, facet number had a negative allometric scaling relationship for each of the three nests (Table 3.1). Comparisons between nests revealed differences in the mean number of facets between nests ($F_{63,57} = 20.58$, $p < 0.001$). Ants from nest #2 had fewer facets per eye than those from nests #1 ($t_{63,57} = 2.73$, $p < 0.01$) or #3 ($t_{63,57} = 2.40$, $p = 0.02$). Ants from nest #1 and nest #3 did not differ in their mean number of facets per eye ($t_{63,57} = 0.16$, $p = 0.87$). Comparisons among all three nests also revealed a significant interaction between the rate of increase in facet number and body size ($F_{63,57} = 6.56$, $p > 0.01$), indicating that it differs for ants from different nests. Pairwise comparisons revealed that the rate of increase in facet number did not differ in ants from nests #1 and #3 ($t_{63,57} = 0.73$, $p = 0.46$). However, ants from nest #2 scaled facet number less steeply than either nest #1 ($t_{63,57} = 2.99$, $p < 0.01$) or #3 ($t_{63,57} = 3.23$, $p < 0.01$).

To establish how facet diameter and facet number contributed to eye area for each nest, we examined how facet number increased as a function of mean facet diameter (Fig. 3.3d). Across all three nests combined, there was a significant increase in facet number with larger mean facet diameters ($F_{63,57} = 12.19$, $p > 0.001$). A significant interaction term between mean facet diameter and nest ($F_{63,57} = 22.25$, $p < 0.001$) indicated that the rate of facet number increase with increasing facet diameter is different

for ants from different nests. Pairwise comparisons revealed that the rate of facet number increase differed between nests #1 and #2 ($t_{63,57} = 4.35$, $p < 0.001$) and nests #2 and #3 ($t_{63,57} = 2.67$, $p < 0.01$), though not between nests #1 and #3 ($t_{63,57} = 1.40$, $p > 0.1$).

Visual examination of the data indicated a subset of putative outliers (Fig. 3.3d). We used PCA combined with cluster analysis to investigate whether these ants formed a distinct group of individuals following different scaling rules from the remainder of nest #2. Again, PCA was used to reduce the three variables of interest (facet number, mean facet diameter and nest affiliation) to two principle components. The first two principle components explained 82.7% of the variance in the data. PC1 was negatively correlated with all three variables to largely equal extents, whereas PC2 was strongly negatively correlated with mean facet diameter (Table 3.2). The cluster analysis revealed three clusters (Fig. 3.7). One cluster was formed from representatives of all three nests and another from ants exclusively from nest #2. There was also a third cluster composed of ants exclusively from nest #3, though there were no obvious outliers (Fig. 3.7). This independent nest #3 cluster is formed from ants that have a facet count higher than predicted from the regression line. The independent nest #2 cluster is formed from the individuals that we identified as putative outliers from nest #2.

3.5 Discussion

By making use of the unique structure of the insect compound eye, we were able to analyse scaling rules that govern organ size. These rules differ among nests from the same population, as well as differing between ants from the same nest. Below we discuss the causes and consequences of these differences in scaling, and the implications for scaling studies more generally.

As wood ant workers' body size increases, so too does the area of their compound eyes, as well as the numbers of facets and their diameters, though they do so with negative allometry. Consequently, smaller ants have compound eyes with relatively larger areas and facet diameters, and relatively more facets than their larger counterparts. These scaling relationships occur in all the nests we studied and, in this respect, they resemble relationships observed in other insect species such as *Formica integroides* (Bernstein and Finn, 1971), *Cataglyphis* sp. (Zollikofer et al., 1995), *Melophorus bagoti* (Schwarz et al., 2011), *Bombus terrestris* (Kapustjanskij et al., 2007) and *Solenopsis* sp. (Baker and Ma, 2006). However, comparison among nests reveals significant differences in their scaling relationships, more typical of those reported among species. Both grade shifts and slope shifts occur depending upon the specific parameter measured. The scaling of eye area primarily differs in intercept among nests, characteristic of grade shifts. Indeed, ants from all three nests differing from each other in terms of eye area. Differences also occurred at the cellular level: mean facet diameters show grade shifts among all three nests; both slope and grade shifts occur in facet number among nests; and both grade and slope shifts occur when facet number scales against mean facet diameter.

Consequently, formulating definitive rules about the allometric scaling of wood ant compound eyes is difficult because no two nests followed similar patterns. Rather than increases in eye area being mediated through either increased facet numbers or diameters, both contribute in a nest-dependent manner. Patterns of eye growth are further complicated by subsets of ants from a given nest using different scaling rules as their eyes develop; ants from nest #2 and #3 contained individuals with a different relationship between facet diameter and number compared with the majority of the

sampled population. Thus, there is considerable plasticity in scaling rules across wood ant populations to which both genetic and environmental factors may contribute.

The nests we compared were all from the same polygynous population, and are likely to have been closely related because polygynous *Formica* sp. alates do not disperse far (Sundström et al., 2005). Nevertheless, there may be substantial genetic variability within the nests because workers may be the progeny of up to 100 queens, and may not be true sisters at all due to polyandry (Sundström, 1993; Sundström et al., 2005). Thus, genetic factors, which are known to affect scaling relationships (Bargum et al., 2004; Shingleton et al., 2009; Sundström, 1993; Sundström et al., 2005), may contribute to scaling differences. Despite being derived from the same locale, the nests may have been subject to different environmental conditions, including nutrition and temperature, which could contribute to differences in scaling relationships. Larval nutrition influences adult body size in insects, with greater access to nutrition giving rise to larger adults (Merry et al., 2011; Thomas, 1993). Temperature likewise affects the growth of insect larvae because they are ectothermic, faster growth in warmer conditions typically resulting in relatively smaller adults (Atkinson, 1994; Mirth and Riddiford, 2007). Both temperature and nutrition influence organ scaling in fruit flies (Shingleton et al., 2009), which like ants are holometabolous, suggesting that these factors may affect scaling.

Nutritional differences among wood ant nests may arise because, following territorial skirmishes at the beginning of the season (Elton, 1932; Skinner, 1980), the trees that they have access to vary in the numbers of aphids from which honeydew can be obtained and other invertebrates (for protein) that they host. This will produce differences in larval nutrition, influencing their growth and, consequently,

resource allocation to developing organs (Emlen, 1997). Differences in nutrition could, therefore, partially explain differences in scaling relationships among wood ant nests.

Temperature differences and fluctuations are also common in natural environments, though *F. rufa* group ants attempt to maintain constant nest temperatures through various mechanisms including site selection to ensure direct access to solar radiation, metabolic heat generation by workers, and from decomposing plant material in larger nests (Jones and Oldroyd, 2006; Kadochová and Frouz, 2013; Rosengren et al., 1987). Wood ant workers also move larvae in the nest, placing them in different thermal environments during development (Rosengren et al., 1987). This suggests that, to some extent, wood ants can compensate for temperature differences and fluctuations within the local environment, though the effectiveness of this buffering is unknown.

Wood ant nests differ not only in the scaling of relative organ size but also in the cellular level rules from which the organs are constructed, so that in some nests larger eyes are primarily composed of more facets whereas in others they are primarily composed of larger facets. In nests #1 and #3 there are increases in both facet number and diameter, implying organ scaling through increases in cell size and number, a phenomenon also described in *Drosophila melanogaster* (Stevenson et al., 1995). Current models of organ growth offer a proximate explanation for such differences. During the non-feeding stage of holometabolous larvae, levels of insulin-like peptides (ILPs) and ecdysone control cell proliferation and growth, respectively, in imaginal discs (Nijhout and Callier, 2015; Nijhout et al., 2014; Wu and Brown, 2006). The release of these hormones is linked with nutrition during the larval feeding stage (Nijhout and Callier, 2015; Nijhout et al., 2014). Thus, increases in facet number may be due to relatively greater levels of ecdysone and increases in facet diameter due to relatively greater levels of ILPs. Genetic background may interact with environmental factors (Sundström et al.,

2005), which can themselves interact, to influence the extent of cellular proliferation or growth resulting in more or larger facets.

Both the number of facets within the compound eye and their diameters affect vision. Increases in facet number provide greater spatial resolution by increasing sampling of the visual field whilst increases in facet diameter improve sensitivity by improving photon capture (Land, 1997). The putative trade-off between increasing facet number and increasing facet diameter implies that nests are engaging in different developmental processes, investing in different aspects of vision.

The rules that govern the scaling of organs are often assumed to be a fundamental characteristic of a particular class of organism (e.g. species, sex). Typically, small numbers of organisms from single populations are used to determine the scaling of a particular trait with the assumption that the entire class conforms to the same relationship (Feener et al., 1988; Goldsmith; Wcislo and Eberhard, 1989). Our study suggests that this assumption does not always hold true. For *F. rufa*, there was considerable variation in allometric scaling relationships even among nests within the same population. Furthermore, allometric scaling studies often focus on the organ level (Eberhard et al., 1998; Emlen, 1997; Gould, 1973), ignoring the cellular level. Our study shows that the structure of organs may vary considerably at the cellular level, changes in organ size being produced by a combination of cell size and number. Our results provide a strong impetus for further investigations examining the interplay of cellular division and growth on the allometry of whole organs, and how these are affected by changes in nutrition and other environmental conditions. Together, our findings emphasise that allometric scaling relationships are highly malleable, at the organ and cellular levels, such malleability presumably allowing organisms to adapt their form to prevailing environmental conditions.

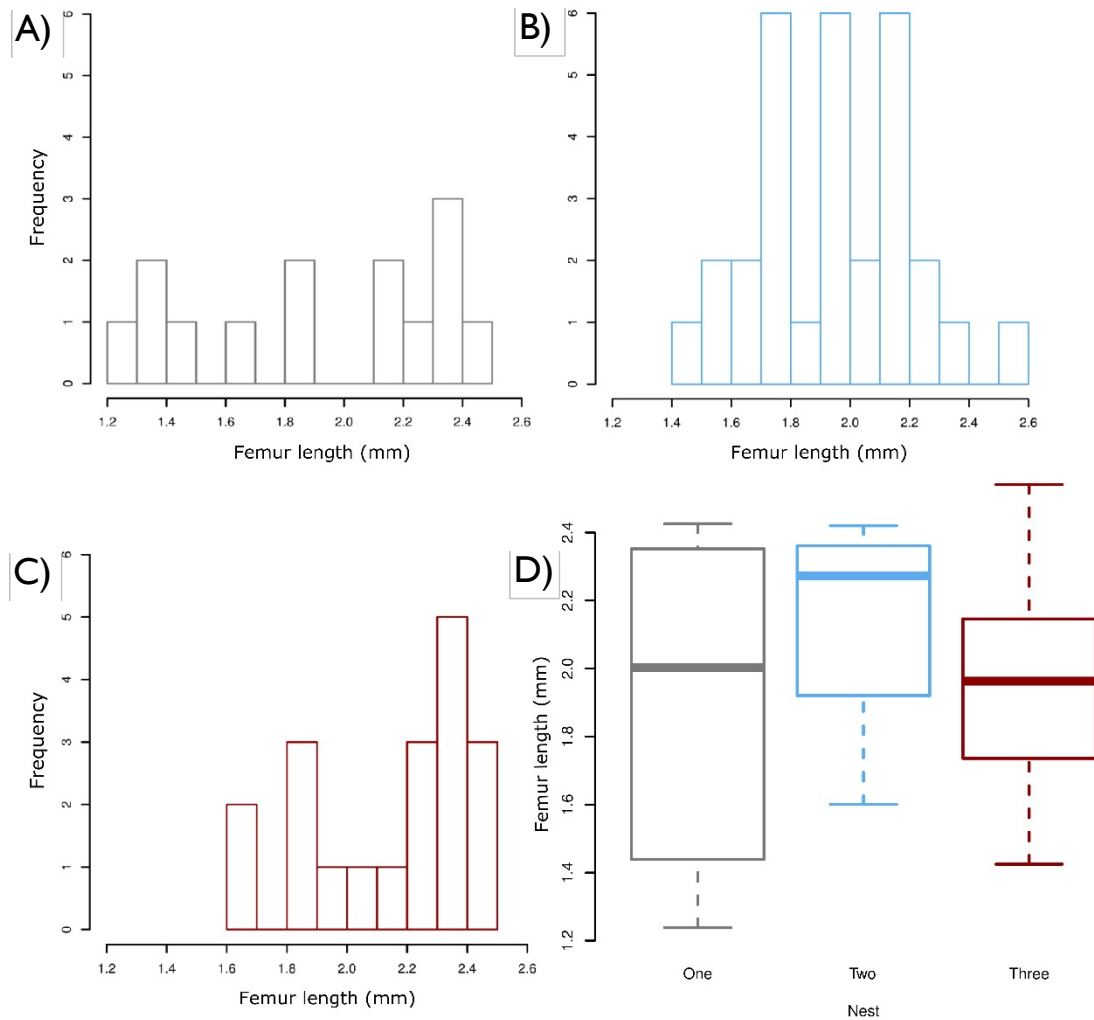


Figure 3.4 The size range and distribution of workers from three nests used in the investigation assessed by femur length. A) Nest #1 (number of ants, $n = 17$), B) Nest #2 ($n = 29$), C) Nest #3 ($n = 19$), and D) Range of femur length distributions from all three nests.

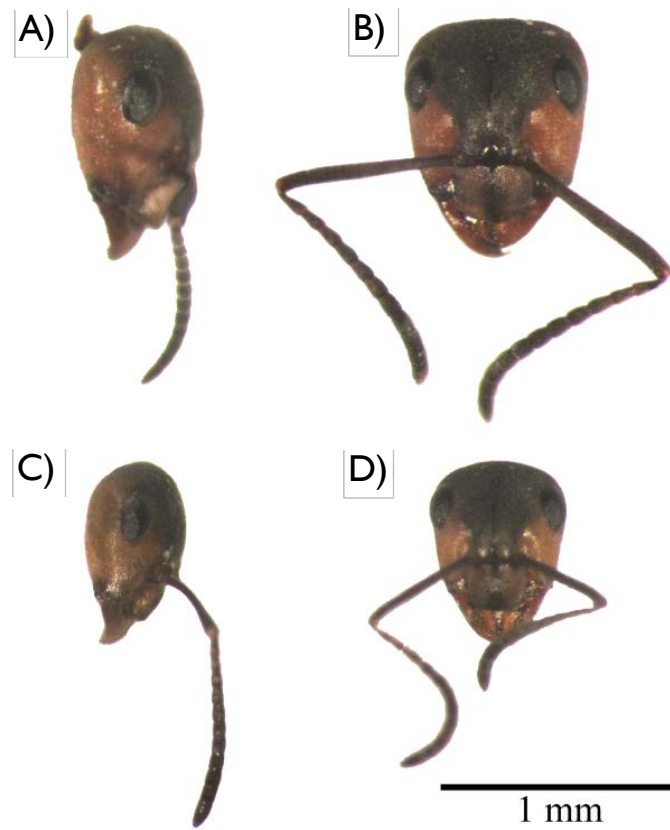


Figure 3.5 Differences in the head size of large and small workers. Lateral A) and frontal B) view of large ant worker head. C), D) as in A) and B) but for the head of a smaller worker.

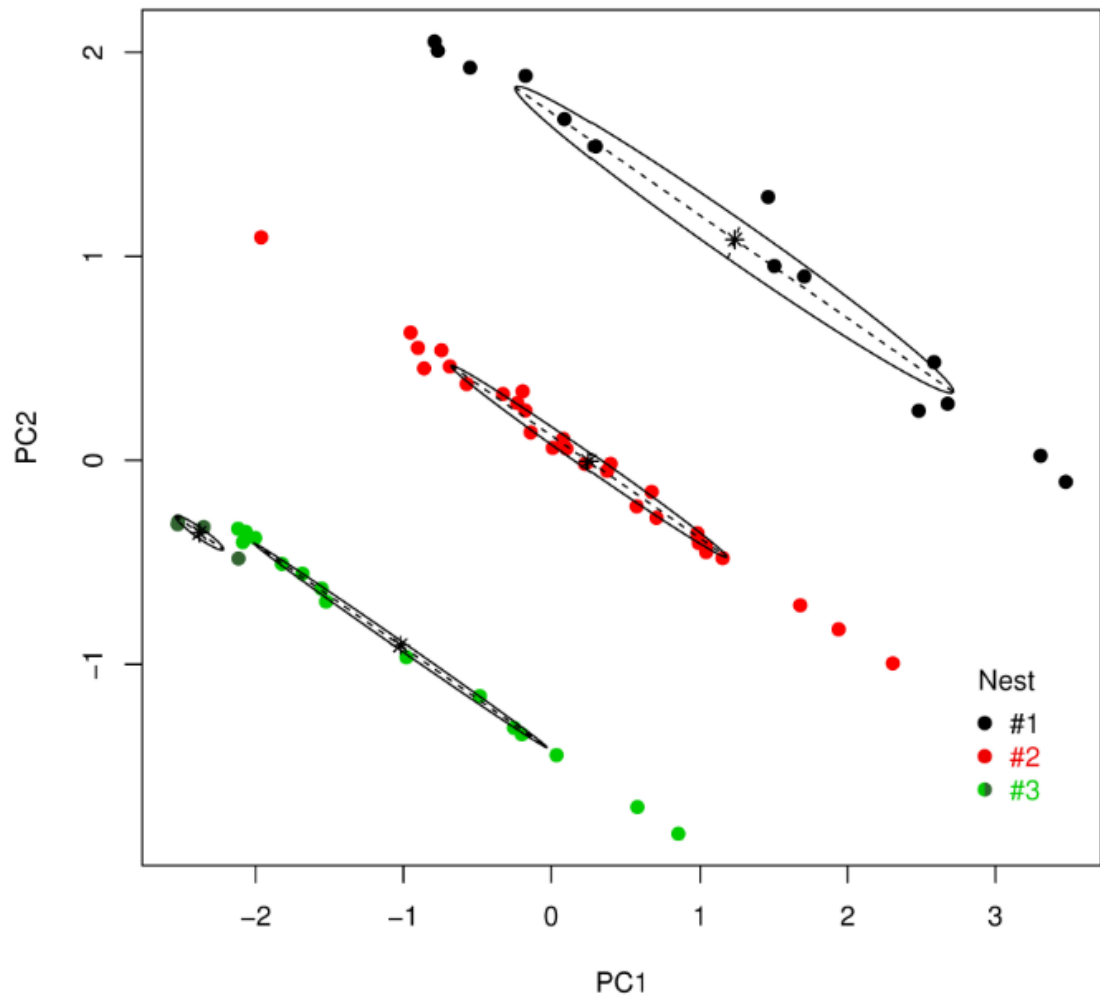


Figure 3.6 Cluster analysis of femur length, eye area and nest after dimension reduction using Principle Component Analysis (PCA).

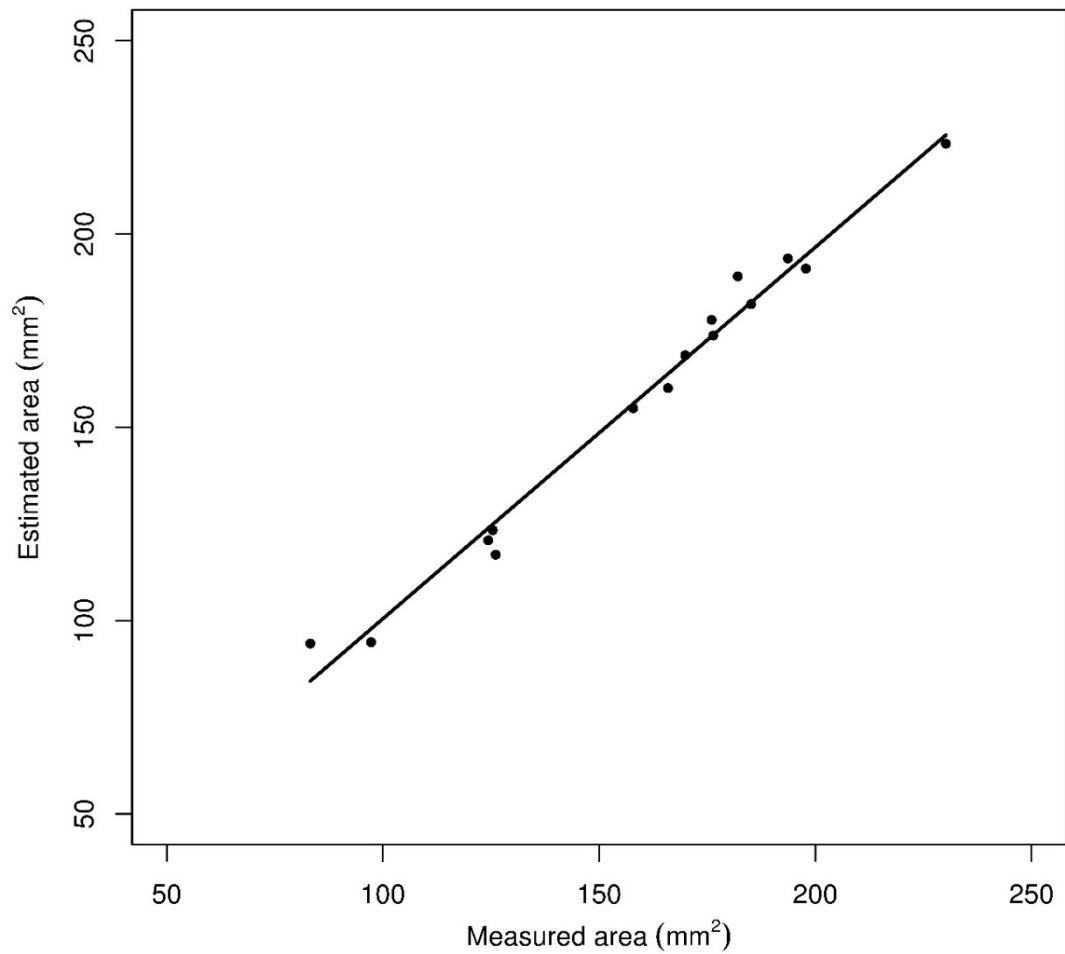


Figure 3.8 Eye area is well approximated by an oval. The area of 15 ant eyes (5 from each nest) estimated by approximating the eye as an oval as a function of area of the same eyes measured with ImageJ. (Intercept = 4.39 ± 5.62 , $p = 0.448$; slope = 0.96 ± 0.03 , $p < 0.0001$; $r^2 = 0.98$).

Table 3.1 *Scaling co-efficients from gross morphological eye measurements. Co-efficients were calculated by fitting a linear model to \log_e transformed data.*

Nest	N	Parameter (vs. femur length)	Slope (α)	\pm standard error	Intercept $\log_e(b)$	\pm standard error	Figure
#1	14	Eye area	0.56	0.12	2.01	0.08	3.3A
#2	30	Eye area	0.68	0.05	2.03	0.03	3.3A
#3	19	Eye area	0.72	0.06	2.04	0.06	3.3A
#1	14	Facet diameter	0.19	0.03	2.66	0.05	3.3B
#2	30	Facet diameter	0.36	0.07	2.63	0.05	3.3B
#3	19	Facet diameter	0.19	0.06	2.72	0.05	3.3B
#1	14	Facet number	0.91	0.04	5.80	0.03	3.3C
#2	30	Facet number	0.52	0.17	6.02	0.11	3.3C
#3	19	Facet number	0.96	0.06	5.86	0.05	3.3C

Table 3.2 *Loadings from principle component analysis used as a precursor to the cluster analysis examining effects of nest, eye area and femur length and loadings from principle component analysis used as a precursor to the cluster analysis examining effects of nest, mean facet diameter and facet number.*

Variable	Component 1	Component 2	Component 3
Femur Length	-0.611	-0.461	0.644
Eye Area	-0.660	-0.152	-0.736
Nest	-0.437	0.874	0.211
Facet Count	-0.579	0.568	0.585
Mean Facet Diameter	-0.534	-0.806	0.254
Nest	-0.616	0.165	-0.770

Chapter 4: Whole-organ and intra-eye scaling among *Formica* species

4.1 Abstract

Static allometries determine how organ size scales in relation to body mass. The extent to which these allometric relationships are free to evolve, and how they differ among closely-related species has been debated extensively and remains unclear; changes in intercept appear common but changes in slope are far rarer. Here we compare the scaling relationships that govern the structure of compound eyes of four closely-related ant species from the genus *Formica*. Comparison among these species revealed changes in intercept but not slope in the allometric scaling relationships governing eye area, facet number and mean facet diameter. Moreover, the scaling between facet diameter and number was conserved across all four species. In contrast, facet diameters from distinct regions of the compound eye differed in both intercept and slope both within a single species and when comparing homologous regions among species. Thus, even when species are conservative in the scaling of whole organs they can differ substantially in regional scaling within organs. This, at least partly, explains how species can produce organs that adhere to genus-wide scaling relationships whilst still being able to differentially invest in particular regions of organs to produce specific features that match their ecology.

4.2 Introduction

Allometric scaling characterizes how organ size changes as organisms themselves increase in size (Huxley and Teissier, 1936). Typically, allometric scaling relationships are power functions defined by two parameters; the intercept (b) and the power (α). Changes can occur in both intercept, referred to as grade shifts, and/or power, referred

to as slope shifts. Scaling relationships can be classified in one of three principle ways (Cock, 1966; Gould, 1966): (1) ontogenetic allometry, which characterises how an organ changes size as an organism develops (e.g. McLellan et al. 2002); (2) static allometry, which compares organ scaling among conspecifics at a given stage of development (typically adulthood) (McCullough et al., 2015); and (3) phylogenetic or evolutionary allometry, which compares the scaling of homologous/analogous structures between related species at a given taxonomic level (e.g. Voje et al. 2014).

The extent to which the intercept and/or the slope of an allometric relationship are evolvable traits has been heavily debated (Egset et al., 2012; Emlen and Nijhout, 2000; Mirth et al., 2016; Pélabon et al., 2014). Functional, developmental or genetic constraints that restrict the morphospace in which organs have the potential to grow have been suggested to limit the extent to which allometries evolve (Bolstad et al., 2015; Pélabon et al., 2014). Pleiotropic effects have also been proposed to contribute to this limitation; changes in the mechanisms that generate allometry causing detrimental changes in other systems, thereby reducing overall fitness (Bolstad et al., 2015). Ontogenetic allometry has also been proposed to act as a developmental constraint limiting evolvability because evolutionary and static allometries are necessarily dependent on variability generated during development (Pélabon et al., 2014).

Despite these proposed limitations, however, there is substantial evidence showing that allometric scaling relationships can evolve (Emlen and Nijhout, 2000; Voje et al., 2014). This is supported by comparisons of static allometries that show they can differ within populations (Perl and Niven, 2016a), and among populations and species (Emlen and Nijhout, 2000; McGuigan et al., 2010; Simmons and Tomkins, 1996; Toju and Sota, 2006; Weber, 1990). Indeed, the idea that allometries can evolve is far from new:

“...allometric trends are as subject to evolutionary alteration as are morphological features” (Gould, 1966).

Grade shifts have been induced by artificial selection demonstrating that some aspects of allometric scaling can evolve rapidly (Bolstad et al., 2015; Frankino et al., 2005; Frankino et al., 2007). In contrast to the wealth of evidence demonstrating that intercepts can evolve, allometric slopes appear more constrained in their evolution, many organs showing remarkably little variation in scaling exponents between species separated by millions of years (Voje et al., 2014). Those experiments that have attempted to artificially select for slope shifts (Bolstad et al., 2015; Egset et al., 2012; Frankino et al., 2007; Stillwell et al., 2016; Tobler and Nijhout, 2010) have been criticized because of the methodology they employ (Mirth et al., 2016; Stillwell et al., 2016). Slope shifts induced by these experiments were often lost rapidly in subsequent generations once selection was eased (Bolstad et al., 2015), or were very minor changes (Stillwell et al., 2016; Voje et al., 2014).

Here we investigate the evolutionary allometry of an organ by comparing the scaling of compound eyes in four species of ant from the genus *Formica*. We examine scaling of the entire compound eye through facet number, facet diameter and eye area. Differences in scaling of facet diameter and facet number are indicative of relative changes in cell size and number, respectively (Chown et al., 2007; Montagne et al., 1999; Perl and Niven, 2016b). Both cell size and number contribute to changes in organ size, the differential contributions of facet number and facet diameter providing information about the mechanistic basis of changes in the size of a compound eye with increasing body size.

We also investigate regional differences within eyes through facet diameter scaling providing insight into how organs change size at a sub-organ (cellular) level (Perl

and Niven, 2016b; Stevenson et al., 1995). By measuring facet diameter scaling in different regions of the compound eye, we can also determine whether an overall change in eye size is produced by uniform changes across the whole eye, or through changes at different rates in different regions. Facet diameter scaling in *Formica rufa* differs among different regions of the compound eye (Perl and Niven, 2016a). By investigating these principles in related ant species, we examine not just the prevalence of evolutionary allometry among the genus but also the extent to which any differences in eye scaling between species can be explained through changes in intra-eye scaling.

We selected ants based on their disparate phylogenetic positions (Fig. 4.1, Goropashnaya et al. 2012), and ecologies. The most derived ants in our study are *F. rufa* and *F. lugubris*, representing the clade *Formica sensu strictu*; both species build large, mound shaped nests in forested regions where they forage along trails for honeydew and invertebrate prey (Collingwood, 1979). In Britain *F. lugubris* is polydomous, unlike the monodomous *F. rufa* (Collingwood, 1979). *Formica sanguinea* represent the Raptiformica; they are facultatively dulotic, raiding for slaves and freely foraging (Mori et al., 2000). *Formica fusca* are the most basal of the ant species we investigated, living in single or double-gyne nests of ~200 freely foraging workers (Collingwood, 1979; Wallis, 1964). Both *Formica sanguinea* and *F. fusca* live in more open field or meadow habitats compared with the *Formica s. s.*

4.3 Materials and Methods

4.3.1 Animals

Formica rufa workers were collected from Ashdown Forest, UK (51.073, 0.043) between June 2013 and August 2014, whereas those of *F. fusca* were collected from University of Sussex campus, UK (50.864, -0.0800) in May 2014. Workers of *F. lugubris* were collected

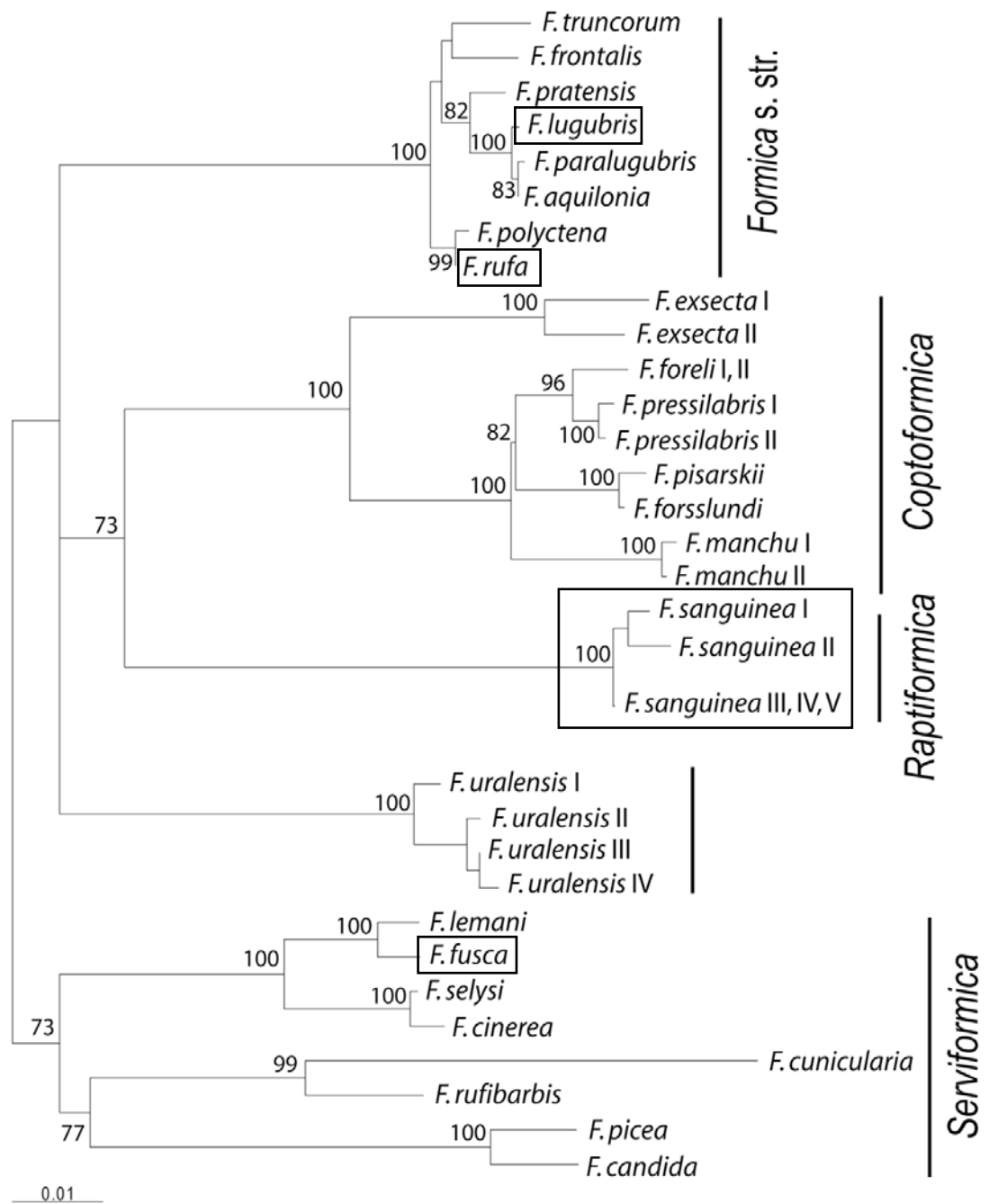


Figure 4.1 Phylogeny of *Formica* sp. Species used in this study are highlighted with a box. Scale bar indicates nucleotide substitutions per site, numbers on branches are bootstrap values. Modified from Goropashnaya et al. (2012).

from forests on North Yorkshire Moors, UK (54.347, -0.883) in September 2014. *Formica sanguinea* workers were collected from north of Cluj-Napoca, Romania (46.862, 23.536) in August 2015. Table 4.1 shows the numbers of animals sampled.

4.3.2 Specimen preparation

Individual worker ants were selected from a colony at random and restrained with Plasticine (Early Learning Centre, UK). Transparent nail varnish (Rimmel London, UK) was applied to both compound eyes using a cocktail stick to create a cast (Ribi et al., 1989). Ants were then stored at 4°C for a minimum of 48 hours to ensure the casts dried completely. These casts were removed, flattened and mounted on to 12.5 mm specimen stubs (Agar Scientific, UK) (Fig. 4.6). The eye casts and the left hind femur (as a proxy for body size) from *F. fusca*, *F. lugubris*, *F. rufa* and *F. sanguinea* were mounted for subsequent measurement. Nail-varnish eye casts and femurs were gold-coated and imaged using a scanning electron microscope (S420 Stereoscan, LEO Electron Microscopy Ltd., Germany) or mounted on a microscope slide (Fig. 4.6) and imaged using a Zeiss Axioskop compound microscope (Carl Zeiss AG, Germany) and photographed using a micropublisher 5.0 RTV (Q-imaging, Canada). Left hind femurs were imaged using a Leica MZI2.5 dissecting microscope (Leica, Germany) and photographed using a Canon EOS 7D SLR camera (Canon, Japan).

Sample sizes can be found in Table 4.1. The mean facet diameter per eye was obtained by measuring 36 facets per individual. Three facets were sampled from three different rows per eye region. The mean facet diameter for each eye region was then ascertained using the mean value of facet diameter from the facets in each specific region. The facet number was measured by counting every facet within an eye. The facet diameter was measured as the diameter of the facet along its longest axis. The eye area was measured by approximating the eye as an oval, which correlates almost exactly with the eye area measured directly (Perl & Niven, 2016a). Facet diameters, femur lengths and facet numbers were all measured and counted from their respective micrographs or

photos using Image J (Schneider et al., 2012).

4.3.3 Statistics

4.3.3.1 *Line fitting*

There is significant debate in the literature concerning the most appropriate line fitting method for allometric data. Some authors (Stillwell et al., 2016), advocate using major axis or standardised (reduced) major axis regression (MA/SMA) on the basis that this accounts best for error in the method of fitting lines to allometric data. Other authors advocate using MA/SMA on the basis that this method accounts for error in the X as well as the Y axis (Warton et al., 2006). Additionally, MA/SMA remove assumptions concerning biological phenomenon being directly related (Stillwell et al., 2016). Major axis or standardised major axis regression lines should only be fitted when both X and Y variables are sampled randomly (Warton et al., 2006), however, we sampled a broad size range of ants to ensure appropriate coverage. The measurement error in our data is likely to be small compared with the (unavoidable) amount of equation error (*i.e.* data points not lying exactly on the regression line). It has been noted that estimating allometric slopes is inaccurate when there is substantial equation error (Egset et al., 2012). Therefore, we have selected ordinary least square regression (OLS) to analyse our data, rather than MA/SMA.

Table 4.1 Number of ants and number of nests used per species for whole-eye scaling and for intra-eye scaling.

	Number of nests	Number of workers for whole eye scaling analysis	Number of workers for intra-eye scaling analysis
<i>F. fusca</i>	2	34	34
<i>F. lugubris</i>	3	52	23
<i>F. sanguinea</i>	3	62	21
<i>F. rufa</i>	3	63	65

4.3.3.2 Statistical test

Eye area, mean facet diameter and facet number were analysed using linear mixed effect models from the ‘nlme’ package (Pinheiro et al., 2016). Using the estimable function from the ‘gmodels’ package (Warnes et al., 2015) and by constructing custom contrast matrices, we made *post hoc* multiple pair-wise comparisons (*t*-tests) of these linear mixed effect models to determine whether changes in slope and/or intercept had occurred. Non-significant model terms were eliminated step-wise until only significant terms remained in the model. All analyses were conducted with log transformed data to allow for valid interpretation of the allometric coefficients. Principle component analysis (PCA) and cluster analysis was conducted using the PCA and HCPC functions from the ‘FactoMineR’ package, which uses agglomerative hierarchical clustering (Husson et al., 2010; Lê et al., 2008).

In addition to gross morphological scaling, we investigated scaling in facet diameters from different regions of the compound eye. These data were also analysed using linear mixed effect models with post-hoc pairwise comparisons. All statistics were

calculated using R v.3.1.2 (R Core Team, 2016) and all model structures can be found in Table 4.2.

Table 4.2 Structure of linear mixed effect models for all analyses. Individual ants were included as a random effect when they contributed more than one data point to a given model.

Response	Fixed effect(s)	Random effect(s)
Mean facet diameter	Femur length	Nest
Facet number	Femur length + species	Nest
Eye area	Femur length + species	Nest
Facet number	Mean diameter	Nest
<i>F. fusca</i> facet diameter	Femur length + eye region	Individual nested in nest
<i>F. lugubris</i> facet diameter	Femur length + eye region	Individual nested in nest
<i>F. sanguinea</i> facet diameter	Femur length x eye region	Individual nested in nest
<i>F. rufa</i> facet diameter	Femur length x eye region	Individual nested in nest
Mean anterior facet diameter	Femur length x species	Nest
Mean dorsal facet diameter	Femur length + species	Nest
Mean posterior facet diameter	Femur length x species	Nest
Mean ventral facet diameter	Femur length x eye region	Nest

4.4 Results

4.4.1 Allometric scaling of compound eyes and facets of *Formica* species

We examined three aspects of the allometric scaling of the compound eyes of workers from four *Formica* ant species: (1) scaling of facet number; (2) scaling of mean facet diameter; and (3) scaling of eye area.

Across the genus facet number increased significantly with increasing hind femur length ($F_{141,128} = 236.94$, $p < 0.001$). The absence of a significant interaction between hind femur length and species ($F_{141,125} = 0.31$, $p = 0.82$) indicated that the slope (i.e. the rate of facet number increase with increasing femur length) did not differ across all four species (Fig. 4.2A, 4.7A; Table 4.5). There was, however, a significant difference in the facet number among species ($F_{141,8} = 4.85$, $p = 0.03$), indicative of a grade shift (or a

change in elevation). Pairwise comparisons revealed that facet number differed between *F. fusca* and the three other species: *F. lugubris* ($t_{141,8} = 2.91$, $p = 0.02$); *F. rufa* ($t_{141,8} = 3.67$, $p < 0.01$); and *F. sanguinea* ($t_{141,8} = 2.88$, $p = 0.02$). There were no differences between the other species pairs ($t_{141,8} < 0.60$, $p > 0.57$). Therefore, for a given body size, *F. fusca* workers have more facets than do workers of the other three species. Despite this difference, the rate of increase in facet number with body size was the same across all four species. Facet number scaled with a negative allometry for all four species, $\alpha < 1$ (Table 4.3), indicating that larger ants had relatively fewer facets than smaller ants.

Mean facet diameter also increased significantly with increasing hind femur length across the genus ($F_{141,128} = 73.86$, $p < 0.001$). There was no significant interaction term between hind femur length and species ($F_{141,125} = 0.21$, $p = 0.89$) and, therefore, the slope did not differ across all four species (Fig. 4.2B, 4.7B; Table 4.5). There was also no significant difference in mean facet diameter between species ($F_{141,8} = 0.21$, $p = 0.89$). Thus, there were no slope or grade shifts between any of the species. The rate of facet diameter increase is the same across workers of all species as is the mean facet diameter for a given size of worker. Mean facet diameter scaled with a negative allometry across all four species, $\alpha < 1$ (Table 4.3), indicating that larger ants had relatively smaller facets than their smaller counterparts.

As expected from the previous analyses, the square root of eye area (used to preserve dimensionality among different response variables) increased significantly with increasing hind femur length across the genus ($F_{141,128} = 646.08$, $p < 0.001$). Again, there was no significant interaction term in the model ($F_{141,128} = 0.66$, $p = 0.58$), indicating that the slope did not differ across all four species (Fig. 4.2C, 4.7C; Table 4.5). There was a significant difference in mean eye area ($F_{141,8} = 8.74$, $p < 0.01$) indicative of a grade shift: *F. fusca* differed from both *F. lugubris* ($t_{141,8} = 3.67$, $p < 0.01$) and *F. rufa* ($t_{141,8} = 4.50$, $p <$

0.01); *F. sanguinea* also differed from both *F. lugubris* ($t_{141,8} = 2.37$, $p < 0.05$) and *F. rufa* ($t_{141,8} = 3.18$, $p = 0.01$). There were no further differences between the species ($t_{141,8} < 1.47$, $p > 0.18$). Thus, *F. rufa* and *F. lugubris* have a similar eye area for a given body size, as do *F. sanguinea* and *F. fusca*. However, though the rate of increase in eye area with increasing body size is similar across all species sampled, *F. fusca* and *F. sanguinea* having a larger area compound eye for a given body size compared with members of *Formica* s. s. Eye area scaled with a negative allometry across all four species, $\alpha < 1$ (Table 4.3), indicating that larger ants have a relatively smaller area eyes than their smaller counterparts.

4.4.2 Scaling of facet number with diameter

By assessing the scaling of facet diameter with facet number, we were able to assess their relative contributions to the overall structure of the compound eye. Facet number increased significantly with increasing mean facet diameter across the genus ($F_{141,128} = 17.61$, $p < 0.001$). There was no significant interaction term in the model, indicating that the slope did not differ across all four species ($F_{141,125} = 0.61$, $p = 0.61$) (Fig. 4.2D, 4.6D; Table 4.5). There were also no significant differences among all four species ($F_{141,8} = 0.12$, $p = 0.95$), indicating that there were no shifts in intercept. Thus, the rate of facet diameter increase with increasing facet number is similar across all the species sampled. Likewise, the mean facet diameter for a given number of facets is the same across all species sampled.

We assessed the differences in facet number and facet diameter with the overall area of the compound eye among the four species using principle component analysis (PCA) followed by cluster analysis (see Materials and Methods; Fig. 4.3; Table 4.4). We used the PCA to reduce the three variables of interest (facet number, mean facet

diameter and eye area) to two principle components. The first two principle components explained 97.4% of the variation in the data: Dimension 1 was strongly positively correlated with eye area, whilst dimension 2 was moderately positively correlated with facet count and moderately negatively correlated with facet diameter. Subsequent agglomerative hierarchical cluster analysis revealed that there were five clusters (Fig. 4.3). Only one cluster consisted of a single species, *F. rufa*. Indeed, *F. rufa* appeared in all five clusters, more than any of the other species (Fig. 4.3). The remaining clusters were all formed from at least two species, with two clusters having representatives from all four species.

4.4.3 Intra-eye scaling

4.4.3.1 Within species

We next examined the scaling of facet diameter in different regions (Fig. 4.6) of the compound eye in each of the four species. For each species we determined the allometric scaling of facet diameter in the anterior, dorsal, posterior and ventral regions of the compound eye.

There were differences in the scaling shifts that occurred within the eyes of different species. Within the *F. fusca* compound eye, there were no slope shifts ($F_{34,96} = 0.11$, $p = 0.95$) indicating that the rate of mean facet diameter increase with increasing body size is the same in each region of the eye. The facet diameters in different regions showed grade shifts relative to one another ($F_{34,99} = 39.52$, $p < 0.0001$) (Fig. 4.4A, 4.8A; Table 4.6). Aside from anterior and dorsal regions ($t_{34,99} = 0.45$, $p = 0.65$) all other regions were grade shifted relative to each other ($t_{34,99} > 5.15$, $p < 0.0001$). Thus, for a given body size, the anterior and dorsal regions have similar mean facet diameters, with the posterior facets being the larger and ventral facets being the smallest.

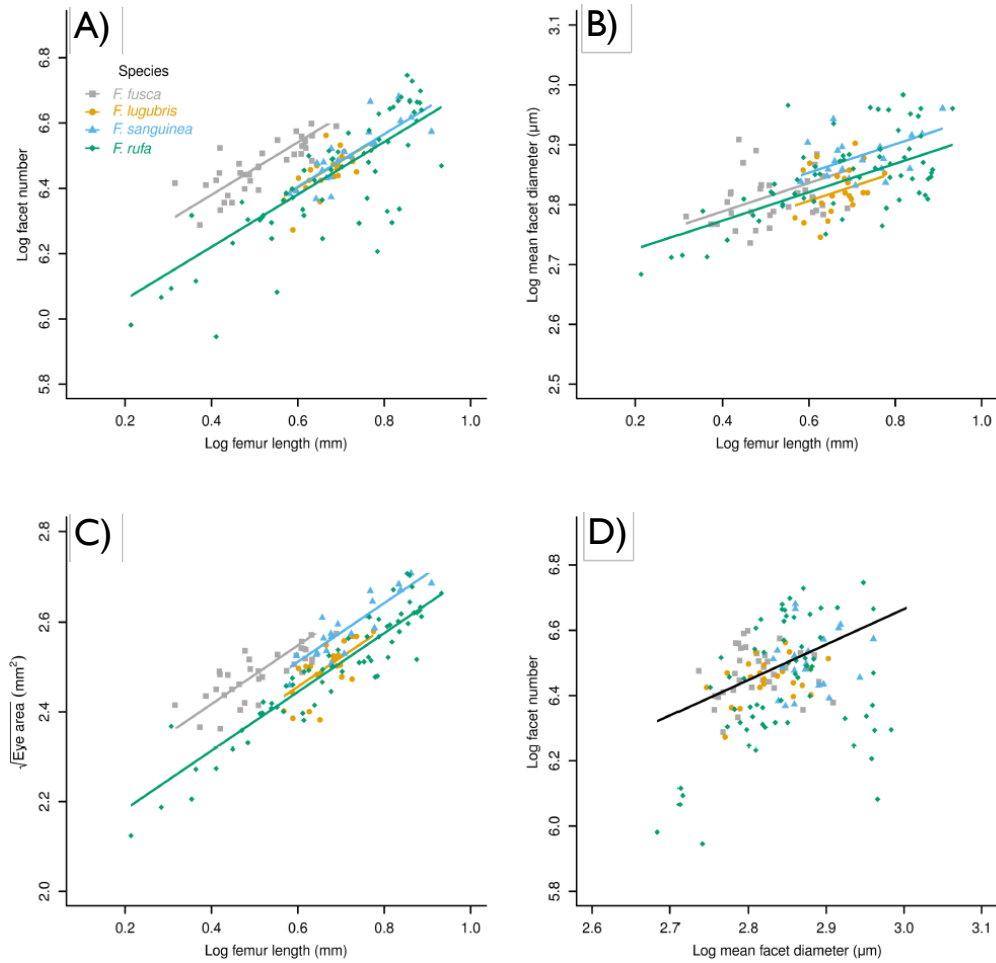


Figure 4.2 Scaling relationships in the four species of *Formica* as derived from linear mixed effect models. A single black regression line indicates no significant difference between species and they are therefore analysed with a common slope. Coloured regression lines are indicative of at least grade shifts between species, hence are analysed with individual regression lines. Data were transformed using \log_e to allow comparison with other allometric analysis. (A) Allometry of facet number per eye as a function of rear left femur length (a proxy of body size). (B) Allometry of mean facet diameter as a function of rear femur length. (C) Allometry of eye area as a function of rear femur length. (D) Scaling of mean facet diameter as a function of number of facets per eye among the four species of *Formica*.

Facet diameters in different regions of the *F. lugubris* compound eye also did not exhibit any slope shifts ($F_{52,150} = 0.02$, $p > 0.99$), only showing grade shifts ($F_{52,153} = 65.41$, $p < 0.0001$). Unlike *F. fusca*, all regions were significantly different from each other ($t_{52,153} > 3.07$, $p < 0.01$) (Fig. 4.4B, 4.8B; Table 4.6). Again, the rate of mean facet diameter increase with increasing body size is similar across all regions of the eye. The posterior

region facets are the largest for a given body size, followed by anterior and dorsal with ventral facets being the smallest.

Table 4.3 Scaling exponents \pm standard error for each species for each scaling relationship. Slope = α , intercept = β . Scaling exponents were extracting from linear models (see supplement), to maintain consistency with other allometric investigations. Supplemental graphs (Fig. 4.6-4.9) show slope and intercept estimates from linear models.

	<i>F. lugubris</i>			<i>F. sanguinea</i>			<i>F. rufa</i>			<i>F. fusca</i>		
	α	$\log\beta$	r^2	α	$\log\beta$	r^2	α	$\log\beta$	r^2	α	$\log\beta$	r^2
Facet												
count	0.85	5.88		0.87	5.88		0.90	5.82		0.66	6.13	
vs.	\pm	\pm	0.47	\pm	\pm	0.68	\pm	\pm	0.66	\pm	\pm	0.63
femur	0.19	0.13		0.13	0.10		0.08	0.06		0.09	0.05	
length												
Mean												
facet												
diam	0.24	2.67		0.16	2.76		0.26	2.67		0.09	2.77	
-ter	\pm	\pm	0.06	\pm	\pm	0.12	\pm	\pm	0.39	\pm	\pm	0.01
vs.	0.15	0.10		0.08	0.06		0.04	0.03		0.08	0.04	
femur												
length												
Eye												
area	0.78	1.97		0.69	2.09		0.69	2.02		0.45	2.25	
vs.	\pm	\pm	0.50	\pm	\pm	0.78	\pm	\pm	0.87	\pm	\pm	0.58
femur	0.16	0.11		0.08	0.06		0.03	0.02		0.07	0.03	
length												

Comparisons among the facet diameters from the four regions in *F. rufa* showed both slope ($F_{65,188} = 4.00$, $p < 0.01$) and grade shifts within the eye ($F_{65,188} = 116.743$, $p < 0.0001$). There were grade shifts between the posterior and all other eye regions; anterior ($t_{65,188} = 2.88$, $p = 0.004$, ventral ($t_{65,188} = 2.98$, $p < 0.01$) and dorsal ($t_{65,188} =$

4.92, $p < 0.0001$). (Fig. 4.4C, 4.8C; Table 4.7). There were no differences between the intercepts of the other pairs ($t_{65,188} < 1.47$, $p > 0.1$). The facet diameters in the anterior ($t_{65,188} = 2.90$, $p < 0.01$) were slope shifted relative to the ventral region. There were no further slope shifts ($t_{65,188} < 1.94$, $p > 0.05$). Thus, the mean diameter of facets in the posterior region is larger than those in the anterior, ventral and dorsal regions. The rate of facet diameter increase with increasing body size is greater in the anterior regions of the eye than in the ventral region.

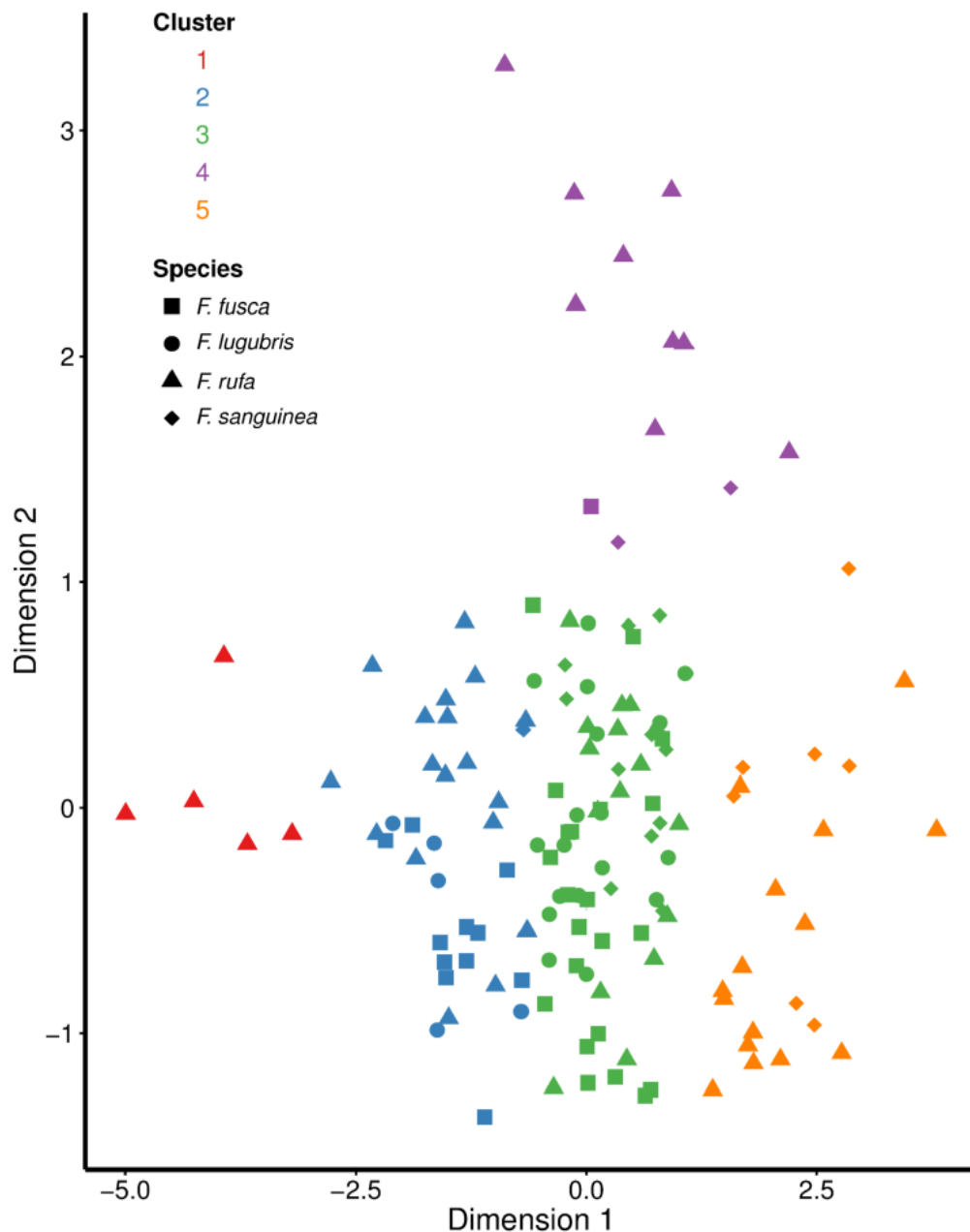


Figure 4.3 Hierarchical cluster analysis of facet number, mean facet diameter and species after dimension reduction using principle component analysis (PCA). Clusters are defined with different colours, whilst different species are represented by using different shapes.

Though there was no significant interaction term for the model ($F_{62,180} = 2.30$, $p = 0.08$), pairwise comparisons between the different regions of the *F. sanguinea* compound eye showed a significant slope shift between facet diameters in the anterior and posterior regions of the eye ($t_{62,180} = 2.38$, $p < 0.02$) (Fig. 4.4D, 4.8D; Table 4.6). There were no further slope shifts between regions ($t_{62,180} < 1.85$, $p > 0.07$) nor were there any grade

Table 4.4 Correlations, eigenvalues and relative contributions of all three factors for all three principle components.

	Principle component 1		Principle component 2		Principle component 3	
	Correlat-ion	Contri-bution	Correlat-ion	Contri-bution	Correlat-ion	Contri-bution
Count	0.84	32.45	-0.52	36.14	0.16	31.41
Diameter	0.72	23.86	0.69	63.37	0.10	12.78
Area	0.98	43.70	-0.06	0.49	-0.21	55.81
Eigenvalues						
Variance	2.181		0.741		0.077	
% of variation	72.72		24.71		2.58	

shifts ($t_{62,180} < 1.93$, $p > 0.05$). Facet diameter scaling is, therefore, similar among all regions of the eye, except between the anterior and posterior regions: The mean facet diameters in the posterior region increase at a greater rate with body size compared with those in the anterior region.

4.4.3.2 Among homologous regions from the compound eyes of different species

Homologous eye regions (Fig. 4.2) scaled differently among the four species. In the anterior region of the eye, there was a significant slope shift among different species (Fig. 4.5A, 4.9A; Table 4.7). Though there was no significant interaction term for the model ($F_{213,197} = 2.57$, $p = 0.055$), pairwise comparisons revealed a significant grade shift between the mean anterior facet diameters of *F. rufa* and *F. sanguinea* ($t_{213,198} = 2.47$, $p < 0.01$). There were no significant differences between the slopes of other species ($t_{213,7} < 2.05$, $p > 0.07$). Likewise, there were no grade shifts between the facets of the anterior region between any of the species ($F_{213,7} = 3.63$, $p = 0.07$). As body size increases, facet

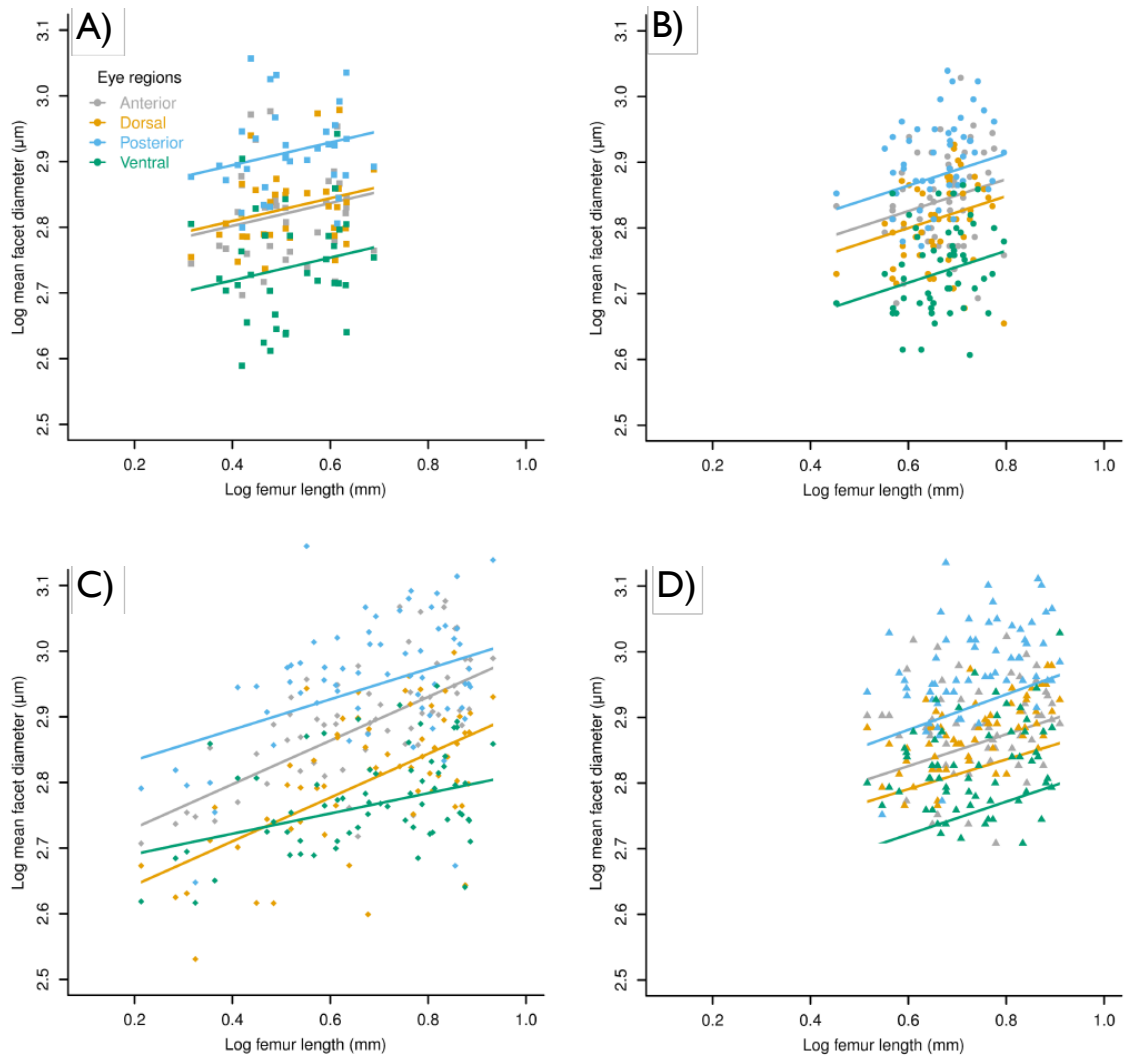


Figure 4.4 Intra-eye facet diameter scaling within species as derived from linear mixed effect models. Data were transformed using \log_e to allow comparison with other allometric analysis. Comparison of the scaling of mean facet diameters in different regions of the compound eyes from: A) *Formica fusca*; B) *F. lugubris*; C) *F. rufa* D) *F. sanguinea*.

diameters in the anterior of the *F. rufa* compound eye increase faster than those of *F. sanguinea*.

There was no significant slope shift in facet diameters from the dorsal region of the compound eye across different species ($F_{213,198} = 0.41$, $p = 0.75$) but there were significant grade shifts ($F_{213,7} = 5.84$, $p = 0.03$; Fig. 4.5B, 4.9B; Table 4.7). The mean dorsal facet diameters were grade shifted between *F. rufa* and *F. fusca* ($t_{213,7} = 3.34$, $p = 0.01$) as well as between *F. rufa* and *F. sanguinea* ($t_{213,7} = 2.76$, $p < 0.03$). There were further grade shifts between *F. lugubris* and both *F. fusca* ($t_{213,7} = 3.12$, $p = 0.02$) and *F. sanguinea* ($t_{213,7} =$

2.40 $p < 0.05$). There were no further grade shifts between the dorsal regions of any of the other species ($t_{213,7} < 1.06$, $p > 0.32$). *Formica fusca* have a larger mean facet diameter in the dorsal region of the compound eye than *F. rufa* for a given body size. The diameters of the dorsal facets of *F. sanguinea* are also larger than those of *F. rufa* for a given body size. *Formica lugubris* have smaller dorsal facet diameters than either *F. fusca* or *F. sanguinea*.

There was no significant slope shift in the posterior region of the eye across different species ($F_{213,198} = 0.48$, $p = 0.69$) nor were there any significant grade shifts ($F_{213,7} = 0.95$, $p > 0.47$) (Fig. 4.5C, 4.9C; Table 4.7). Consequently, there were no differences between species in terms of either mean facet diameter for a given body size nor in the rate of facet diameter increase with body size in the posterior regions of the eye.

As with the dorsal and posterior regions of the compound eye, there was no significant slope shift in the ventral region of the eye across different species ($F_{213,198} = 0.38$, $p = 0.77$) but there were significant grade shifts ($F_{213,201} = 15.23$ $p < 0.0001$) (Fig. 4.5D, 4.9D; Table 4.7). The mean ventral facet diameters were grade shifted between *F. sanguinea* and *F. lugubris* ($t_{213,7} = 3.16$, $p = 0.02$). There were no further grade shifts in ventral facet diameters between any of the other species ($t_{213,7} < 1.89$, $p > 0.10$). The mean diameter of facets in the ventral region eye region of *F. sanguinea* are larger for a given body size than those from *F. lugubris*.

4.5 Discussion

By comparing the static allometric scaling relationships governing compound eye size, facet number and diameter across closely related species, our findings demonstrate that evolutionary shifts exist in the allometric scaling of organs. At the whole-eye level changes in static allometric scaling relationships are restricted to grade shifts, with slope

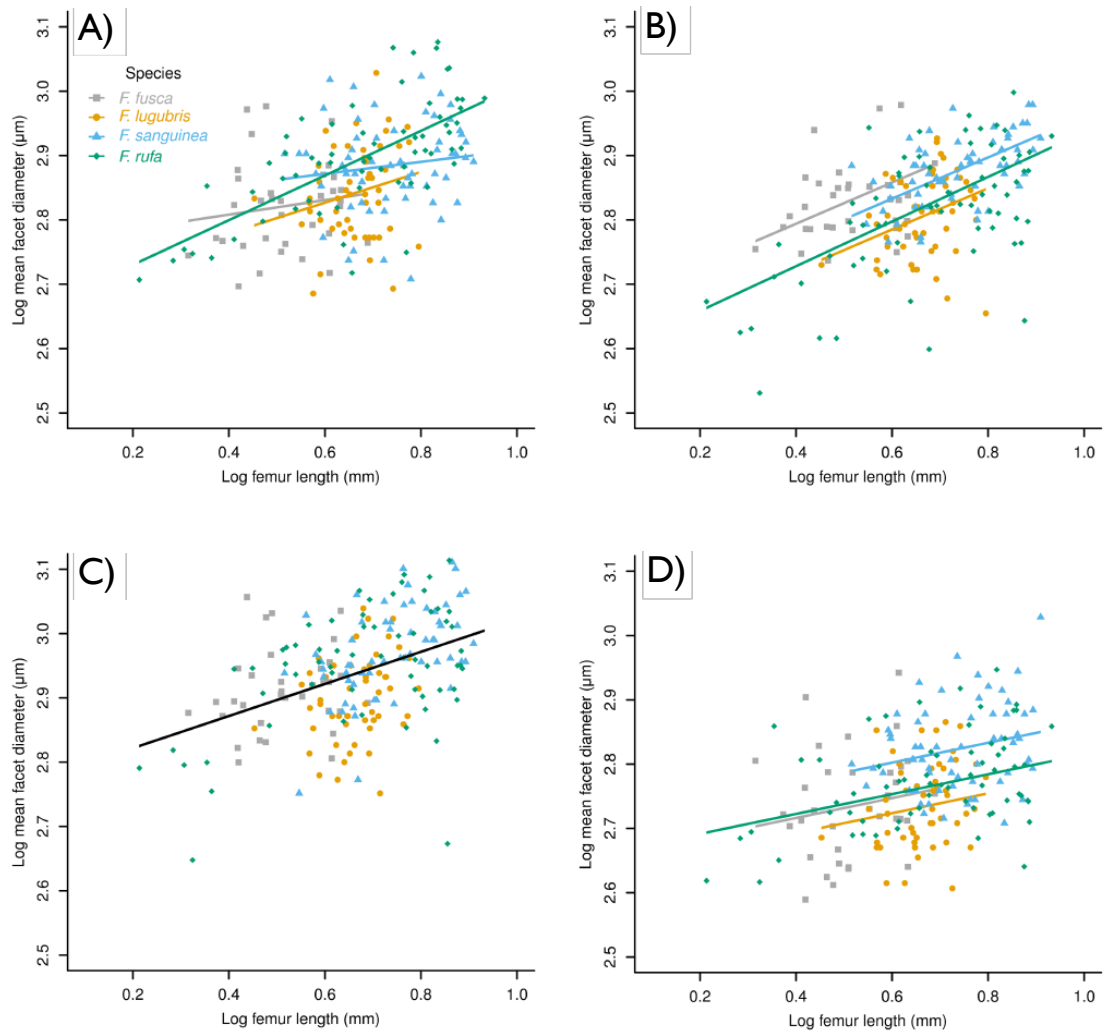


Figure 4.5 Intra-eye facet diameter scaling among species as derived from linear mixed effect models. Comparison of the scaling of mean facet diameters from homologous regions of the compound eyes of the four *Formica* species. Data were transformed using \log_e to allow comparison with other allometric analysis. A single black regression line indicates no significant difference between species and they are therefore analysed with a common slope. Coloured regression lines are indicative of at least grade shifts between species, hence are analysed with individual regression lines. Mean facet diameter scaling of: A) the anterior region; B) the dorsal region; C) the posterior region; D) the ventral region.

shifts entirely absent. This supports previous claims based upon comparisons among species that allometric relationships can evolve but that grade shifts are easier to achieve than slope shifts (Bolstad et al., 2015; Emlen and Nijhout, 2000; Frankino et al., 2005;

Frankino et al., 2007; Pélabon et al., 2014; Tobler and Nijhout, 2010; Toju and Sota, 2006; Voje et al., 2014).

Despite grade shifts in the allometric scaling of the compound eye among the four *Formica* species in terms of eye scaling, the scaling of mean facet diameter with facet number is remarkably consistent; no grade or slope shifts occurred among the species. This is supported by the PCA/cluster analysis in which workers do not cluster based entirely upon their species. The high degree of conservation of the relationship between mean facet diameter and facet count may indicate that developmentally or functionally related traits are not necessarily as free to vary as those same traits are with body size. Under artificial selection, Frankino et al. (2005, 2007) demonstrated that a genetically and functionally linked trait (hind-wing size of a butterfly) can be forced into alternative scaling regimes, indicating that the restrictions on functionally-linked morphological traits are not necessarily developmental/genetic (Mirth et al., 2016). Pélabon et al. (2013) also concluded that constraints on evolutionary allometry are the consequence of selection, rather than due to a developmental limitation. If this is the case for the relationship between facet diameter and facet number in the present study, it implies that the relationship is maintained through selection across the genus and that deviating from this reduces fitness.

Changes in scaling across the entire organ are not the only way in which changes can occur in static allometric scaling relationships, they can also occur at the sub-organ level (Perl and Niven, 2016a). In contrast to the relatively conservative changes in the allometric scaling relationships of the whole compound eye among the four *Formica* species, we found substantial variability in the allometric scaling relationships of facets in specific regions of the compound eye. Both grade shifts and slope shifts occur among regions. The patterns of facet diameter scaling between eye regions appear unique to

each species, as well as to any particular region among species. As such, these differences could explain species-specific adaptations whilst adhering to genus-wide relationships at the level of the entire eye.

The intra-eye differences are mediated primarily through grade shifts so that for a given body size facet diameter depends upon the eye region in which that facet resides, but the rate at which facet diameter increases is the same across the different eye regions. However, slope shifts occur between one or more regions in two species; *F. rufa* and *F. sanguinea*. This demonstrates that the way in which evolutionary changes occur in static allometries is far more nuanced than implied by mean measurements sampled from across the entire organ.

To expand on this further; the scaling of eye area is consistent between different species of the genus. However, the means through which they all arrive at the same scaling rules does differ. From the analysis of mean facet diameter, there is no difference in mean facet diameter scaling between species. However, intra-eye facet diameter scaling differs vastly depending on the region the facets are in and the species to which they belong. Grade shifts in facet number scaling also occur between some species. Therefore, consistency in eye area scaling is maintained through differential scaling of facet diameters and relative investment in facet number. A fixed eye area can be obtained through either changes in facet number or diameter. Two eyes may have the same area, one with large numbers of small facets and the other with fewer, larger facets. Among our four species, some may change the scaling in the anterior portion of their eye relative to the other regions, whereas another may scale the posterior region instead. This is combined with grade shifts in facet number. Through this mechanism, the scaling of eye area is the same across the genus, whilst individual species display differential facet diameter scaling in different regions of the eye.

The differences in intra-eye scaling between species are further emphasised when examining scaling shifts between homologous regions of different species. In two of the four regions investigated (ventral and dorsal) at least one species pair demonstrated grade shifts, though the patterns of grade shifts were different between regions. Between the dorsal region of different species, there were ample grade shifts with only *F. fusca* and *F. sanguinea* being similar along with *F. lugubris* and *F. rufa*. In contrast, anterior facet diameters show only slope shifts, but only between a single pair of species; *F. rufa* and *F. sanguinea*. This implies that allometric shifts across evolutionary timescales are not simple changes that affect entire organs or even parts of organs in the same way. Thus, even though slope shifts did not occur between species when looking at scaling at a whole organ level, slope shifts do occur between homologous regions within the compound eyes of different species. Furthermore, grade shifts that are not apparent when examining whole organ allometry become obvious when examining within-organ scaling.

Slope shifts are purportedly less common than grade shifts in evolutionary allometry (Egset et al., 2012) and difficult to maintain across generations even when induced through strong artificial selection (Bolstad et al., 2015; Stillwell et al., 2016). However, our analysis demonstrates that slope shifts do occur, even between closely related species, though not at a whole organ level. Thus, species with different life histories and foraging habits have similar investment in mean facet diameter as a function of facet number but differ in facet diameter scaling relationships between the homologous eye regions. This implies that the internal proportions of an organ are far freer to vary than the rate of organ size increase with body size, explaining how compound eyes can be specifically adapted to particular visual ecologies whilst conforming to specific scaling relations at a genus wide level.

Though our findings demonstrate changes in the static allometric scaling among and within the compound eyes of closely-related species, there is a lack of phylogenetic consistency in these scaling relationships. Moreover, allometric shifts do not appear to be related to life history or ecology irrespective of whether they are at the whole-eye or intra-eye level. This may be a consequence of the relatively sparse sampling of species or a lack of sufficiently detailed descriptions of the visual ecologies of the species we studied. Detailed studies of the behaviour, physiology and morphology of single species have shown that eye regionalisation of this sort is very common in insects (Land, 1997), and that it is often associated with specific behavioural requirements that provide a strong selective incentive, such as mate (Collett and Land, 1975; Kirschfeld and Wenk, 1976) or prey detection (Labhart and Nilsson, 1995). Even though we cannot attribute regional changes in facet diameter to specific behavioural and ecological requirements, our results show that not only do regions scale differentially within a species (Perl and Niven, 2016a) but that closely related species can evolve substantial differences in homologous regions.

4.6 Supplementary materials

This supplement details the eye casts used in the study and demonstrates the homology between eye regions (Fig. 4.6). It also contains Fig. 4.7-4.9, which show the same data as in the main text but with the regression lines plotted using the estimates from linear models rather than the linear mixed effect models. These figures indicate the allometric relationships without accounting for nest affiliation. The estimates of these slopes and intercepts (allometric exponents) can be found in Table 4.3 of the main text.

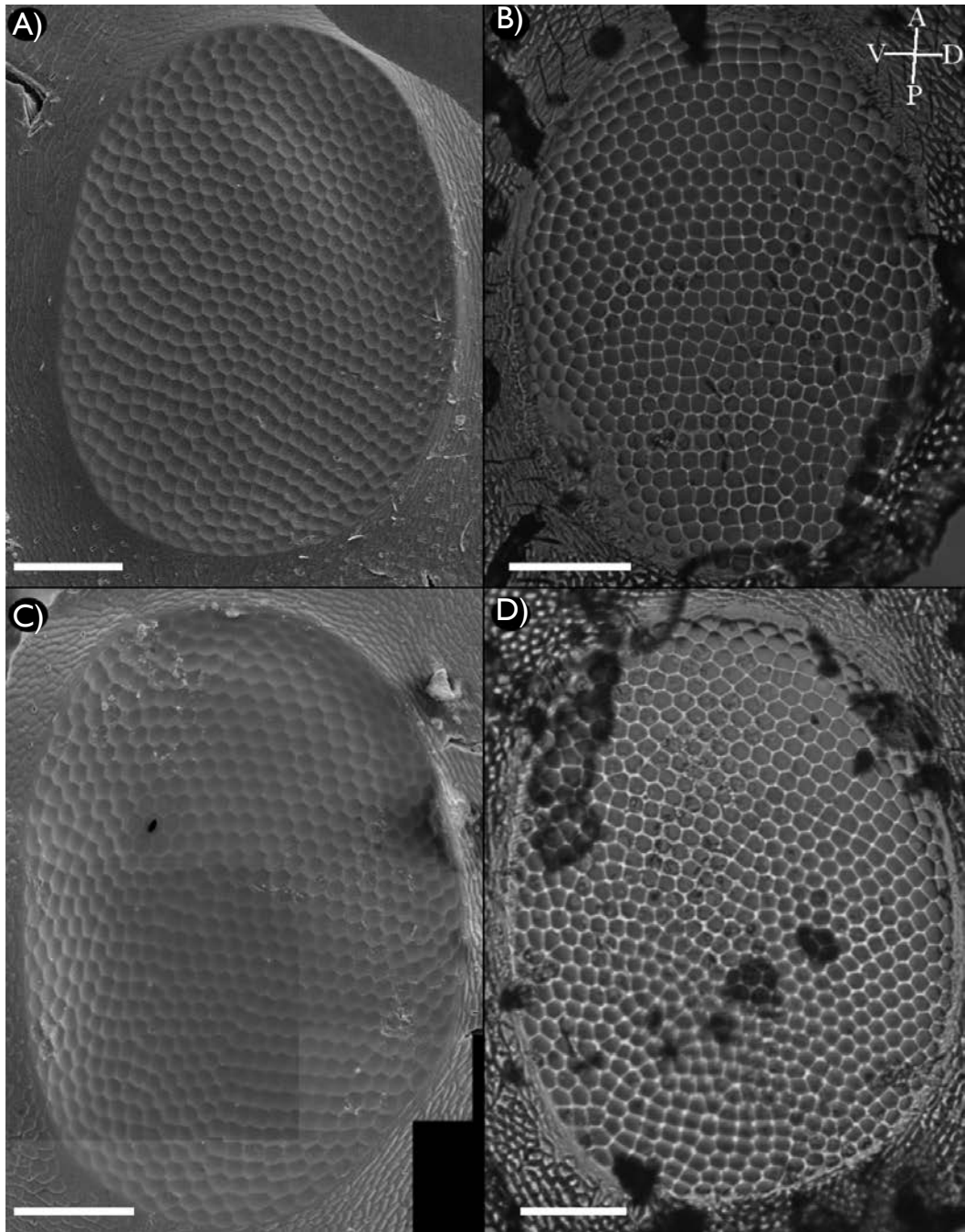


Figure 4.6 Eye casts from the four species used in this study. A) A representative electron micrograph of an eye cast from *Formica fusca*. B) A representative photo an eye case from *F. lugubris*. C) A representative electron micrograph of an eye cast from *F. rufa*. D) A representative photo an eye cast from *F. sanguinea*. Scale bars = 100 μ m. A,D,V,P refer to anterior, dorsal, ventral and posterior, respectively. Visible smudges on the casts of *Formica lugubris* B) and *F. rufa* D) are artefacts caused by casts having been removed from SEM stubs and re-mounted to microscope slides. Where present, the artefacts did not hinder data collection.

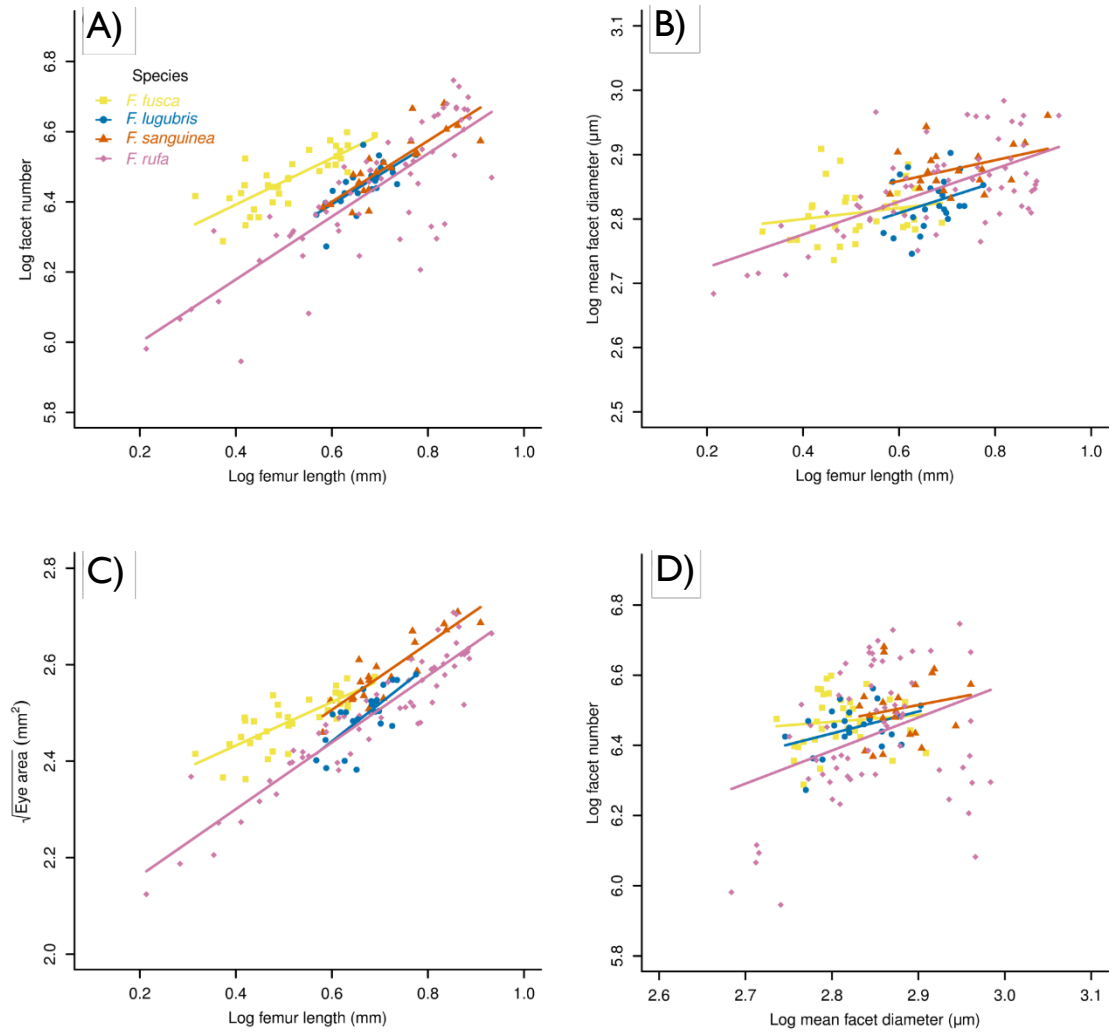


Figure 4.7 Allometric scaling relationships in the four species of *Formica* as derived from linear models. Data were transformed using \log_e to allow comparison with other allometric analysis. A) Allometry of facet number per eye as a function of rear left femur length (a proxy of body size). B) Allometry of mean facet diameter as a function of rear femur length. C) Allometry of eye area as a function of rear femur length. D) Scaling of mean facet diameter as a function of number of facets per eye among the four species of *Formica*.

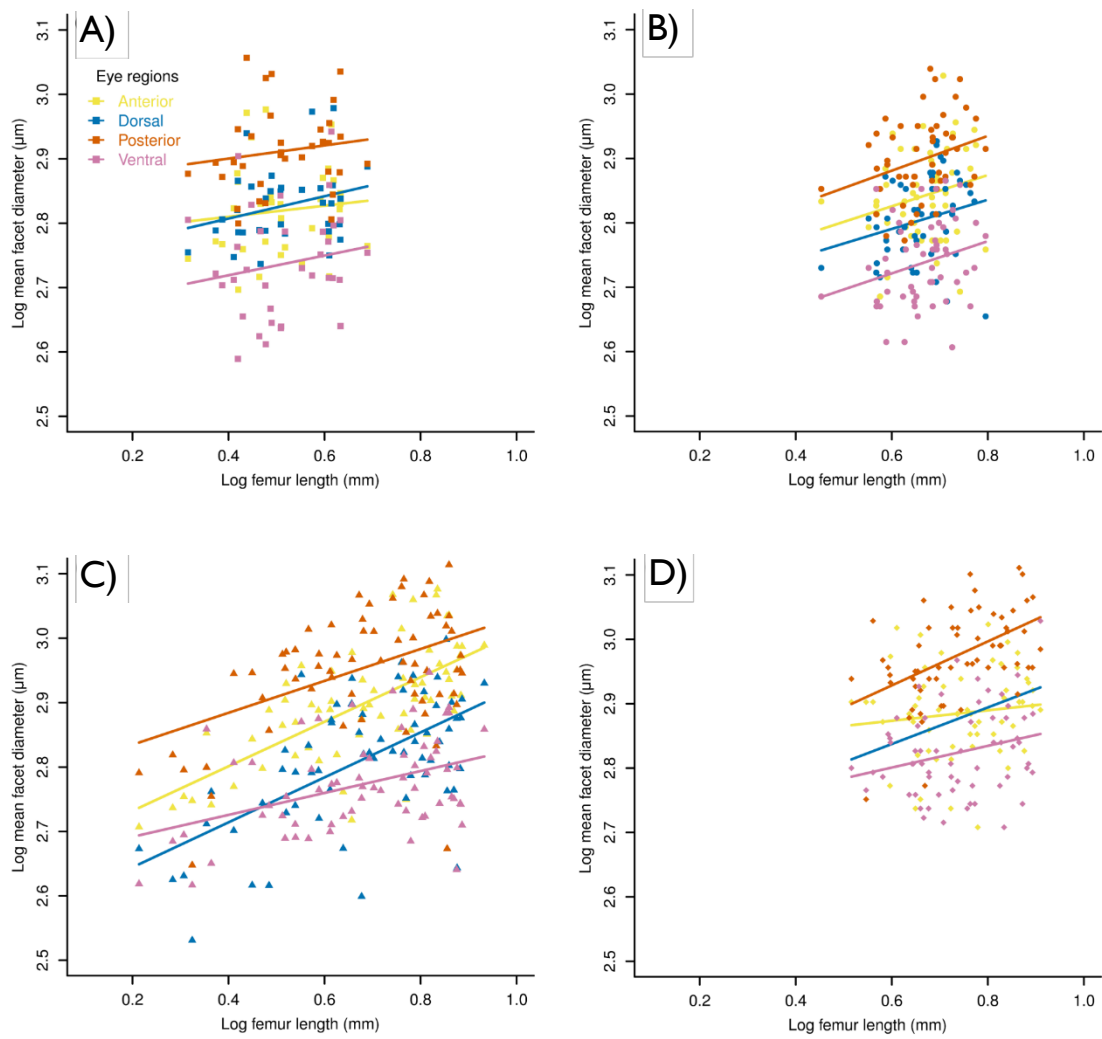


Figure 4.8 Intra-eye facet diameter allometric scaling within species as derived from linear models. Data were transformed using \log_e to allow comparison with other allometric analysis. Comparison of the allometric scaling of mean facet diameters in different regions of the compound eyes from: A) *Formica fusca*; B) *F. lugubris*; C) *F. sanguinea*; D) *F. rufa*.

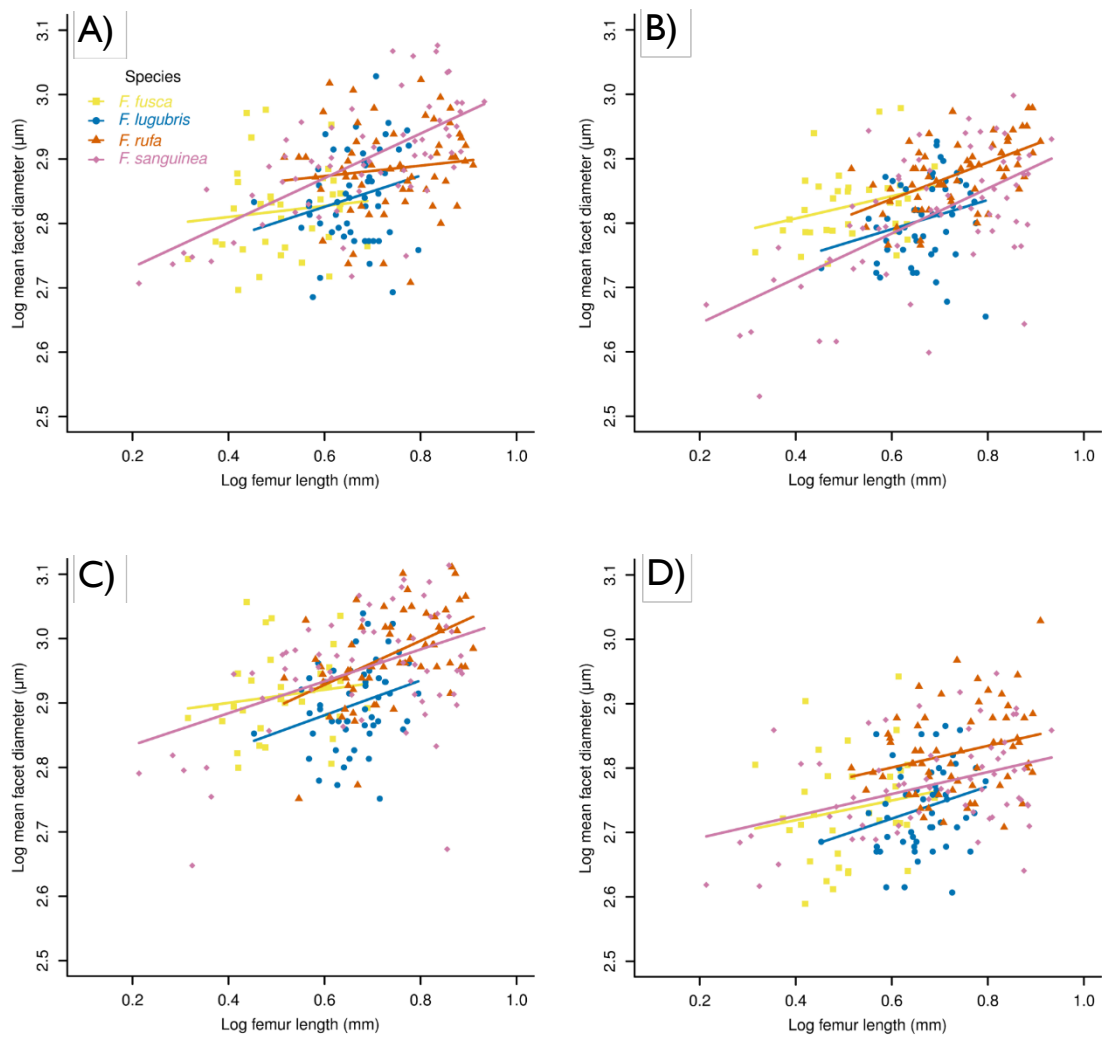


Figure 4.9 Intra-eye facet diameter allometric scaling among species as derived from linear models. Data were transformed using \log_e to allow comparison with other allometric analysis. Comparison of the allometric scaling of mean facet diameters from homologous regions of the compound eyes of the four Formica species. Mean facet diameter scaling of: A) the anterior region; B) the dorsal region; C) the posterior region; D) the ventral region.

Table 4.5 Slope and intercept estimates derived from linear mixed effect models analysing differences between Formica species. in terms of whole organ eye scaling.

	Estimate	± SE
Facet count vs femur length		
Slope	0.80	0.05
<i>F. fusca</i> intercept	6.06	0.04
<i>F. lugubris</i> intercept	5.92	0.05
<i>F. rufa</i> intercept	5.90	0.04
<i>F. sanguinea</i> intercept	5.92	0.05
Mean facet diameter vs femur length		
Slope	0.24	0.03
<i>F. fusca</i> intercept	2.69	0.02
<i>F. lugubris</i> intercept	2.66	0.03
<i>F. rufa</i> intercept	2.68	0.02
<i>F. sanguinea</i> intercept	2.71	0.03
Eye area vs femur length		
Slope	0.65	0.03
<i>F. fusca</i> intercept	2.16	0.02
<i>F. lugubris</i> intercept	2.06	0.02
<i>F. rufa</i> intercept	2.05	0.02
<i>F. sanguinea</i> intercept	2.12	0.03
Facet number vs mean facet diameter		
Slope	1.09	0.20
Intercept	3.41	0.56

Table 4.6 Slope and intercept estimates derived from linear mixed effect models analysing differences in facet diameter scaling between different regions of the eye within different *Formica* species.

	Estimate	± SE
<i>F. fusca</i>		
Slope	0.17	0.08
Anterior intercept	2.73	0.04
Dorsal intercept	2.74	0.04
Posterior intercept	2.83	0.04
Ventral intercept	2.65	0.04
<i>F. lugubris</i>		
Slope	0.24	0.14
Anterior intercept	2.68	0.09
Dorsal intercept	2.65	0.09
Posterior intercept	2.72	0.09
Ventral intercept	2.57	0.09
<i>F. sanguinea</i>		
Anterior intercept	2.68	0.09
Dorsal intercept	2.65	0.09
Posterior intercept	2.72	0.09
Ventral intercept	2.57	0.09
Anterior slope	0.24	0.14
Dorsal slope	0.23	0.18
Posterior slope	0.27	0.18
Ventral slope	0.25	0.18
<i>F. rufa</i>		
Anterior intercept	2.66	0.04
Dorsal intercept	2.58	0.04
Posterior intercept	2.79	0.04
Ventral intercept	2.66	0.04
Anterior slope	0.33	0.05
Dorsal slope	0.33	0.06
Posterior slope	0.23	0.06
Ventral slope	0.15	0.06

Table 4.7 Slope and intercept estimates derived from linear mixed effect models analysing differences in facet diameter scaling within homologous regions of the eye between different *Formica* species.

	Estimate	± SE
Ventral region		
Slope	0.15	0.04
<i>F. fusca</i> intercept	2.65	0.03
<i>F. lugubris</i> intercept	2.63	0.03
<i>F. rufa</i> intercept	2.66	0.03
<i>F. sanguinea</i> intercept	2.71	0.03
Posterior region		
Slope	0.25	0.04
Intercept	2.77	0.03
Anterior region		
<i>F. fusca</i> intercept	2.76	0.07
<i>F. lugubris</i> intercept	2.68	0.10
<i>F. rufa</i> intercept	2.66	0.03
<i>F. sanguinea</i> intercept	2.82	0.07
<i>F. fusca</i> slope	0.11	0.13
<i>F. lugubris</i> slope	0.24	0.20
<i>F. rufa</i> slope	0.35	0.14
<i>F. sanguinea</i> slope	0.09	0.16
Dorsal region		
Slope	0.32	0.04
<i>F. fusca</i> intercept	2.67	0.03
<i>F. lugubris</i> intercept	2.59	0.03
<i>F. rufa</i> intercept	2.59	0.03
<i>F. sanguinea</i> intercept	2.64	0.03

Chapter 5: Metabolic rate scaling, ventilation patterns and respiratory water loss in red wood ants

5.1 Abstract

Metabolic rate and its relationship with body size is a fundamental determinant of organismal fitness. Alongside various environmental and physiological factors, the metabolic rate of insects is linked to distinct ventilation patterns. Despite significant attention, however, the precise role of these ventilation patterns remains uncertain. Here we determine the allometric scaling of metabolic rate and respiratory water loss in the red wood ant, as well as assessing the effect of movement upon metabolic rate and ventilation pattern. Metabolic rate and respiratory water loss are both negatively allometric. We observed both continuous and cyclic ventilation associated with relatively higher and lower metabolic rates, respectively. In wood ants, however, movement not metabolic rate is the primary determinant of which ventilation pattern is performed. Conversely, metabolic rate not ventilation pattern is the primary determinant of respiratory water loss. Our statistical models produced a range of relatively shallow intraspecific scaling exponents between 0.47 and 0.60, emphasising the dependency upon model structure. Moreover, metabolic rate scaling is invariant among wood ant nests unlike some aspects of morphological scaling, suggesting that these two forms of scaling respond to environmental factors in different ways.

5.2 Introduction

Metabolism is the sum of anabolic and catabolic processes that enable the formation of biological material and fuel cellular and physiological work (Alexander, 1999; Chown and Nicolson, 2004). As such, the rate at which metabolism occurs is intrinsically linked to how much energy and how many resources are available for movement, growth and

reproduction (Brown et al., 2004; Glazier, 2015). These fundamental properties affect other life history traits, such as senescence, and more holistic traits, such as survivability (Burton et al., 2011; Speakman, 2005). Thus, metabolic rate is a key determinate of organismal fitness.

Movement, growth and reproduction increase metabolic rates in most animals (though see Glazier, 2015), including insects (Basson and Terblanche, 2010; Chown and Nicolson, 2004; Clusella-Trullas et al., 2010; Glazier, 2009; Hammond and Diamond, 1997; Heglund and Taylor, 1988; Lighton, 1990; Niven and Scharlemann, 2005; Weibel and Hoppeler, 2005). Larger body masses also generate higher metabolic rates, though smaller individuals have a higher mass-specific metabolic rate, a pattern reflected for both intra- and interspecific relationships (Bartholomew et al., 1988; Brown et al., 2004; Chown et al., 2007; Gillooly et al., 2001; Glazier, 2005). The ultimate cause of this negative allometry is undetermined despite intensive scrutiny, which has left interspecific metabolic rate relatively under-examined (Brown et al., 2004; Burton et al., 2011; Chown et al., 2007; Glazier, 2005; Terblanche et al., 2007; West et al., 1997; 1999). Yet any population with significant variation in adult body size will experience intraspecific metabolic rate scaling (Glazier, 2005). Therefore, any differences in intraspecific metabolic rate scaling form an important part of explaining behavioural and fitness differences between individuals (Burton et al., 2011).

The metabolic rate of insects is linked to the pattern of ventilation (Contreras and Bradley, 2009; 2010; Gibbs and Johnson, 2004). There are three principle ventilation patterns observed in insects; continuous, discontinuous and cyclic (Chown and Nicolson, 2004), though the adaptive value of these patterns remains unclear (Chown et al., 2006; Marais et al., 2005). To date, most studies have focussed on discontinuous gas exchange cycles (DGC) (Buck and Kesiter, 1955; Chown et al., 2006; Lighton, 1994; Quinlan and

Gibbs, 2006), though the majority of insects do not use DGC (Marais et al., 2005). Therefore, it is important to understand the causes and consequences of the other more prevalent ventilation patterns: cyclic and continuous (Contreras and Bradley, 2009). Increases in metabolic rate induce changes in ventilation pattern; from DGC at the lowest metabolic rates, switching to cyclic ventilation as metabolic rate increases and continuous ventilation at the highest (Basson and Terblanche, 2010; Contreras and Bradley, 2010; Klok and Chown, 2005).

Increases in temperature and activity increase metabolic rate, which has been shown to drive changes in ventilation pattern (Contreras and Bradley, 2010). However, another popular hypothesis links ventilation types to respiratory water loss (Chown et al., 2006; Lighton, 1994). Discontinuous gas exchange cycles were originally proposed to be an adaptation to reduce respiratory water loss (Buck and Kesiter, 1955), though contemporary studies have questioned this (Chown and Holter, 2000; Hetz and Bradley, 2005; Lighton and Berrigan, 1995; Matthews and White, 2010). Surprisingly, there is relatively little information addressing the effects of other ventilatory patterns on respiratory water loss, though there is a consensus that spiracular opening is major route of water loss (Chown, 2002).

Ants are often ecologically influential (Hölldobler and Wilson, 1990), so their metabolic rate and water loss, along with the factors that affect them, are of broad significance. Metabolic rates in ants scale with negative allometry, both intra- and interspecifically (Bartholomew et al., 1988; Chown et al., 2007; Lighton, 1989; Lighton and Wehner, 1993). Investigations of the ventilation patterns performed by ants have shown DGC, cyclic and continuous ventilation patterns (Gibbs and Johnson, 2004; Lighton, 1989; Lighton and Berrigan, 1995; Lighton and Garrigan, 1995; Quinlan and Lighton, 1999). Respiratory water loss in ants has been positively correlated with

metabolic rate (Chown, 2002; Schilman et al., 2005), and shown to change with differing ventilation types (Gibbs and Johnson, 2004).

Wood ants, in particular, are keystone species that exert effects upon aphid, invertebrate and plant populations (Domisch et al., 2009; Hawes et al., 2002; Skinner and Whittaker, 1981), as well as having a role in nutrient cycling (Finér et al., 2013). This impact manifests primarily through workers foraging for honeydew and scavenging other invertebrates (Skinner, 1980). Consequently, the metabolic rate of wood ant workers, among whom body size varies considerably, has a direct impact on their environment. Here we study the metabolic rates, respiratory water loss and ventilation patterns of wood ant foragers covering a broad range of naturally occurring body sizes. We show that metabolic rate and respiratory water loss both scale with negative allometry. Foragers' respiratory water loss can be explained solely through increases in metabolic rate. The ventilation pattern performed by ants is strongly associated with metabolic rate, independent of any changes induced by increasing mass. However, changes in metabolic rate are not necessary to cause changes in ventilation type, instead activity is the trigger that induces changes in ventilation pattern in wood ants.

5.3 Materials and methods

5.3.1 Animals

Formica rufa L. (Hymenoptera: Formicidae) foragers were collected from Ashdown Forest, Sussex, UK (51.0780, 0.0300) on five separate dates from the 25th August to the 19th September 2016. Four nests were visited and approximately 30 ants were collected from each nest on each collection date. Nest locations were as follows: Nest #1: 51.07555, 0.02962; Nest #2: 51.07531, 0.03524; Nest #3: 51.07552, 0.03475; Nest #4: 51.07549, 0.03019. Not all the ants collected were analysed, ants were kept for a

maximum of 10 days. Upon collection, workers from each nest were housed separately and provided with a diet of water and sugar water *ad libitum*. Subsequently, the whole of Nest #3 was collected and moved to the laboratory on 25th May 2017. The nest was kept under 12h:12h light:dark regime and fed sugar water and frozen crickets *ad libitum*.

5.3.2 Metabolic rate and water loss measurement

5.3.2.1 Experiment 1

The metabolic rate and water loss of individual ants were measured using a LI-7000 flow through respirometer (LI-COR, USA). Air at room temperature (22-24°C) was pumped through two pairs of scrubbing columns; the first pair contained soda lime to remove carbon dioxide and the second pair contained Drierite (W.A. Hammond Drierite, USA) to remove water vapour. The scrubbed air was pumped at 100 ml min⁻¹ into two chambers of approximately 0.03 L connected in parallel. One chamber served as the reference chamber, the other the test chamber. The reference chamber remained empty and the test chamber contained a single ant. The metabolic rate of the ant was measured as the difference in the volume of carbon dioxide between the two chambers. Individual ants remained in the test chamber for between 30 minutes and one hour.

A total of 107 ants were used for the analysis of Experiment 1, 29 ants from Nest #1, 25 ants from Nest #2, 26 ants from Nest #3 and 27 ants from Nest #4.

5.3.2.2 Experiment 2

Ants from Nest #3 were placed in the test chamber (see above) twice; once restrained with modelling clay (Plasticine®) and once unrestrained. The order of the treatments was randomised. During the restrained treatment only, an equal mass of modelling clay

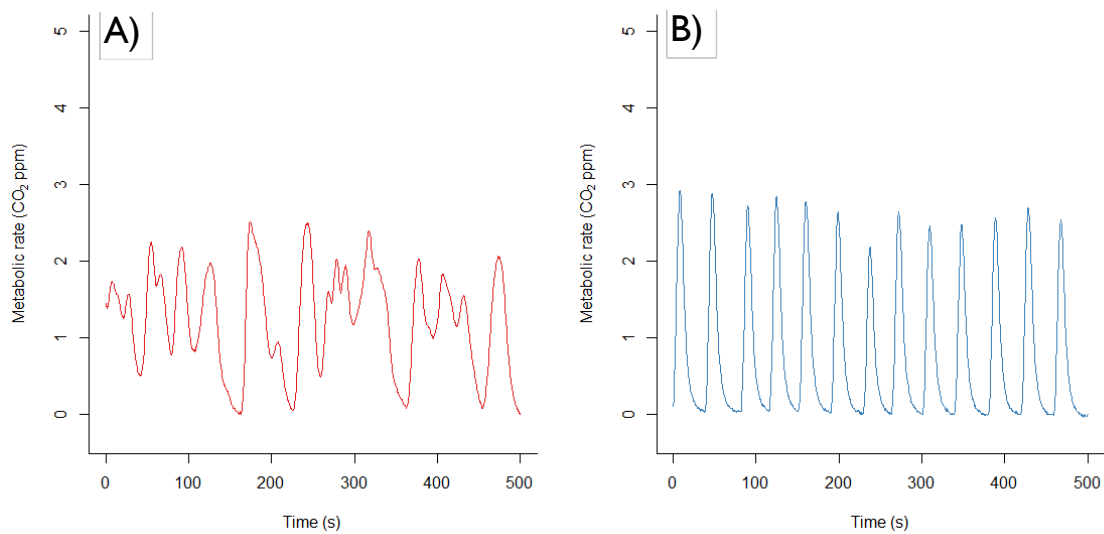


Figure 5.1 *Formica rufa* workers used two different ventilation types for gas exchange. Traces of carbon dioxide excretion during: A) Continuous gas exchange; B) Cyclic gas exchange.

was placed in the reference chamber to ensure that the volume of both chambers was equal. A total of 24 ants were used for Experiment 2.

5.3.3 Ventilation type classification and exclusions

Ventilation type was classified from the metabolic rate traces. Ants showed one of two ventilation types; continuous or cyclic (Fig. 5.1). Ventilation patterns were classified following Gray and Bradley, 2006; Lighton, 1994: cyclic ventilation was characterised by pronounced, regular peaks and troughs (Fig. 5.1), whereas continuous ventilation had no obvious regularity and carbon dioxide was continuously excreted (Fig. 5.1). Some ants demonstrated both ventilation types whilst in the chamber. In such cases, the metabolic rate of the ant was calculated from the most prevalent ventilation type.

Four ants had intermediate ventilation types that could not be confidently assigned to one category and, consequently, were excluded from Experiments 1 and 2. Four recordings that showed baseline drift were also excluded from Experiment 1. In

two cases, ants died during or shortly after the end of a recording. These too were excluded from our analysis.

5.3.4 Dry mass

Ants were dried in a Gallenkamp Hotbox Oven Size 1 (Weiss Technik UK, UK) for seven days at 50°C. Individual ants were then weighed to the nearest 0.1 mg using an AV264C Adventurer Pro Analytical Balance (OHAUS, Switzerland).

5.3.5 Analysis

The total rate of carbon dioxide production and water loss for each ant was calculated using Origin 2016 (OriginLab Corporation, USA) and a mean rate calculated. All statistical analyses were conducted using R v. 3.3.3. (R Core Team).

5.3.5.1 Experiment 1

The allometric scaling of metabolic rate and water loss on nest and ventilation type was assessed with linear mixed effects models using the lme function from the 'nlme' package (Pinheiro et al., 2016). A maximal model (Table 5.1) incorporating all biologically relevant interactions was fitted initially. Model simplification occurred through stepwise removal of non-significant factors until the minimal adequate model remained (Table 5.1). Data were log transformed to ensure comparability with previous studies into the allometric scaling of metabolic rate (e.g. Lighton and Bartholomew, 1988; Nicholls et al., 2017; Vogt and Appel, 1999) and to normalise the data. Collection date was included as a random factor to account for any temporal effects on metabolic rate (Table 5.1).

To determine how scaling exponents might depend upon the structure of the statistical model, we compared the scaling exponent derived from the linear mixed

Table 5.1 Structure of linear mixed effects models used for assessing metabolic and water loss rate scaling of *Formica rufa*. Data were transformed using loge to allow comparison with other allometric analysis.

Model	Response	Fixed effects	Random effects
Maximal model	Log (Metabolic rate)	(Nest + log (dry weight) + ventilation type) ²	Collection date
Minimal adequate model	Log (Metabolic rate)	Log (dry weight) + ventilation type	Collection date
Maximal model	Log (Water loss)	(Nest + log (dry weight) + ventilation type) ²	Collection date
Minimal adequate model	Log (Water loss)	Log (dry weight) + ventilation type	Collection date
Maximal model	Log (Water loss)	(Nest + log (dry weight) + ventilation type + log(metabolic rate)) ²	Collection date
Minimal adequate model	Log (Water loss)	Log (metabolic rate)	Collection date

effects model (Table 5.1) with linear models in which the size of workers was the only independent variable, and metabolic rate the dependent variable. One contained all the ants regardless of ventilation type, another with ants only using continuous ventilation, and yet another with ants using only cyclic ventilation (Table 5.2).

Table 5.2 Different models used to assess variability of metabolic rate scaling exponents.

Model type	Dependent variable(s)	Scaling exponent	Standard error
Linear mixed effects model	Continuous and cyclic ventilation + dry weight	0.48	0.12
Linear model	Continuous and cyclic ventilation + dry weight	0.60	0.17
Linear model	Continuous ventilation	0.52	0.17
Linear model	Cyclic ventilation	0.56	0.23

5.3.5.2 Experiment 2

The likelihood of restraint affecting ventilation type was assessed using a contingent logistic regression, modelled using the ‘survival’ package (Therneau, 2015). Cyclic and continuous ventilation were scored as 0 and 1, respectively. Changes in metabolic rate were analysed using *t*-tests and Wilcoxon signed-rank tests from the R base package.

5.4 Results

5.4.1 Experiment 1

5.4.1.1 Metabolic rate scaling

We assessed the metabolic rates of wood ant workers selected from four nests within the same population (see Materials and Methods). The dry mass of workers ranged from 1.2 to 8.0 mg and, despite being sampled from four distinct nests, the range of worker sizes did not differ significantly (ANOVA, $F_{3,107} = 1.49$, $p > 0.20$; Fig. 5.9). To determine their metabolic rates, we placed individual ants in a respirometry chamber and recorded

continuously the amount of carbon dioxide that they excreted (see Materials and Methods). Whilst in the respirometry chamber ants displayed two distinct ventilation patterns: cyclic and continuous (Fig. 5.1).

Metabolic rate increased with increasing ant body mass ($t_{100,107} = 3.84$, $p < 0.001$). Overall, ant metabolic rate was negatively allometric, scaling with an exponent of 0.48 ± 0.12 (standard error), indicating that larger ants have relatively lower metabolic rates

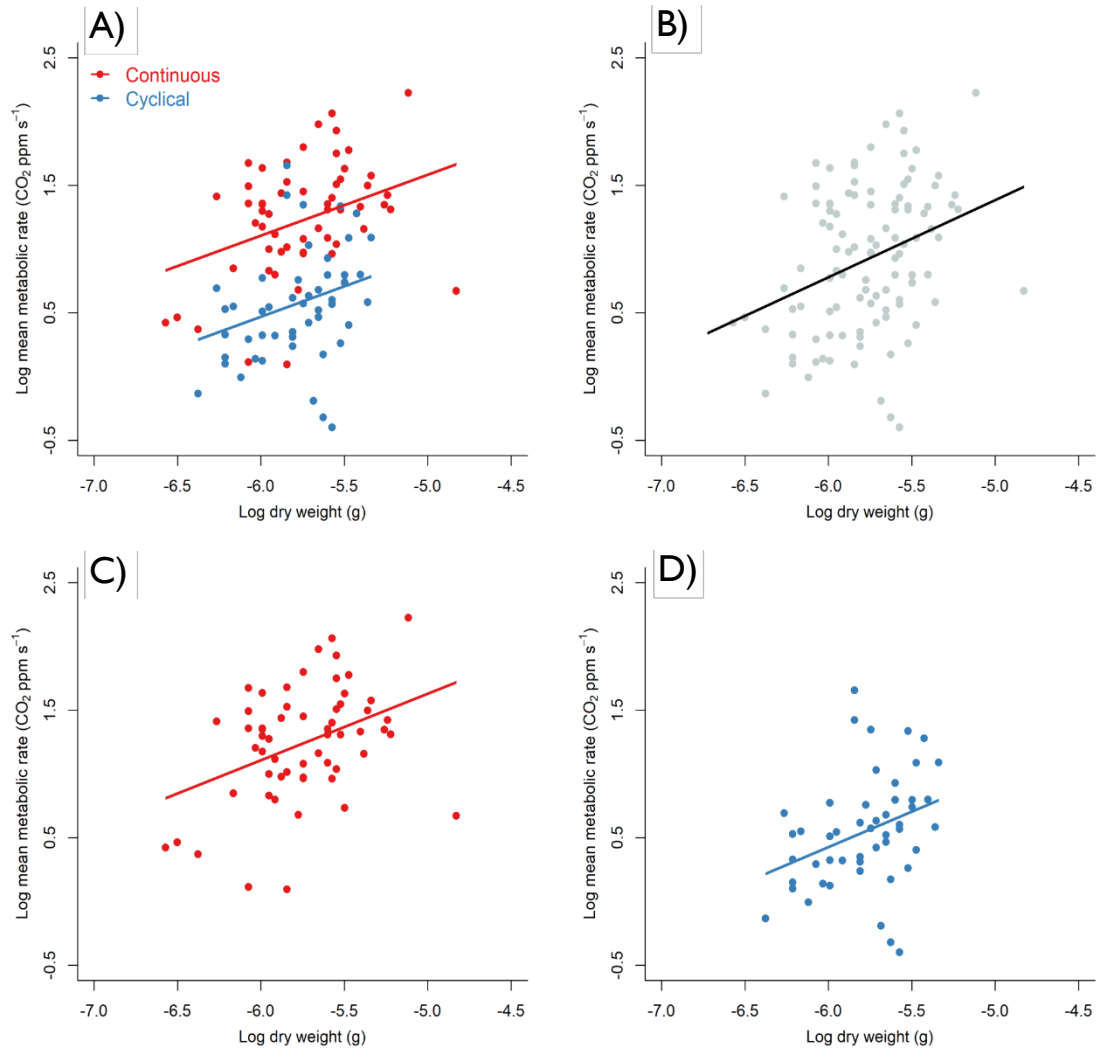


Figure 5.2 *Formica rufa* worker metabolic rate is higher when ventilating continuously than cyclically and scales with negative allometry. Metabolic rates during different ventilation patterns do not differ in their response to body size increases. A) Parameters estimated from a linear mixed effects model using data from all ants. B) Parameters estimated from a linear model using data from all ants. C) Parameters estimated from a linear model using data from ants using only continuous gas exchange. D) Parameters estimated from a linear model using data from ants using only continuous gas exchange.

than their smaller counterparts. Continuously ventilating ants had a higher metabolic rate than those using cyclic ventilation (Fig. 5.2A, $t_{100,107} = 8.21$, $p < 0.001$) but metabolic rate increased with size in a similar way irrespective of the type of ventilation being conducted ($F_{6,111} = 0.24$, $p = 0.63$). Mean metabolic rate did not differ between nests (Fig. 5.3, $F_{3,107} = 0.64$, $p = 0.59$), nor was there any difference in the response of metabolic

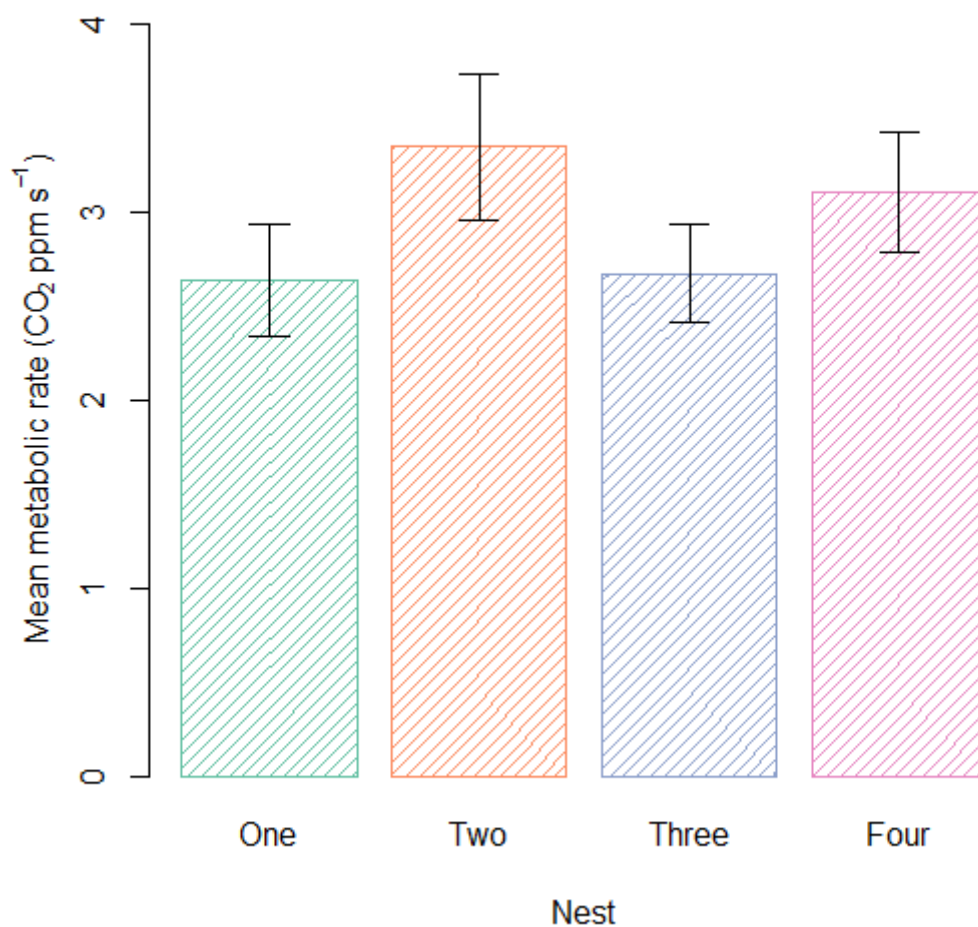


Figure 5.3 *Formica rufa* metabolic rate does not differ between nests. Mean metabolic rate (\pm standard error).

rate to size among all four nests ($F_{3,107} = 0.43$, $p = 0.73$). Mean metabolic rate of the different ventilation types was also similar among nests ($F_{3,107} = 0.49$, $p = 0.69$).

5.4.1.2 Effect of different models on metabolic scaling exponent

Several different methods are available for estimating allometric scaling exponents. To determine the extent to which different methods and statistical models influenced scaling exponents, we constructed several different biologically relevant models. We found a difference in the allometric scaling exponents estimated by different statistical models

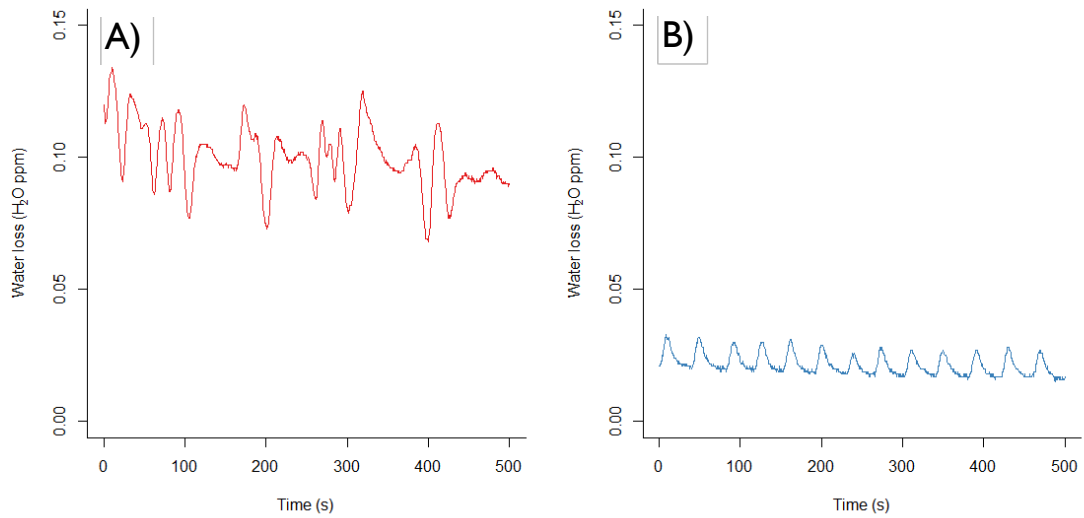


Figure 5.4 *Formica rufa* workers used two different ventilation types for gas exchange, which is reflected in the patterns of respiratory water loss. Respiratory water loss during: A) Continuous gas exchange and B) Cyclic gas exchange.

(Fig. 5.2, Table 5.2). The linear mixed effects model, which included ventilation type as a factor, predicted slopes of $0.48 (\pm 0.12 \text{ standard error})$ (Fig. 5.2A), whereas, a linear model, in which ventilation type was ignored, predicted a slope of $0.60 (\pm 0.17)$ (Fig. 5.2B). Two additional linear models that analysed continuously and cyclically ventilating ants independently also produced different scaling exponents. The linear model with only continuously ventilating ants predicted a slope of $0.52 (\pm 0.17)$ (Fig. 5.2C) and the linear model with only cyclically ventilating ants predicted a slope of $0.56 (\pm 0.23)$ (Fig. 5.2D). Consequently, the structure of the statistical model strongly affects the estimated scaling exponents.

5.4.1.3 Water loss scaling

We simultaneously monitored water loss for each ant (Fig. 5.4) in addition to the carbon dioxide excreted. Water loss increased with increasing ant mass (Fig. 5.5; $t_{3,107} = 2.13$, p

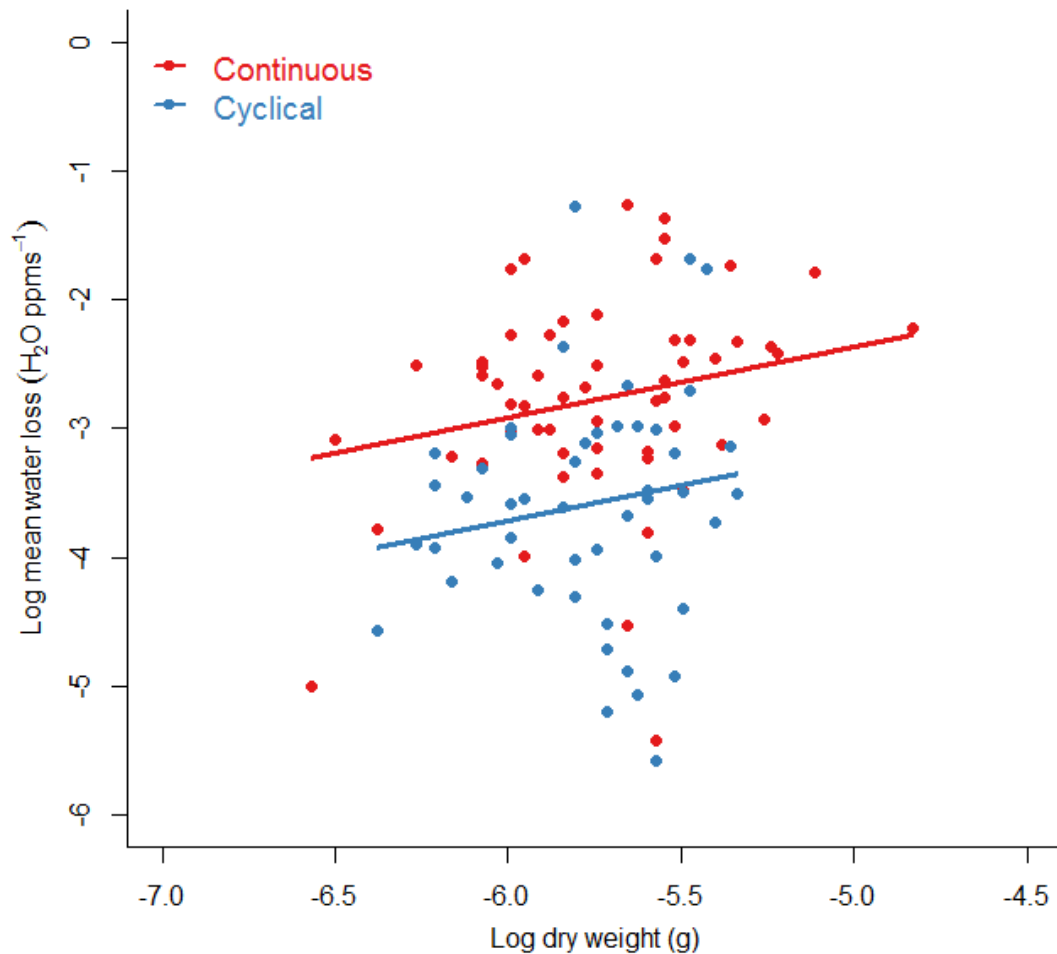


Figure 5.5 Respiratory water loss of *Formica rufa* workers scales with negative allometry and is higher when ventilating continuously compared with cyclically. Water loss rates during different ventilation patterns not differ in response to body size increases.

= 0.04). Overall, water loss was negatively allometric, scaling with an exponent of 0.55 ± 0.26 (standard error) indicating that larger ants lose relatively less water than their smaller conspecifics. Mean water loss was higher when ants were ventilating continuously than when they were ventilating cyclically (Fig. 5.5, $t_{3,107} = 4.99$, $p < 0.001$), though the scaling of water loss with increasing mass did not differ between the two ventilation types ($F_{1,107} = 0.24$, $p = 0.62$). Mean water loss ($F_{3,107} = 1.39$, $p = 0.25$) and the scaling of water loss ($F_{3,107} = 0.37$, $p = 0.77$) was non-significantly different among all four nests. Mean water loss also did not differ among nests when performing the two different ventilation types ($F_{3,107} = 1.37$, $p = 0.26$).

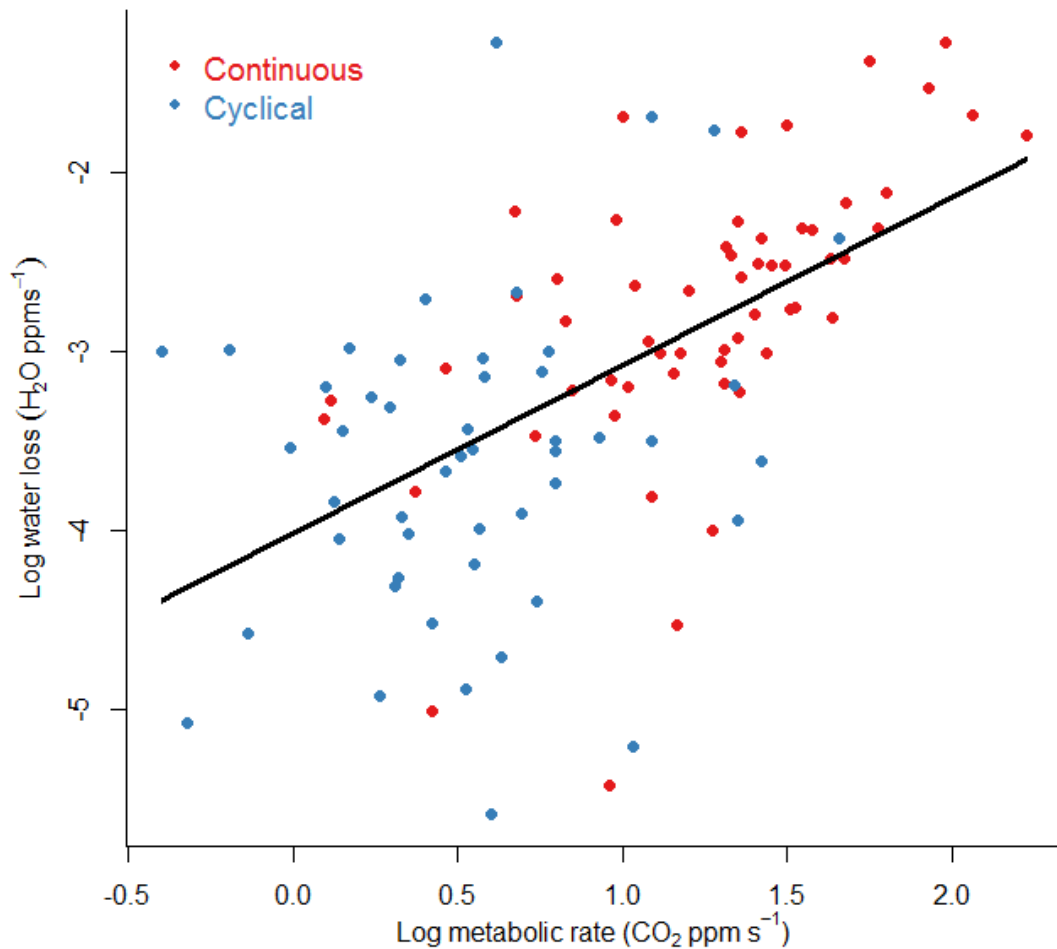


Figure 5.6 Respiratory water loss is driven entirely by increases in metabolic rate. *Formica rufa* worker water lost through ventilation as a function of metabolic rate.

5.4.1.4 Response of water loss to metabolic rate

Having determined that water loss and metabolic rate responded similarly to body size, nest affiliation and ventilation type, we assessed the response of water loss to metabolic rate. Given that ventilation type and size proved important in the previous models, we fitted a maximal model that included dry weight, nest affiliation and ventilation type to ascertain the response of water loss in the context of all potentially relevant variables

(Table 5.1). Metabolic rate was, however, the only significant predictor of water loss (Fig. 5.6, $F_{1,107} = 51.04$, $p < 0.001$).

Water loss increased with increasing metabolic rate (Fig. 5.6, $t_{101,107} = 7.14$, $p > 0.001$) with an almost isometric relationship (slope = 0.88 ± 0.15 (s.e.)) showing that respiratory water loss is driven primarily by metabolic rate. Mean water loss did not differ significantly between nests ($F_{3,107} = 0.95$, $p = 0.42$) or ventilation types ($F_{3,107} = 1.22$, $p = 0.31$). The scaling of water loss with metabolic rate ($F_{1,107} = 0.55$, $p = 0.65$) and mass ($F_{3,107} = 0.44$, $p = 0.72$) also did not differ among nests. Mean water loss was non-significantly different between ants using cyclic and continuous ventilation ($F_{1,107} = 2.41$, $p = 0.12$). Water loss scaling with both metabolic rate ($F_{1,107} = 0.55$, $p = 0.65$) and mass ($F_{1,107} = 0.10$, $p = 0.75$) was non-significantly different among ventilation types. Increases in mass did not cause an increase in water loss ($F_{1,107} = 0.51$, $p = 0.48$). Further, increases in water loss in response to mass were unaffected by simultaneous increases in metabolic rate ($F_{1,107} = 0.17$, $p = 0.69$).

5.4.2 Experiment 2

The differences in metabolic rate and water loss between ants performing continuous and cyclical ventilation may have been due to activity within the respiratory chamber. To test whether movement was indeed linked to changes in ventilation type, we examined the effects of restraining ants within the chamber. Unrestrained ants were $1.1 (\pm 1.04$ s.e.) times more likely to engage in continuous gas exchange than cyclical gas exchange (contingent logistic regression; $z_{1,48} = 2.30$, $p < 0.02$), suggesting that continuous gas exchange is linked to activity whereas cyclic gas exchange occurs when ants are stationary. Surprisingly, there was no difference in the mean metabolic rate of restrained and unrestrained ants (Fig. 5.7, paired t -test, $t_{23,48} = 0.17$, $p = 0.86$) and

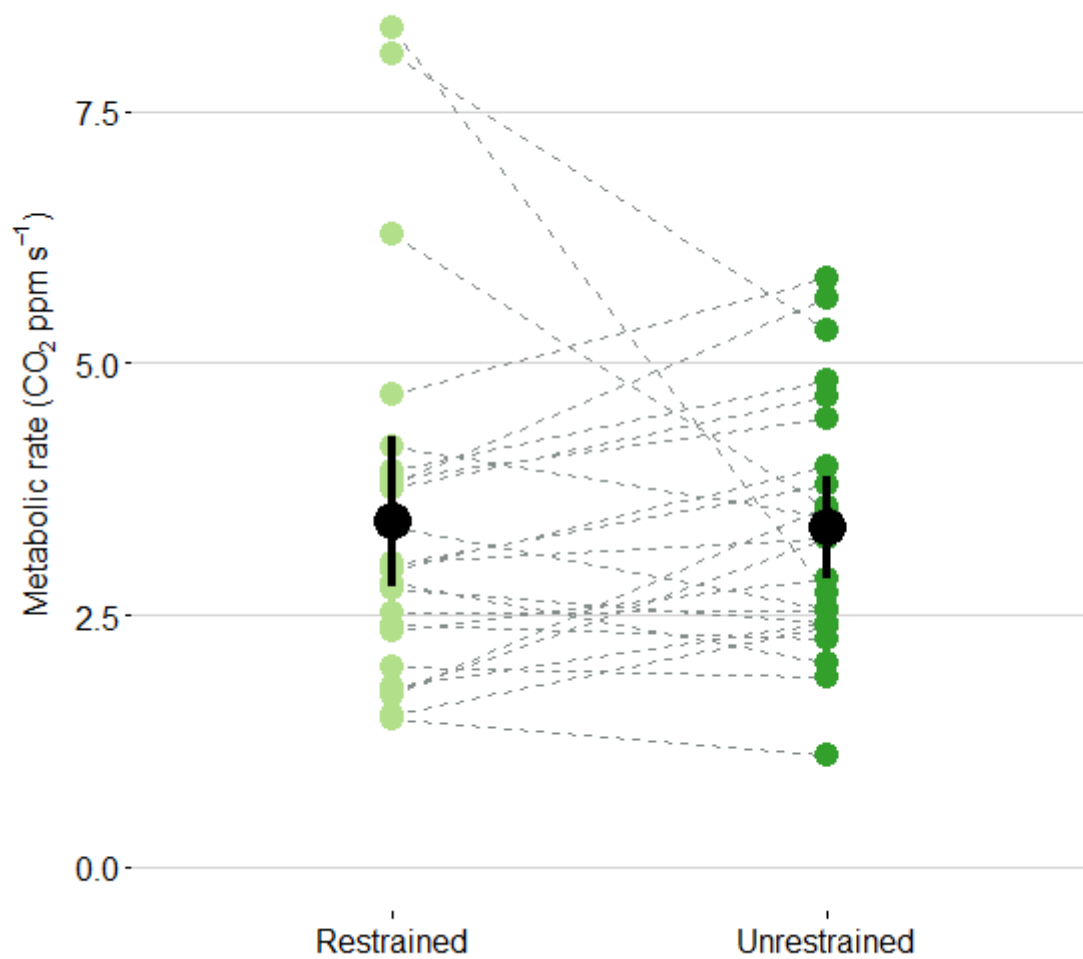


Figure 5.7 *Formica rufa* workers do not change their metabolic rate when restrained or unrestrained. Large dot and bar show mean metabolic rate (\pm standard error) and paired dots show individual ant metabolic rate.

individual ants did not increase their metabolic rate when restrained compared with when they were unrestrained (Fig. 5.7, Wilcoxon signed-rank test, $V = 181$, $p = 0.39$). Moreover, water loss was also not significantly different when ants were restrained or

unrestrained (paired t -test, $t_{23,48} = 0.001$, $p > 0.99$). Consequently, changes in ventilation type are driven by activity but are independent of increases in metabolic rate.

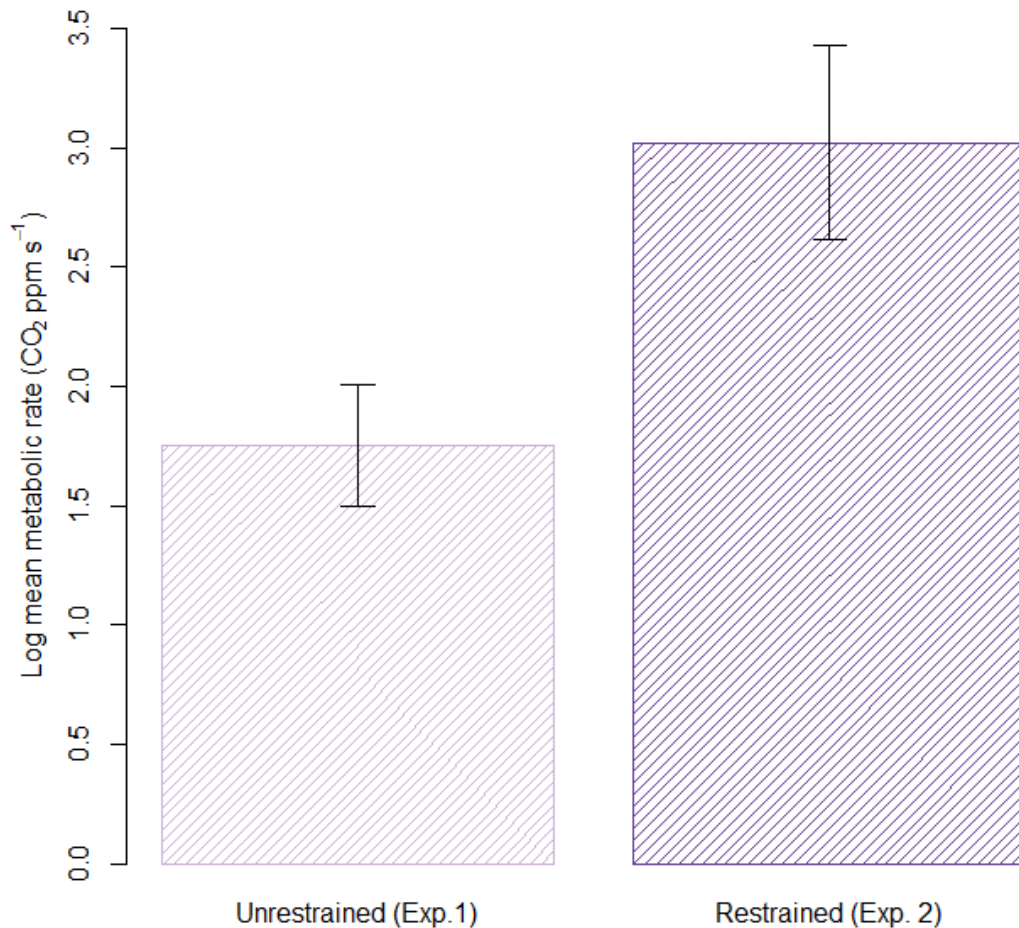


Figure 5.8 *Formica rufa* metabolic rate differed between restrained or unrestrained cyclically ventilating ants. Mean metabolic rate (\pm standard error) of unrestrained *F. rufa* workers from Experiment 1 compared with restrained *F. rufa* workers from Experiment 2.

To explain the absence of a significant difference in metabolic rate in the restrained versus the unrestrained ants, we compared the mean metabolic rate of the restrained ants performing cyclic ventilation ($N = 16$) from Experiment 2 with an equal number of size-matched unrestrained cyclically ventilating ants from Experiment 1. There was a significant difference in the mean metabolic rate between the two groups of ants (Fig. 5.8, t -test, $t_{29,32} = 3.50$, $p = 0.002$); restrained ants from Experiment 2 had a

higher mean metabolic rate than unrestrained ants from Experiment I (Fig. 5.8). This suggests that restraining ants elevated their metabolic rate compared with stationary but unrestrained ants, emphasizing that activity not elevated metabolic rate causes the change in ventilation type.

5.5 Discussion

The relationships between metabolic rate, water loss, ventilation pattern, activity and size in insects remain uncertain with numerous, sometimes conflicting, interpretations (Chown, 2002; Chown et al., 2006; Gibbs and Johnson, 2004). We explored the interactions between these factors in foragers of the red wood ant, *Formica rufa*. Although large foragers had higher metabolic rates than their smaller counterparts, metabolic rate was negatively allometric so that larger ants had relatively lower metabolic rates than small ants. Foragers performed either cyclic or continuous ventilation patterns. Ants performing cyclic ventilation had lower metabolic rates than equivalently sized ants performing continuous ventilation but an increase in metabolic rate did not drive the switch between these two ventilation patterns. Rather, the switch from cyclic to continuous ventilation is driven by activity; foragers perform cyclic ventilation whilst stationary at the same mean metabolic rate as they perform continuous ventilation whilst active.

Respiratory water loss, like metabolic rate, was negatively allometric so that larger ants lost absolutely more, but relatively less water than their smaller counterparts. Ants performing cyclic ventilation lost less water than equivalently sized ants performing continuous ventilation. However, metabolic rate was the primary driver for respiratory water loss, higher metabolic rates causing greater water loss. The independence of water loss from the ventilation pattern was confirmed by comparing restrained and

unrestrained ants; similar rates of water loss occurred at similar metabolic rates despite differences in ventilation pattern. This emphasises that within wood ant foragers both metabolic rate and respiratory water loss are independent of ventilation pattern, which is itself driven by activity.

5.5.1 Ventilation pattern is independent of metabolic rate

Higher metabolic rates in insects have been associated with distinct ventilation patterns (Contreras and Bradley, 2010; Gibbs and Johnson, 2004), continuous ventilation being used during periods of high metabolic demand to ensure adequate gas exchange for cellular respiration (Chown, 2002; Gibbs and Johnson, 2004). In wood ant foragers too, continuous ventilation is associated with higher metabolic rates than is cyclical ventilation. However, increasing metabolic demand in wood ants occurs simultaneously with switching from cyclic to continuous ventilation because both are induced by the onset of movement, an interpretation supported by comparisons of restrained and unrestrained ventilation patterns. Unrestrained workers are far more likely to perform continuous gas exchange than restrained workers despite having similar metabolic rates, implying that ventilation pattern is independent of metabolic rate, and is driven by activity. Thus, stationary workers perform cyclic ventilation, movement triggering continuous ventilation rather than an increase in metabolic rate.

Previous investigations that explicitly examined the switching from cyclic to continuous ventilation concluded that it is driven primarily by increasing metabolic rate (Basson and Terblanche, 2010; Gibbs and Johnson, 2004; Käfer et al., 2015; Moerbitz and Hetz, 2010; Nicholls et al., 2017). The activity-induced switching we observed in wood ant foragers cannot explain all these observations. For example, experimenters studying tsetse flies removed periods of activity from their analysis but still observed

switching from cyclic to continuous ventilation (Basson and Terblanche, 2010). Consequently, metabolic rate in these flies appears to be the primary cause of switching ventilation patterns (Basson and Terblanche, 2010). Studies of other taxa are more equivocal. Harvester ant queens have higher metabolic rates when ventilating continuously than cyclically (Gibbs and Johnson, 2004). However, their activity was not recorded raising the possibility that differences in activity drive changes in ventilation pattern.

Although we show a discrete switch between cyclic and continuous ventilation, the role of activity in directly driving ventilatory patterns could be subtler. There is evidence to suggest that ventilation patterns vary along a continuum rather than being discrete entities (Contreras and Bradley, 2010), and therefore differential activity could drive changes in cycle frequency, which would also account for our observations of discrete ventilation pattern switching. European paper wasps also change their ventilation pattern with increasing metabolic rate (Käfer et al., 2015). The increase in the frequency of ventilatory cycles with increasing metabolic rate coincided with an increase in activity (Käfer et al., 2015). This raises the possibility that movement could be responsible for the changes in ventilation pattern of paper wasps, just as in wood ants. Similar observations were made of weevils (Klok and Chown, 2005) where changes in cycle frequency, caused by increasing temperatures, were accompanied by concurrent changes in activity (Klok and Chown, 2005). Activity drives metabolic rate, rather than the reverse (Glazier, 2015). Consequently, increased activity should trigger changes in ventilation pattern simultaneously, to ensure that adequate gas exchange can occur.

5.5.2 The absence of discontinuous gas exchange

Formica rufa workers did not perform discontinuous gas exchange cycles (DGC) despite it being found in the workers (Lighton and Wehner, 1993; Quinlan and Lighton, 1999) and queens (Gibbs and Johnson, 2004; Lighton and Berrigan, 1995) of other ant species. This may seem surprising given that *F. rufa* workers' mean mass-specific metabolic rate is $0.20 \text{ ml CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ (± 0.10), similar to the workers of other ant species that perform DGC (Table 5.3). However, several aspects of wood ant ecology suggest DGC may be unnecessary. For example, wood ant workers are not at risk from dehydration because their mesic habitats have relatively high rainfall and their diet consists of honeydew (Collingwood, 1979) allowing lost water to be swiftly replaced. *Formica rufa* nests are formed of large mounds of twigs, leaves and needles, very little of the nest is subterranean, unlike many other ants (Hölldobler and Wilson, 1990). Workers spend time on the surface of the nest or foraging, thus, workers are unlikely to suffer from poor respiratory gas diffusion due to claustral living (Chown et al., 2006; Lighton and Berrigan, 1995; White et al., 2007a).

Table 5.3 Dry mass specific metabolic rate of different ant workers under different ventilation regimes.

Species	Metabolic rate ($\text{ml CO}_2 \text{ g}^{-1} \text{ h}^{-1}$)	Standard deviation	Gas exchange	Reference
<i>Pogonomyrmex californicus</i>	0.29	0.07	Discontinuous	Quinlan and Lighton, 1999
<i>P. rugosus</i>	0.33	0.13		
<i>P. occidentalis</i>	0.28	0.14		
<i>Messor pergandei</i>	0.27	0.20	Continuous	Lighton and Berrigan, 1995
<i>M. julianus</i>	0.19	0.07	Discontinuous	
<i>Camponotus vicinus</i>	0.19	0.03	Discontinuous	Lighton and Garrigan, 1995
<i>Formica rufa</i>	0.20	0.10	Cyclic	This study

It is feasible that there are no adaptive functions of DGC (or cyclic gas exchange) (Contreras and Bradley, 2010) and that all ventilation patterns are a continuum, responding to fixed gaseous set-points (Chown and Holter, 2000). The set-point hypothesis posits that the opening and closing of spiracles respond to local concentrations of carbon dioxide and oxygen independently producing the observed ventilation patterns. However, this hypothesis cannot explain changes in ventilation pattern without an increase in metabolic rate. If spiracles were only responding to set-points, increased metabolism would be required to change the partial pressures of carbon dioxide and oxygen within the tracheae and induce changes in ventilation type. Instead, we observe changes in ventilation type independent of metabolic rate.

5.5.3 Metabolic rate drives respiratory water loss independent of ventilation pattern

There has been considerable debate about the cause of respiratory water loss in insects, though there is a consensus that larger insects with higher metabolic rates incur greater losses than smaller insects, and that open spiracles are associated with higher amounts of water loss (Addo-Bediako et al., 2001; Chown, 2002). Respiratory water loss in wood ant foragers increased with body mass, scaling nearly isometrically. Continuous ventilation in these ants incurred greater water loss than cyclic ventilation but the primary driver is metabolic rate. The lower rates of water loss during cyclic ventilation are due to lower metabolic rates not to any reduction caused by the pattern itself (Gibbs and Johnson, 2004). This is consistent with the strong link between water loss and metabolic rate reported across insects (Chown, 2002).

5.5.4 Homogeneous physiological scaling among colonies

The homogeneity of scaling in metabolic rate and respiratory water loss among workers from *F. rufa* nests contrasts with the heterogeneity in morphological scaling demonstrated by the implementation of different growth rules for compound eye growth (facet size *versus* facet number) in different nests (Perl and Niven, 2016b). An explanation for this difference is that environmental effects causing variation in morphological scaling do not affect metabolic scaling. Morphological variability of ant workers is likely a consequence of exposure to different environments (e.g. nutrition) during growth and development. However, in honey bees, larval starvation has no effect on imago metabolic rates under normal feeding conditions (Wang et al., 2016), raising the possibility that metabolic scaling is resilient to environmental factors that generate variability in morphological scaling. Furthermore, the homogeneity of metabolic rate scaling among colonies indicates that any differences in fitness between nests is unlikely to be mediated by forager metabolic rate.

Some studies have suggested that metabolic rate scaling is caused by differential increases in body sizes through cell number or cell size (Davison, 1955; Kozłowski et al., 2003). Growth mediated by increased cell number produces isometric scaling whereas growth through increased cell size produces an exponent of 0.67 (Chown et al., 2007; Davison, 1955; Kozłowski et al., 2003). This idea has been tested within ants, predicated on the idea that relative cell number (facet number) and cell size (facet size) in compound eyes is an accurate proxy for the entire body (Chown et al., 2007). Were this true in *F. rufa*, heterogeneity in the scaling of worker eyes among nests (Perl and Niven, 2016b) would reflect scaling of their entire bodies producing different metabolic scaling exponents among nests. The absence of differences in metabolic scaling among nests despite differences in the contributions of facet number and size to compound eye

scaling (Perl and Niven, 2016b), suggest that this does not apply to wood ants. However, direct measurements of both metabolic and morphological scaling from the same nests are necessary to confirm this fully.

5.5.5 Intraspecific metabolic scaling in wood ant foragers

The metabolic rate of wood ant foragers is negatively allometric. However, depending on the statistical model fitted, the scaling exponent differed numerically. Fitting a linear mixed effect model incorporating ventilation pattern estimated a scaling exponent of 0.47, lower than intraspecific metabolic rate scaling exponents of ant workers (Table 5.4) and other invertebrate taxa, aside from ribbon worms (0.48) (Glazier, 2005). The exponents for individual linear models for each ventilation type (0.52 and 0.56 for continuously and cyclically ventilating ants, respectively; Table 5.2) were also lower than estimates from other invertebrates (Glazier, 2005) but were similar to estimates from *Camponotus fulvopilosus*. (Table 5.4; Chown et al., 2007; Lighton, 1989). However, a linear model estimated a scaling exponent of 0.60, which is consistent with metabolic rates previously reported for workers of *F. rufa* and other species (Table 5.4; Chown et al., 2007). All the scaling exponents are also lower than interspecific scaling relationships among ants (Table 5.4; Chown et al., 2007; Lighton and Wehner, 1993) and other insects (Addo-Bediako et al., 2002; Chown et al., 2007; Lighton et al., 2001; Niven and Scharlemann, 2005), a common pattern when comparing inter- and intraspecific metabolic rate scaling (Glazier, 2005; Harrison, 2017). The probable cause of relatively lower intraspecific metabolic rate scaling exponents is the smaller range of body sizes available within a species, compared with the ranges available interspecifically. The numerical differences between exponent estimates are important if scaling relationships are to be used as a predictive tool (Glazier, 2005). One explanation for the differences

in scaling exponent is whether the statistical model incorporated the ventilation pattern. This demonstrates that estimates of scaling exponents that ignore ventilation pattern (Vogt and Appel, 1999) and do not control for activity (Lighton and Bartholomew, 1988) should be treated with caution.

Table 5.4 Summary of scaling exponents derived from different ant workers.

Species	Scaling exponent	Ventilation pattern	Ant activity	Reference
<i>Camponotus fulvopilosus</i>	0.55	Undefined	Uncontrolled	Lighton, 1989
<i>Eciton hamatum</i>	0.84	Undefined	Uncontrolled	Bartholomew et al., 1988
<i>Pogonomyrmex rugosus</i>	0.70	Undefined	Controlled	Lighton and Bartholomew, 1988
<i>Anoplolepis steinergroeveri</i>	0.61			
<i>Atta columbica</i>	0.64			
<i>Camponotus fulvopilosus</i>	0.56	Undefined	Uncontrolled	Chown et al., 2007
<i>C. maculatus</i>	0.60			
<i>Eciton hamatum</i>	0.84			

Table 5.4 continued Summary of scaling exponents derived from different ant workers.

Species	Scaling exponent	Ventilation pattern	Ant activity	Reference
<i>Formica rufa</i>	0.69			
<i>Messor capensis</i>	1.28	Undefined	Uncontrolled	Chown et al., 2007
<i>M. pergandei</i>	0.61			
Interspecific scaling (15 species of ant)	0.76	-	-	Mason et al., 2015
Interspecific scaling (24 species of ant)	0.93	-	-	Lighton and Wehner, 1993
Interspecific scaling (83 species of insect)	0.80	-	-	Vogt and Appel, 1999

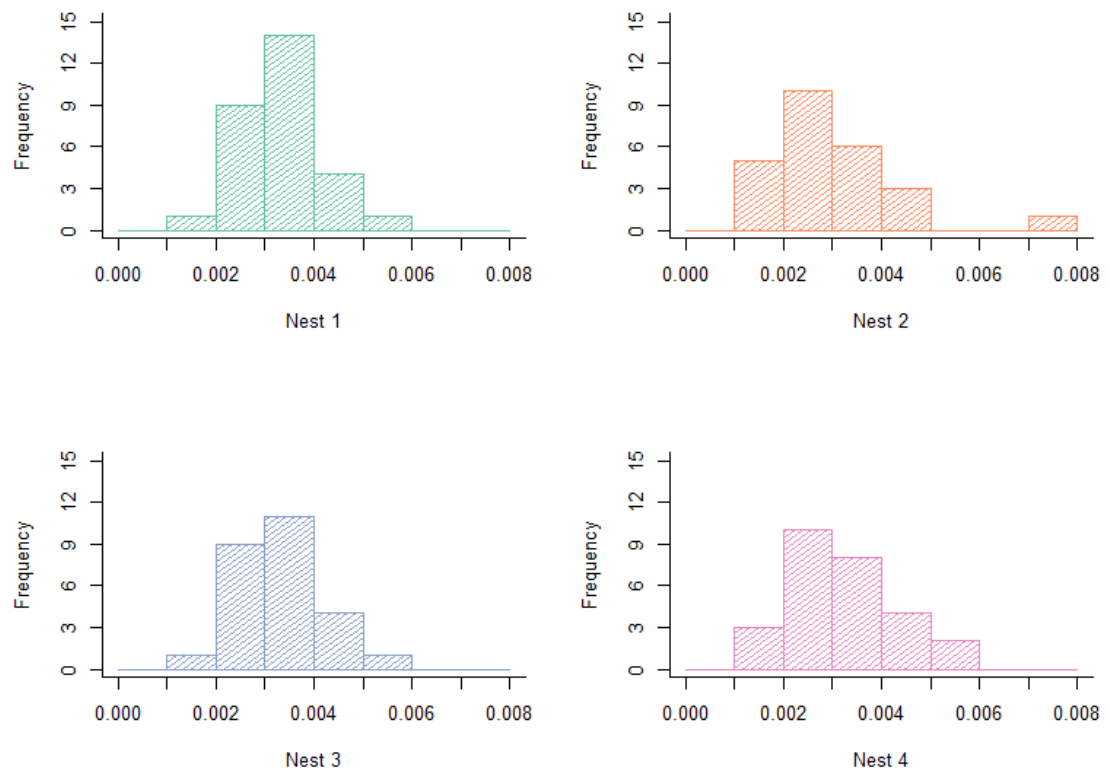


Figure 5.9 *Formica rufa* worker size did not differ among nests. Size distributions of workers from all four nests: A) Nest 1; B) Nest 2; C) Nest 3, D) Nest 4.

Chapter 6: General discussion

6.1 The contributions of this thesis

This thesis makes several novel contributions to the field of scaling in insects and has further implications for scaling in animals generally. In chapter two, I demonstrate that morphological scaling does not necessarily occur uniformly across an entire organ. Regional grade-shifts in facet diameters are an important mechanism for improving sensitivity and acuity in a compound eye, but this process of regionalisation is different from differential scaling. The slope shifts in facet diameter are truly novel, they indicate that distinct parts of an organ can differ in their response to increases in body size. These data can be extrapolated beyond implications for compound eyes, which were a useful system for investigation, and provide insight into regulation within a developmental compartment.

In chapter three, I show that scaling rules in *Formica rufa* also differ between nests in the same population. *Formica rufa* are a eusocial species and their colonial living made it possible to compare scaling relationships within a single population. Workers from some nests enlarge their compound eye primarily through increases in facet diameter, whereas workers from other nests increase compound eye size through increases in ommatidia number. Increasing the number of ommatidia will improve resolution, and larger facets will improve sensitivity. The differences between nests are surprising because it is a reasonable assumption that eye structure is determined by visual ecology (Land and Nilsson, 2002). If so, we would expect ants from the same environment to use the same scaling rules for constructing their eyes, to maintain the same visual adaptations. The difference between nests indicates that there are other factors involved in eye scaling. If increases in the relative size and number of cells in an organ are representative of how entire body growth (Chown et al., 2007; Stevenson et al., 1995),

the differences between nests may be mechanistic. Cell size increase is mediated by insulin-like peptides, however, cell number increase is mediated by ecdysone. These hormones are regulated by nutrition (Andersen et al., 2013; Callier and Nijhout, 2013; Nijhout and Callier, 2015) and, therefore, differential access to nutrition among nests could explain why eye scaling rules are not uniform throughout the population.

In chapter four, I explore differential intra-eye scaling among members of the *Formica* genus. Slope shifts similar to those observed in *F. rufa* are found within one other member of the genus, *F. sanguinea*, but not within two other species that were investigated (*F. fusca*, *F. lugubris*). All species demonstrate grade shifted facet diameters versus femur length. Given the close phylogenetic relationship between *F. rufa* and *F. lugubris*, it is unlikely that there is a phylogenetic component to intra-eye scaling. Likewise, due to the similarity of habitats between *F. rufa* and *F. lugubris* a strong ecological component is also unlikely to be the source of intra-eye scaling. Despite the dearth of slope shifts within species, homologous regions among species do show slope shifts.

Interestingly, the scaling of facet number against facet diameter revealed no difference between any of the species. This implies that across the genus, there is a fixed rule that determines the mean facet diameter to facet number ratio. However, the variability in regional scaling might provide a mechanism by which specific eye modifications can be made whilst still conforming to overall genus-wide scaling rules.

In chapter five, I examined continuous and cyclic gas exchange patterns, as well as metabolic rate scaling, of *F. rufa* workers from different nests. Unlike morphological scaling, there are no differences in the scaling of metabolic rate or water loss of workers from different nests. Water loss in *F. rufa* workers is driven by metabolic rate only, independent of body size. We also demonstrate that movement is the key factor that

initiates switching of ventilation patterns in *F. rufa* workers. Previous investigations state that increases in metabolic rate are responsible for causing changes in ventilation type (Gibbs and Johnson, 2004). Metabolic rate is probably indirectly responsible for causing changes in ventilation pattern, because both metabolic rate increase and ventilation pattern changes are induced by the onset of movement.

6.2 Importance of differential scaling

The capacity for slope shifts to exist within a single organ has important implications for our understanding of development and growth. Global resources are distributed unevenly among developing organs, allowing some organs to grow larger than others with increases in body size (Emlen et al., 2012). There is evidently an analogous process occurring within the compound eye – and by extension, developmental compartments of other organs. Some organ regions get access to more resources than others, hence cellular growth is differentially enhanced. The nature of ommatidial development made the compound eye a useful organ with which to test differential resource distribution. Once ommatidial development has initiated from a progenitor cell (Friedrich et al., 1996), cells do not merge or cross into adjacent ommatidia (Wolff and Ready, 1991). Therefore, resources are restricted to each ommatidia, demonstrating that differential resource allocation does occur within an organ.

Differential resource allocation within an organ may be mediated in the same way as it is for whole organs within a body; via expression of insulin receptors. Greater expression of insulin receptors in cells from one region will promote a larger response to increases in insulin-like peptides (ILPs), compared with another region in which cells express fewer receptors. Larval nutrition is responsible for the release of ILPs and adult body mass, hence, different regions of a single organ can respond differently to increases

in size. This mechanism allows organs to diversify and fulfil a specific ecological function. This is evident from the results of Chapter 3 in which it is shown that there is a fixed ratio of facet number to mean facet diameter across all four *Formica* species, indicative of a potential functional constraint. However, there is variability among species in terms of differential facet diameter scaling. The capacity to modify selective parts of the eye may allow species to adapt to their own specific visual ecologies, whilst still adhering to genus wide scaling constraints.

Differential scaling may also provide a mechanism through which morphological diversity can be generated. Ant workers do not directly produce offspring, and therefore the negative consequences of deleterious morphological mutations may not have any fitness effects. Hence, worker ant organs may experience a greater degree of developmental flexibility. In other species, there are organs where diversification and modification are a critical component of species or subspecies reproductive isolation. Part of this isolation is generating morphological novelty. This is often the case with the a portion of male genitalia (aedeagus), where closely related species may only be distinguishable through aedeagus morphology (Franco et al., 2006; Hosken and Stockley, 2004; Kulikov et al., 2004). Another example can be found in *Onthophagus* beetles where horns are highly modified at a species specific level (Emlen et al., 2005; McCullough et al., 2015). The mechanism used to scale organs differentially across an entire body (relative insulin receptor expression) may also be responsible for within organ scaling. If so, it becomes a matter of merely changing the patterns of insulin expression within an organ to create differences in how many prongs, folds or wedges a horn or aedeagus expresses. Differential scaling could be a mechanism by which changes in organ morphology can be modified through simple developmental changes.

6.3 Variability in morphological and physiological scaling

There is a striking contrast in the plasticity of morphological scaling and the conservation of physiological scaling between nests. Workers from different colonies scale their eyes differently, investing in facet number or diameter. However, there are no significant differences in the metabolic rate scaling of workers between nests. There are several potential reasons for this. Firstly, metabolic rate is susceptible to environmental and physiological variability, increasing when insects are moving rapidly or ambient temperature increases (Chown and Nicolson, 2004). When placed in a homogenous laboratory environment, it is possible that environmental conditions that might affect workers from different nests no longer apply. Thus, the workers scale their metabolic rate similarly. Morphological scaling is also susceptible to environmental variation. During ontogeny, changes in temperature can affect adult organ scaling (Shingleton et al., 2009). Even though resting metabolic rate is often viewed as an invariant property, metabolic rate is constantly in flux, whereas adult organs are a fixed size; they can no longer respond to environmental change. Hence, the fluctuations in environmental variables that affect metabolic rate can no longer affect adult organ scaling.

It is possible that the lack of difference in metabolic rate scaling among nests is because physiological scaling is tightly restricted. There are many competing explanations for why and how metabolic rate scales with body size across different taxa (Brown et al., 2004; Chown and Nicolson, 2004; Glazier et al., 2015; Hirst et al., 2014; West et al., 1997). Many interspecific metabolic rate scaling hypotheses posit that fundamental physical laws, such as; the fractal nature of nutrient supply networks (West et al., 1997; West et al., 1999), growth through cell number compared with cell size (Chown et al., 2007) or resource flow and relative surface area (Glazier et al., 2015) are responsible for invariant metabolic rate scaling across taxa. We would expect intraspecific scaling to

have a different exponent compared with interspecific scaling (Glazier, 2005; Harrison, 2017), principally because the range of body sizes is much smaller. However, fundamental physical laws should apply equally within as across species. Both instances depend upon increasing body sizes. If metabolic rate scaling is restricted, even moderately, by fundamental physical laws it is unsurprising that we do not observe differences in metabolic rate scaling among nests; all nests would be subject to the same underlying constraints.

6.4 Insect ventilation patterns and their causes

The consensus on insect ventilation patterns is that switching is driven by increases in metabolic rate (Contreras and Bradley, 2010; Gibbs and Johnson, 2004). In many cases where metabolic rate increases are cited as causing changes in ventilation pattern, it is possible that movement is the actual trigger (Gibbs and Johnson, 2004; Käfer et al., 2015; Klok and Chown, 2005)). Changes in metabolic rate and ventilation pattern are concurrent rather than causal. Metabolic rate increases are the ultimate cause of changes in ventilation pattern, because of the increased requirement for gas exchange, but in wood ant workers the proximate mechanism by which the pattern changes is the onset of movement. This proximate mechanism may not be true for all insect species (Basson and Terblanche, 2010).

Integrating movement as the proximate cause of ventilation pattern changes with previous investigations is difficult because there has been a strong focus on discontinuous gas exchange cycles (DGC), which we did not observe in *Formica rufa* workers. Explanations from the chthonic and hygric hypotheses, which suggest that DGC preserve water and optimise gas concentrations respectively, do not apply here (Chown et al., 2006; Gibbs and Johnson, 2004; Lighton, 1998). However, movement

induced ventilation pattern changes is consistent with the hypothesis that DGC are used to avoid generating free radicals (Hetz and Bradley, 2005). The onset of movement indicates that more oxygen is going to be consumed, lowering the risk of oxygen over-exposure, and so it follows that spiracles will spend less time closed. Changes in spiracular opening time manifest as changes in ventilation pattern. Furthermore, our paradigm is not mutually exclusive with another DGC hypothesis: DGC being the default gas exchange pattern, controlled by ganglia (Matthews and White, 2010; Matthews and White, 2013). Worker ants are highly active and, therefore, may never lower brain activity enough to devolve spiracular control to local ganglia. Testing the ants before and after decapitation would provide a useful experiment with which to test this. Insect ventilation types may not be truly discrete entities, and may instead exist on a continuous scale (Contreras and Bradley, 2010). If so movement could still be critical for increasing cycle rates (Klok and Chown, 2005).

6.5 Ecological implications of homogenous metabolic rate among nests

Metabolic rate is hypothesised to influence several aspects of animal ecology, many of which have a direct or indirect impact on fitness. Relative fitness among *Formica rufa* nests is unlikely to be mediated by worker metabolic rate because there is no difference in metabolic rate scaling between nests. This is surprising because in some animals, certain behavioural traits, like aggressiveness, are linked with metabolic rate (Careau et al., 2008). Territory is fought over in the spring (Elton, 1932) and therefore, workers with higher metabolic rates and more aggression might secure more territory, which could influence fitness through access to aphid resources. It is feasible that worker metabolic rate changes throughout the season. Ants were collected towards the end of the summer, and so it is possible that their metabolic rates were lowering in preparation

for overwintering. An interesting future experiment would first track worker metabolic rate across the season, to assess any early-season nest differences, and then correlate this with nest foraging territory.

Worker metabolic rate is important because it forms part of the mechanism modulating how resources enter the nest. However, in the majority of species, individual caste scaling exponents are different from the metabolic rate scaling of whole colonies (Vogt and Appel, 1999). This is because the ecology of adult insects differs vastly from that of larvae and pupae and so they have different metabolic demands (e.g. movement *versus* growth). The metabolic costs of the entire nest may be more pertinent to determining colony fitness than the metabolic rates of individual workers. Colony fitness might be determined by number of workers instead. Differences in worker number could have a role in determining colony metabolic rate. Furthermore, workers may be selected to be as energetically efficient as possible (Bartholomew et al., 1988; Feener et al., 1988; Wright et al., 2000), to maximise resource collection. All colonies within the population may be constrained in how efficient their workers are. Due to their size whole wood ant nests cannot be put into a respirometer, though it may be possible to estimate whole nest metabolic rate by estimating caste populations and their mass specific metabolic rates.

6.6 Open questions

This thesis has generated many questions that remain to be addressed. The function, if any, of differential intra-organ scaling is currently unknown. Though there is differential investment in distinct parts of the ant eye, the behavioural or ecological benefits of this remain to be discovered. It is possible that investment in some parts of an eye are more beneficial than others. This type of regionalisation is well documented in compound eyes

and allows locally high acuity without overly large compound eyes (Land, 1997). Differential scaling takes this hypothesis further and implies that some eye regions are increasingly beneficial with increasing size *i.e.* it is better for some regions to be bigger but only when the animal itself is large.

Examination of the visual properties of the eye would first be needed to assess if intra-organ scaling was adaptive. Without a measure of visual acuity and sensitivity it is difficult to estimate whether the changes in facet diameter are functionally adaptive (Land, 1997). Ants are strongly driven by olfaction and so their vision is general poorer than other members of the Hymenoptera (Lunau et al., 2009; Macuda et al., 2001; Spaethe and Chittka, 2003; Zollikofer et al., 1995). Less reliance on poorer vision may mean that it is not important what the facet diameters are, within a certain range. If the facet diameter changes are so small as to not have an impact on vision, there may be developmental deregulation instead. Compound eye development, at least in *Drosophila melanogaster*, is tightly regulated to ensure that facets are hexagonal and pack together closely into a neat facet array (Kim et al., 2016; Ready et al., 1976). Comparing the facet array of an ant with that of a bee (Jander and Jander, 2002), a locust (Homberg and Paech, 2002) or a fruit fly (Kim et al., 2016) reveals that ant facets do not conform well to a stereotypical hexagonal shape (Baker and Ma, 2006). Ant facets are often misshapen, many have too many or too few sides, forming irregular pentagons or even octagons. This implies that developmental control of facet formation and packing has been deregulated. If so, the rate of facet diameter increase with body size may be similarly deregulated and the distribution of resources within the developing eye may be uneven, not by design but by neglect. Though this may explain how differential intra-organ scaling arose, it is insufficient to explain why intra-organ scaling is found in some *Formica* species but not others.

It is unknown how pervasive the phenomenon of differential scaling is. Given that slope shifts do not occur in the compound eyes of all *Formica* species it is certainly restricted to some degree. It remains to be seen whether other groups of insects also have differential scaling in their eyes, especially those with better vision than ants. If this exists in a species that relies on highly acute vision, that would indicate an adaptive function rather than developmental deregulation.

It is also unknown if differential scaling is present in other organs. It is difficult to test in organs that do not have distinctly differentiated functional subunits. One potentially suitable candidate organ is the insect ovary, which is composed of subunits called ovarioles. The size of an ovary could be changed by making ovarioles larger or more numerous, an analogy to increasing eye area with larger facets or more ommatidia. Thus, if differential scaling exists in other organs, ovaries are an ideal candidate.

References

- Addo-Bediako, A., Chown, S. L. and Gaston, K. J.** (2001). Revisiting water loss in insects: A large scale view. *J. Insect Physiol.* **47**, 1377–1388.
- Addo-Bediako, A., Chown, S. L. and Gaston, K. J.** (2002). Metabolic cold adaptation in insects: A large-scale perspective. *Funct. Ecol.* **16**, 332–338.
- Agutter, P. S. and Wheatley, D. N.** (2004). Metabolic scaling: Consensus or controversy? *Theor. Biol. Med. Model.* **1**, 1–13.
- Alatalo, R. V., Höglund, J. and Lundberg, A.** (1988). Patterns of variation in tail ornament size in birds. *Biol. J. Linn. Soc.* **34**, 363–374.
- Alerstam, T., Rosén, M., Bäckman, J., Ericson, P. G. P. and Hellgren, O.** (2007). Flight Speeds among bird species: Allometric and phylogenetic effects. *PLoS Biol.* **5**, e197.
- Alexander, R. M.** (1999). *Energy for animal life*. Oxford University Press.
- Andersen, D. S., Colombani, J. and Léopold, P.** (2013). Coordination of organ growth: Principles and outstanding questions from the world of insects. *Trends Cell Biol.* **23**, 336–344.
- Atanasov, A. T.** (2007). The linear allometric relationship between total metabolic energy per life span and body mass of mammals. *Biosystems* **90**, 224–233.
- Atkinson, D.** (1994). Temperature and organism size: A biological law for ectotherms? *Adv. Ecol. Res.* **25**, 1–58.
- Baker, G. T. and Ma, P. W. K.** (2006). Morphology and number of ommatidia in the compound eyes of *Solenopsis invicta*, *Solenopsis richteri*, and their hybrid (Hymenoptera: Formicidae). *Zool. Anz.* **245**, 121–125.
- Baker, G. S. and Wilkinson, R. H.** (2001). Phylogenetic analysis of sexual dimorphism and eye-span allometry in stalk-eyed flies (Diopsidae). *Evolution.* **55**, 1373–1385.
- Bargum, K., Boomsma, J. J. and Sundström, L.** (2004). A genetic component to size in queens of the ant, *Formica truncorum*. *Behav. Ecol. Sociobiol.* **57**, 9–16.
- Bartholomew, G. A., Lighton, J. R. B. and Feener Jr., D. H.** (1988). Energetics of trail running, load carriage, and emigration in the column-raiding army ant *Eciton hamatum*. *Physiol. Zool.* **61**, 57–68.
- Basson, C. H. and Terblanche, J. S.** (2010). Metabolic responses of *Glossina pallidipes* (Diptera: Glossinidae) puparia exposed to oxygen and temperature variation: Implications for population dynamics and subterranean life. *J. Insect Physiol.* **56**, 1789–1797.
- Basson, C. H. and Terblanche, J. S.** (2011). Respiratory pattern transitions in three species of *Glossina* (Diptera, Glossinidae). *J. Insect Physiol.* **57**, 433–443.
- Bauer, T. and Kredler, M.** (1993). Morphology of the compound eyes as an indicator of life-style in carabid beetles. *Can. J. Zool.* **71**, 799–810.
- Bejan, A., Marden, J. H. and Ansell** (2006). Unifying constructal theory for scale effects in running, swimming and flying. *J. Exp. Biol.* **209**, 238–48.
- Bernstein, S. and Finn, C.** (1971). Ant compound eye: Size-related ommatidium differences within a single wood ant nest. *Experientia* **27**, 708–710.
- Berrigan, D.** (1991). The allometry of egg size and number in insects. *Oikos* **60**, 313–321.

- Berrigan, D. and Pepin, D. J.** (1995). How maggots move: Allometry and kinematics of crawling in larval Diptera. *J. Insect Physiol.* **41**, 329–337.
- Bickler, P. E. and Buck, L. T.** (2007). Hypoxia Tolerance in reptiles, amphibians, and fishes: Life with variable oxygen availability. *Annu. Rev. Physiol.* **69**, 145–170.
- Biro, P. A. and Stamps, J. A.** (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends Ecol. Evol.* **25**, 653–659.
- Blackmer, A. L., Mauck, R. A., Ackerman, J. T., Huntington, C. E., Nevitt, G. A. and Williams, J. B.** (2005). Exploring individual quality: Basal metabolic rate and reproductive performance in storm-petrels. *Behav. Ecol.* **16**, 906–913.
- Blueweiss, L., Fox, H., Kudzma, V., Nakashima, D., Peters, R. and Sams, S.** (1978). Relationships between body size and some life history parameters. *Oecologia* **37**, 257–272.
- Boardman, L., Terblanche, J. S., Hetz, S. K., Marais, E. and Chown, S. L.** (2012). Reactive oxygen species production and discontinuous gas exchange in insects. *Proc. R. Soc. B Biol. Sci.* **279**, 893–901.
- Bolstad, G. H., Cassara, J. A., Márquez, E., Hansen, T. F., van der Linde, K., Houle, D. and Pélabon, C.** (2015). Complex constraints on allometry revealed by artificial selection on the wing of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 13284–9.
- Bonduriansky, R.** (2007). Sexual selection and allometry: A critical reappraisal of the evidence and ideas. *Evolution*. **61**, 838–849.
- Bonduriansky, R. and Day, T.** (2003). The evolution of static allometry in sexually selected traits. *Evolution* **57**, 2450–8.
- Brooke, M. D. L., Hanley, S. and Laughlin, S. B.** (1999). The scaling of eye size with body mass in birds. *Proc. R. Soc. London B Biol. Sci.* **226**, 405–412.
- Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B.** (2004). Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789.
- Buck, J. and Kesiter, M.** (1955). Cyclic CO₂ release in diapausing *Agapema* pupae. *Biol. Bull.* **109**, 144–163.
- Burton, R. F.** (2006). A new look at the scaling of size in mammalian eyes. *J. Zool.* **269**, 225–232.
- Burton, B. G. and Laughlin, S. B.** (2003). Neural images of pursuit targets in the photoreceptor arrays of male and female houseflies *Musca domestica*. 3963–3977.
- Burton, B. G., Tatler, B. W. and Laughlin, S. B.** (2001). Variations in photoreceptor response dynamics across the fly retina. *J. Neurophysiol.* **86**, 950–60.
- Burton, T., Killen, S. S., Armstrong, J. D. and Metcalfe, N. B.** (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc. R. Soc. London B* **278**, 3465–3473.
- Byrne, D. N., Buchmann, S. L. and Spangler, H. G.** (1998). Relationship between wing loading, wingbeat frequency and body mass in homopterous insects. *J. Exp. Biol.* **135**, 9–23.
- Callier, V. and Nijhout, H. F.** (2013). Body size determination in insects: A review and synthesis of size- and brain-dependent and independent mechanisms. *Biol. Rev.* **88**, 944–954.
- Campione, N. E. and Evans, D. C.** (2012). A universal scaling relationship between body

- mass and proximal limb bone dimensions in quadrupedal terrestrial tetrapods. *BMC Biol.* **10**, 60.
- Careau, V., Thomas, D., Humphries, M. M. and Réale, D.** (2008). Energy metabolism and animal personality. *Oikos* **117**, 641–653.
- Cariveau, D. P., Nayak, G. K., Bartomeus, I., Zientek, J., Ascher, J. S., Gibbs, J. and Winfree, R.** (2016). The allometry of bee proboscis length and its uses in ecology. *PLoS One* **11**, e0151482.
- Cheng, K., Narendra, A. and Wehner, R.** (2006). Behavioral ecology of odometric memories in desert ants: Acquisition, retention, and integration. *Behav. Ecol.* **17**, 227–235.
- Cheverud, J. M.** (1982). Relationships among ontogenetic, static, and evolutionary allometry. *Am. J. Phys. Anthropol.* **59**, 139–149.
- Chown, S. L.** (2002). Respiratory water loss in insects. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **133**, 791–804.
- Chown, S. L. and Gaston, K. J.** (2010). Body size variation in insects: a macroecological perspective. **85**, 139–169.
- Chown, S. L. and Holter, P.** (2000). Discontinuous gas exchange cycles in *Aphodius fossor* (Scarabaeidae): a test of hypotheses concerning origins and mechanisms. *J. Exp. Biol.* **203**, 397–403.
- Chown, S. and Nicolson, S. W.** (2004). *Insect physiological ecology: Mechanisms and patterns*. Oxford University Press.
- Chown, S. L., Gibbs, A. G., Hetz, S. K., Klok, C. J., Lighton, J. R. B. and Marais, E.** (2006). Discontinuous gas exchange in insects: A clarification of hypotheses and approaches. *Physiol. Biochem. Zool.* **79**, 333–43.
- Chown, S. L., Marais, E., Terblanche, J. S., Klok, C. J., Lighton, J. R. B. and Blackburn, T. M.** (2007). Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Funct. Ecol.* **21**, 282–290.
- Christiansen, P.** (1999). Scaling of the limb long bones to body mass in terrestrial mammals. *J. Morphol.* **239**, 167–190.
- Clarke, A. and Fraser, K. P. P.** (2004). Why does metabolism scale with temperature? *Funct. Ecol.* **18**, 243–251.
- Clarke, A. and Johnston, N. M.** (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *J. Anim. Ecol.* **68**, 893–905.
- Clarke, A., Rothery, P. and Isaac, N. J. B.** (2010). Scaling of basal metabolic rate with body mass and temperature in mammals. *J. Anim. Ecol.* **79**, 610–619.
- Clusella-Trullas, S., Terblanche, J. S. and Chown, S. L.** (2010). Phenotypic plasticity of locomotion performance in the seed harvester *Messor capensis* (Formicidae). *Physiol. Biochem. Zool.* **83**, 519–30.
- Cock, A. G.** (1966). Aspects of metrical growth and form in animals. *Q. Rev. Biol.* **41**, 131–190.
- Collett, T. S. and Land, M. F.** (1975). Visual control of flight behaviour in the hoverfly *Syritta pipiens* L. *J. Comp. Physiol. A* **99**, 1–66.
- Collett, M., Collett, T. S. and Wehner, R.** (1999). Calibration of vector navigation in desert ants. *Curr. Biol.* **9**, 1031–4.

- Collingwood, C. A.** (1979). *The Formicidae (Hymenoptera) of Fennoscandia and Denmark*. Klampenborg, Denmark: Scandinavian Science Press.
- Contreras, H. L. and Bradley, T. J.** (2009). Metabolic rate controls respiratory pattern in insects. *J. Exp. Biol.* **212**, 424–428.
- Contreras, H. L. and Bradley, T. J.** (2010). Transitions in insect respiratory patterns are controlled by changes in metabolic rate. *J. Insect Physiol.* **56**, 522–528.
- Contreras, H. L. and Bradley, T. J.** (2011). The effect of ambient humidity and metabolic rate on the gas-exchange pattern of the semi-aquatic insect *Aquarius remigis*. *J. Exp. Biol.* **214**, 1086–91.
- Cuervo, J. J. and Møller, A. P.** (2009). The allometric pattern of sexually size dimorphic feather ornaments and factors affecting allometry. *J. Evol. Biol.* **22**, 1503–1515.
- Currie, D. A., Milner, M. J. and Evans, C. W.** (1988). The growth and differentiation in vitro of leg and wing imaginal disc cells from *Drosophila melanogaster*. *Development* **102**,.
- D’Ettorre, P. and Heinze, J.** (2001). Sociobiology of slave-making ants. *Acta Ethol.* **3**, 67–82.
- Dander, U. and Jander, R.** (1994). Numerical allometric growth of the ommatidia, antennal sensilla, and teeth of foretibial combs in the milkweed bug *Oncopeltus fasciatus* Dallas (Heteroptera: Lygaeidae). *Int. J. Insect Morphol. Embryol.* **23**, 329–344.
- Davison, J.** (1955). Body weight, cell surface, and metabolic rate in anuran Amphibia. *Biol. Bull.* **109**, 407–419.
- Derting, T. L.** (1989). Metabolism and food availability as regulators of production in juvenile cotton rats. *Ecology* **70**, 587–595.
- Dial, K. P., Greene, E. and Irschick, D. J.** (2008). Allometry of behavior. *Trends Ecol. Evol.* **23**, 394–401.
- Dodds, P. S., Rothman, D. H. and Weitz, J. S.** (2001). Re-examination of the “3/4-law” of Metabolism. *J. Theor. Biol.* **209**, 9–27.
- Domisch, T., Finér, L., Neuvonen, S., Niemelä, P., Risch, A. C., Kilpeläinen, J., Ohashi, M. and Jurgensen, M. F.** (2009). Foraging activity and dietary spectrum of wood ants (*Formica rufa* group) and their role in nutrient fluxes in boreal forests. *Ecol. Entomol.* **34**, 369–377.
- Döring, T. F. and Spaethe, J.** (2009). Measurements of eye size and acuity in aphids (Hemiptera: Aphididae). *Entomol. Gen.* **32**, 77–84.
- Dudley, R. and Srygley, R.** (1994). Flight physiology of neotropical butterflies: allometry of airspeeds during natural free flight. *J. Exp. Biol.* **191**, 125–139.
- Duncan, F. D., Krasnov, B. and McMaster, M.** (2002). Metabolic rate and respiratory gas-exchange patterns in tenebrionid beetles from the Negev Highlands, Israel. *J. Exp. Biol.* **205**, 791–8.
- Earle, M. and Lavigne, D. M.** (1990). Intraspecific variation in body size, metabolic rate, and reproduction of deer mice (*Peromyscus maniculatus*). *Can. J. Zool.* **68**, 381–388.
- Eberhard, W. G.** (2009). Static allometry and animal genitalia. *Evolution* **63**, 48–66.
- Eberhard, W. G., Huber, B. A., S., R. L. R., Briceno, R. D., Salas, I. and 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**, 415–431.

- Edwards, H. A. and Miller, P. L.** (1986). Patterns of intermittent ventilation and responses to perfusing gas mixtures in quiescent *Blaberus craniifer*. *Physiol. Entomol.* **11**, 263–272.
- Egelhaaf, A., Altenfeld, H. and Hoffmann, H.-U.** (1988). Evidence for the priming role of the central retinula cell in ommatidium differentiation of *Ephesia kuehniella*. *Dev. Genes Evol.* **197**, 184–189.
- Egset, C. K., Hansen, T. F., LE Rouzic, A., Bolstad, G. H., Rosenqvist, G. and Pélabon, C.** (2012). Artificial selection on allometry: Change in elevation but not slope. *J. Evol. Biol.* **25**, 938–48.
- Ellis, S. and Robinson, E. J. H.** (2014). Polydomy in red wood ants. *Insectes Soc.* **61**, 111–122.
- Ellis, S., Franks, D. W. and Robinson, E. J. H.** (2014). Resource redistribution in polydomous ant nest networks: Local or global? *Behav. Ecol.* **25**, 1183–1191.
- Elton, C.** (1932). Territory among wood ants (*Formica rufa* L.) at Picket Hill. *J. Anim. Ecol.* **1**, 69.
- Emlen, D. J.** (1997). Diet alters male horn allometry in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proc. R. Soc. B* **264**, 567–574.
- Emlen, D. J.** (2008). The evolution of animal weapons. *Annu. Rev. Ecol. Evol. Syst.* **39**, 387–413.
- Emlen, D. J. and Allen, C. E.** (2003). Genotype to phenotype: Physiological control of trait size and scaling in insects. *Integr. Comp. Biol.* **43**, 617–634.
- Emlen, D. J. and Nijhout, H. F.** (2000). The development and evolution of exaggerated morphologies in insects. *Annu. Rev. Entomol.* **45**, 661–708.
- Emlen, D. J., Marangelo, J., Ball, B. and Cunningham, C. W.** (2005). Diversity in the weapons of sexual selection: horn evolution in the beetle genus *Onthophagus* (Coleoptera: Scarabaeidae). *Evolution*. **59**, 1060–1084.
- Emlen, D. J., Warren, I. A., Johns, A., Dworkin, I. and Lavine, L. C.** (2012). A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science*. **337**, 860–864.
- Espadaler, X. and Gómez, C.** (2001). Formicine ants comply with the size-grain hypothesis. *Funct. Ecol.* **15**, 136–138.
- Everett, A., Tong, X., Briscoe, A. D. and Monteiro, A.** (2012). Phenotypic plasticity in opsin expression in a butterfly compound eye complements sex role reversal. *BMC Evol. Biol.* **12**, 232.
- Feener, D. H., Lighton, J. R. B. and Bartholomew, G. A.** (1988). Curvilinear allometry, energetics and foraging ecology: A comparison of leaf-cutting ants and army ants. *Funct. Ecol.* **2**, 509.
- Fernandes, A. S. D., Philippides, A., Collett, T. S. and Niven, J. E.** (2015). Acquisition and expression of memories of distance and direction in navigating wood ants. *J. Exp. Biol.* **218**, 3580–3588.
- Finér, L., Jurgensen, M. F., Domisch, T., Kilpeläinen, J., Neuvonen, S., Punttila, P., Risch, A. C., Ohashi, M. and Niemelä, P.** (2013). The role of wood ants (*rufa* group) in carbon and nutrient dynamics of a boreal norway spruce forest ecosystem. *Ecosystems* **16**, 196–208.
- Fischer, S., Müller, C. H. G. and Meyer-rochow, V. B.** (2011). How small can small be: The compound eye of the parasitoid wasp *Trichogramma evanescens* (Westwood 1833) (Hymenoptera, Hexapoda), an insect of 0.3- to 0.4-mm total body size. *Vis. Neurosci.* **28**, 295–308.

- Ford, N. B. and Seigel, R. A.** (1989). Relationships among body size, clutch size, and egg size in three species of oviparous snakes. *Herpetologica* **45**, 75–83.
- Förster, T. D. and Hetz, S. K.** (2010). Spiracle activity in moth pupae—The role of oxygen and carbon dioxide revisited. *J. Insect Physiol.* **56**, 492–501.
- Fraley, C., Raftery, A. E. and Murphy, T. B.** (2012). mclust version 4 for R: Normal mixture modeling for model-based clustering, classification, and density estimation.
- Franco, F. F., Prado, P. R. R., Sene, F. M., Costa, L. F. and Manfrin, M. H.** (2006). Aedeagus morphology as a discriminant marker in two closely related cactophilic species of *Drosophila* (Diptera; Drosophilidae) in South America. *An. Acad. Bras. Cienc.* **78**, 203–12.
- Frankino, W. A., Zwaan, B. J., Stern, D. L. and Brakefield, P. M.** (2005). Natural selection and developmental constraints in the evolution of allometries. *Science* **307**, 718–20.
- Frankino, W. A., Zwaan, B. J., Stern, D. L. and Brakefield, P. M.** (2007). Internal and external constraints in the evolution of morphological allometries in a butterfly. *Evolution*. 2958–2970.
- Friedrich, M., Rambold, I. and Melzer, R. R.** (1996). The early stages of ommatidial development in the flour beetle *Tribolium castaneum* (Coleoptera; Tenebrionidae). *Dev. Genes Evol.* **206**, 136–146.
- García-Barros, E.** (2000). Body size, egg size, and their interspecific relationships with ecological and life history traits in butterflies (Lepidoptera: Papilionoidea, Hesperioidea). *Biol. J. Linn. Soc.* **70**, 251–284.
- Garland, T.** (1984). Physiological correlates of locomotory performance in a lizard: an allometric approach. *Am. J. Physiol.* **247**, R806–15.
- Gatesy, S. M.** (1991). Hind limb scaling in birds and other theropods: Implications for terrestrial locomotion. *J. Morphol.* **209**, 83–96.
- Gayon, J.** (2000). History of the concept of allometry. *Am. Zool.* **40**, 748–758.
- Gibbs, A. G. and Johnson, R. A.** (2004). The role of discontinuous gas exchange in insects: The chthonic hypothesis does not hold water. *J. Exp. Biol.* **207**, 3477–3482.
- Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. and Charnov, E. L.** (2001). Effects of size and temperature on metabolic rate. *Science*. **293**, 2248–2251.
- Glazier, D. S.** (2005). Beyond the 3/4 variation in the intra- and interspecific scaling of metabolic rate in animals. *Biol. Rev.* **80**, 611–662.
- Glazier, D. S.** (2008). Effects of metabolic level on the body size scaling of metabolic rate in birds and mammals. *Proc. R. Soc. B* **275**, 1405.
- Glazier, D. S.** (2009). Activity affects intraspecific body-size scaling of metabolic rate in ectothermic animals. *J. Comp. Physiol. B* **179**, 821–828.
- Glazier, D. S.** (2010). A unifying explanation for diverse metabolic scaling in animals and plants. *Biol. Rev.* **85**, 111–138.
- Glazier, D. S.** (2015). Is metabolic rate a universal “pacemaker” for biological processes? *Biol. Rev.* **90**, 377–407.
- Glazier, D. S., Hirst, A. G. and Atkinson, D.** (2015). Shape shifting predicts ontogenetic changes in metabolic scaling in diverse aquatic invertebrates. *Proc. R. Soc. B* **282**, 20142302.

- Goldsmith, S. K.** Male diimorphism in *Dendrobias mandibularis* Audinet-Serville (Coleoptera: Cerambycidae). *J. Kansas Entomol. Soc.* **58**, 534–538.
- Goropashnaya, A. V., Fedorov, V. B., Seifert, B. and Pamilo, P.** (2012). Phylogenetic relationships of Palaearctic *Formica* species (Hymenoptera, Formicidae) based on mitochondrial cytochrome B sequences. *PLoS One* **7**, e41697.
- Gorr, T. A., Wichmann, D., Hu, J., Hermes-lima, M., Welker, A. F., Terwilliger, N., Wren, J. F., Viney, M., Morris, S., Nilsson, G. E., et al.** (2010). Hypoxia tolerance in animals: Biology and application. *Physiol. Biochem. Zool. Ecol. Evol. Approaches Physiol. Biochem. Zool.* **83**, 733–752.
- Gould, S. J.** (1966). Allometry and size in ontogeny and phylogeny. *Biol. Rev.* **41**, 587–638.
- Gould, S. J.** (1973). Positive allometry of antlers in the “Irish Elk”, *Megaloceros giganteus*. *Nature* **244**, 375–376.
- Graham, P. and Collett, T. S.** (2002). View-based navigation in insects: How wood ants (*Formica rufa* L.) look at and are guided by extended landmarks. *J. Exp. Biol.* **205**, 2499–2509.
- Graham, P., Fauria, K. and Collett, T. S.** (2003). The influence of beacon-aiming on the routes of wood ants. *J. Exp. Biol.* **206**, 535–541.
- Gray, E. M. and Bradley, T. J.** (2006). Evidence from mosquitoes suggests that cyclic gas exchange and discontinuous gas exchange are two manifestations of a single respiratory pattern. *J. Exp. Biol.* **209**, 1603–11.
- Griebeler, E. M. and Werner, J.** (2016). Mass, phylogeny, and temperature are sufficient to explain differences in metabolic scaling across mammalian orders? *Ecol. Evol.* **6**, 8352–8365.
- Gullan, P. J. and Cranston, P. S.** (2005). *The Insects: An outline of entomology*. 3rd ed. Hoboken, New Jersey, USA: Blackwell Publishing Ltd.
- Hadley, N. F.** (1994). Ventilatory patterns and respiratory transpiration in adult terrestrial insects. *Physiol. Zool.* **67**, 175–189.
- Hammond, K. A. and Diamond, J.** (1997). Maximal sustained energy budgets in humans and animals. *Nature* **386**, 457–462.
- Harris, R. A., Graham, P. and Collett, T. S.** (2007). Visual cues for the retrieval of landmark memories by navigating wood ants. *Curr. Biol.* **17**, 93–102.
- Harrison, J. F.** (2017). Do performance–safety tradeoffs cause hypometric metabolic scaling in animals? *Trends Ecol. Evol.* **32**, 653–664.
- Harrison, J. F., Kaiser, A. and VandenBrooks, J. M.** (2010). Atmospheric oxygen level and the evolution of insect body size. *Proc. R. Soc. B* **277**, 1937–46.
- Hawes, C., Stewart, A. J. A. and Evans, H.** (2002). The impact of wood ants (*Formica rufa*) on the distribution and abundance of ground beetles (Coleoptera: Carabidae) in a Scots pine plantation. *Oecologia* **131**, 612–619.
- Hayssen, V. and Lacy, R. C.** (1985). Basal metabolic rates in mammals: Taxonomic differences in the allometry of BMR and body mass. *Comp. Biochem. Physiol. A* **81**, 741–754.
- Heglund, N. C. and Taylor, C. R.** (1988). Speed, stride frequency and energy cost per stride: How do they change with body size and gait? *J. Exp. Biol.* **138**, 301–318.
- Heglund, N. C., Richard Taylor, C. and McMahon, T. A.** (1974). Scaling stride frequency and gait to animal size: Mice to horses. *Science*. **186**, 1112–1113.

- Hetz, S. K. and Bradley, T. J.** (2005). Insects breathe discontinuously to avoid oxygen toxicity. *Nature* **433**, 516–519.
- Heusner, A. A.** (1982). Energy metabolism and body size. I. Is the 0.75 mass exponent of Kleiber's equation a statistical artifact? *Respir. Physiol.* **48**, 1–12.
- Hirst, A. G., Glazier, D. S. and Atkinson, D.** (2014). Body shape shifting during growth permits tests that distinguish between competing geometric theories of metabolic scaling. *Ecol. Lett.* **17**, 1274–1281.
- Hoback, W. W. and Stanley, D. W.** (2001). Insects in hypoxia. *J. Insect Physiol.* **47**, 533–542.
- Hölldobler, B. and Wilson, E. O.** (1990). *The Ants*. Berlin: Springer.
- Holm, S., Davis, R. B., Javoiš, J., Ōunap, E., Kaasik, A., Molleman, F. and Tammaru, T.** (2016). A comparative perspective on longevity: The effect of body size dominates over ecology in moths. *J. Evol. Biol.* **29**, 2422–2435.
- Homberg, U. and Paech, A.** (2002). Ultrastructure and orientation of ommatidia in the dorsal rim area of the locust compound eye. *Arthropod Struct. Dev.* **30**, 271–280.
- Hoppeler, H. and Weibel, E. R.** (2005). Scaling functions to body size: Theories and facts. *J. Exp. Biol.* **208**, 1573–4.
- Hosken, D. J. and Stockley, P.** (2004). Sexual selection and genital evolution. *Trends Ecol. Evol.* **19**, 87–93.
- Huey, R. B.** (1982a). Temperature, physiology, and the ecology of reptiles. In *Biology of the Reptilia* (eds. C. Gans and F. H. Pough), pp. 25–74. London: Academic Press.
- Huey, R. B.** (1982b). Phylogenetic and ontogenetic determinants of sprint performance in some diurnal Kalahari lizards. *Koedoe* **25**, 43–48.
- Hulbert, A. J., Pamplona, R., Buffenstein, R. and Buttemer, W. A.** (2007). Life and death: Metabolic rate, membrane composition, and life span of animals. *Physiol. Rev.* **87**, 1175–213.
- Hunt, J. and Simmons, L. W.** (2000). Maternal and paternal effects on offspring phenotype in the dung beetle *Onthophagus taurus*. *Evolution*. **54**, 936–41.
- Hunt, J. and Simmons, L. W.** (2001). Status-dependent selection in the dimorphic beetle *Onthophagus taurus*. *Proc. R. Soc. B* **268**, 2409–2414.
- Hurlbert, A. H., Ballantyne, F. and Powell, S.** (2008). Shaking a leg and hot to trot: The effects of body size and temperature on running speed in ants. *Ecol. Entomol.* **33**, 144–154.
- Husson, F., Josse, J. and Pages, J.** (2010). *Agrocampus Principal component methods - hierarchical clustering -partitional clustering: Why would we need to choose for visualizing data?*
- Huxley, J. S.** (1924). The variation in the width of the abdomen in immature fiddler crabs considered in relation to its relative growth-rate. *Am. Nat.* **58**, 468–475.
- Huxley, J. S. and Teissier, G.** (1936). Terminology of relative growth. *Nature* **137**, 780–781.
- Jander, U. and Jander, R.** (2002). Allometry and resolution of bee eyes (Apoidea). *Arthropod Struct. Dev.* **30**, 179–193.
- Jones, J. C. and Oldroyd, B. P.** (2006). Nest thermoregulation in social insects. *Adv. In Insect Phys.* **33**, 153–191.
- Judd, S. P. D. and Collett, T. S.** (1998). Multiple stored views and landmark guidance in ants. *Nature* **392**, 710–714.

- Julian, G. E. and Gronenberg, W.** (2002). Reduction of brain volume correlates with behavioral changes in queen ants. *Brain. Behav. Evol.* **60**, 152–64.
- Kadochová, S. and Frouz, J.** (2013). Thermoregulation strategies in ants in comparison to other social insects, with a focus on red wood ants (*Formica rufa* group). *FI000Research* **2**, 280.
- Käfer, H., Kovac, H., Oswald, B. and Stabentheiner, A.** (2015). Respiration and metabolism of the resting European paper wasp (*Polistes dominulus*). *J Comp Physiol B* **185**, 647–658.
- Kalinkat, G., Jochum, M., Brose, U. and Dell, A. I.** (2015). Body size and the behavioral ecology of insects: linking individuals to ecological communities. *Curr. Opin. Insect Sci.* **9**, 24–30.
- Kapustjanskij, A., Streinzer, M. and Paulus, H. F.** (2007). Bigger is better: Implications of body size for flight ability under different light conditions and the evolution of alloethism in bumblebees. 1130–1136.
- Kawano, K.** (2000). Genera and allometry in the stag beetle family Lucanidae, Coleoptera. *Ann. Entomol. Soc. Am.* **93**, 198–207.
- Keister, M. and Buck, J.** (1964). Respiration: some exogenous and endogenous effects on rate of respiration. In *The Physiology of Insecta* (ed. Rockstein, M.), pp. 617–658. New York: Academic Press.
- Kelber, A., Warrant, E. J., Pfaff, M., Wallén, R., Theobald, J. C., Wcislo, W. T. and Raguso, R. A.** (2006). Light intensity limits foraging activity in nocturnal and crepuscular bees. *Behav. Ecol.* **17**, 63–72.
- Keller, L.** (1991). Queen number, mode of colony founding, and queen reproductive success in ants (Hymenoptera: Formicidae). *Ethol. Ecol. Evol.* **3**, 307–316.
- Killen, S. S., Atkinson, D. and Glazier, D. S.** (2010). The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecol. Lett.* **13**, 184–193.
- Kim, S., Cassidy, J. J., Yang, B., Carthew, R. W. and Hilgenfeldt, S.** (2016). Hexagonal patterning of the insect compound eye: Facet area variation, defects, and disorder. *Biophys. J.* **111**, 2735–2746.
- King, R. B.** (2000). Analyzing the relationship between clutch size and female body size in reptiles. *J. Herpetol.* **34**, 148.
- Kirschfeld, K. and Wenk, P.** (1976). The dorsal compound eye of simuliid flies: An eye specialized for the detection of small, rapidly moving objects. *Z. Naturforsch* **31**, 764–765.
- Kleiber, M.** (1932). Body size and metabolism. *Hilgardia* **6**, 315–353.
- Klok, C. J. and Chown, S. L.** (2005). Temperature-and body mass-related variation in cyclic gas exchange characteristics and metabolic rate of seven weevil species: Broader implications. *J. Insect Physiol.* **51**, 789–801.
- Klok, C. J. and Harrison, J. F.** (2009). Atmospheric hypoxia limits selection for large body size in insects. *PLoS One* **4**, e3876.
- Klotz, J. H., Reid, B. L. and Gordon, W. C.** (1992). Variation of ommatidia number as a function of worker size in *Camponotus pennsylvanicus* (DeGeer) (Hymenoptera: Formicidae). **236**, 233–236.
- Knaden, M. and Graham, P.** (2016). The sensory ecology of ant navigation: From natural environments to neural mechanisms. *Annu. Rev. Entomol* **61**, 63–76.

- Kodric-Brown, A., Sibly, R. M. and Brown, J. H.** (2006). The allometry of ornaments and weapons. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 8733–8.
- Kozłowski, J., Konarzewski, M. and Gawelczyk, A. T.** (2003). Cell size as a link between noncoding DNA and metabolic rate scaling. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 14080–5.
- Kulikov, A. M., Melnikov, A. I., Gornostaev, N. G., Lazebny, O. E. and Mitrofanov, V. G.** (2004). Morphological analysis of male mating organ in the *Drosophila virilis* species group: a multivariate approach. *J. Zool. Syst. Evol. Res.* **42**, 135–144.
- Kunze, P.** (1979). *Comparative Physiology and Evolution of Vision in Invertebrates*. pp. 441–502. Berlin: Springer.
- Labhart, T. and Meyer, E. P.** (1999). Detectors for Polarized Skylight in Insects : A Survey of Ommatidial Specializations in the Dorsal Rim Area of the Compound Eye. **379**, 368–379.
- Labhart, T. and Nilsson, D.-E.** (1995). The dorsal eye of the dragonfly *Sympetrum*: specializations for prey detection against the blue sky. *J. Comp. Physiol. A* **176**, 437–453.
- Land, M. F.** (1997). Visual acuity in insects. *Annu. Rev. Entomol* **42**, 147–77.
- Land, M. F. and Nilsson, D. E.** (2002). *Animal Eyes*. New York: Oxford University Press.
- Lavine, L., Gotoh, H., Brent, C. S., Dworkin, I. and Emlen, D. J.** (2015). Exaggerated trait growth in insects. *Annu. Rev. Entomol.* **60**, 453–472.
- Lê, S., Rennes, A., Josse, J. and Husson, F.** (2008). FactoMineR: An R package for multivariate analysis. *JSS J. Stat. Softw.* **25**, 1–18.
- Lease, H. M. and Wolf, B. O.** (2010). Exoskeletal chitin scales isometrically with body size in terrestrial insects. *J. Morphol.* **271**, 759–768.
- Lighton, J. R. B.** (1989). Individual and whole-colony respiration in an African Formicine ant. *Funct. Ecol.* **3**, 523.
- Lighton, J. R. B.** (1990). Slow discontinuous ventilation in the namib dune-sea ant *Camponotus detritus* (Hymenoptera, Formicidae). *J. Exp. Biol.* **151**, 71–82.
- Lighton, J. R. B.** (1994). Discontinuous ventilation in terrestrial insects. *Physiol. Zool.* **67**, 142–162.
- Lighton, J. R. B.** (1996). Discontinuous gas exchange in insects. *Annu. Rev. Entomol.* **41**, 309–324.
- Lighton, J. R. B.** (1998). Notes from underground: towards ultimate hypotheses of cyclic, discontinuous gas-exchange in tracheate arthropods. *Am. Zool.* **38**, 483–491.
- Lighton, J. R. and Bartholomew, G. A.** (1988). Standard energy metabolism of a desert harvester ant, *Pogonomyrmex rugosus*: Effects of temperature, body mass, group size, and humidity. *Proc. Natl. Acad. Sci. U. S. A.* **85**, 4765–9.
- Lighton, J. R. B. and Berrigan** (1995). Questioning paradigms: Caste-specific ventilation in harvester ants, *Messor pergandei* and *M. julianus* (Hymenoptera: Formicidae). *J. Exp. Biol.* **198**, 521–30.
- Lighton, J. and Garrigan, D.** (1995). Ant breathing: Testing regulation and mechanism hypotheses with hypoxia. *J. Exp. Biol.* **198**, 1613–1620.
- Lighton, J. R. B. and Wehner, R.** (1993). Ventilation and respiratory metabolism in the thermophilic desert ant, *Cataglyphis bicolor* (Hymenoptera, Formicidae). *J. Comp. Physiol. B* **163**, 11–17.

- Lighton, J., Brownell, P., Joos, B. and Turner, R.** (2001). Low metabolic rate in scorpions: Implications for population biomass and cannibalism. *J. Exp. Biol.* **204**, 607–613.
- Lunau, K., Unseld, K. and Wolter, F.** (2009). Visual detection of diminutive floral guides in the bumblebee *Bombus terrestris* and in the honeybee *Apis mellifera*. *J. Comp. Physiol. A* **195**, 1121–1130.
- Macuda, T., Gegear, R. J., Lavery, T. M. and Timney, B.** (2001). Behavioural assessment of visual acuity in bumblebees (*Bombus impatiens*). *J. Exp. Biol.* **204**, 559–64.
- Marais, E., Klok, C. J., Terblanche, J. S. and Chown, S. L.** (2005). Insect gas exchange patterns: A phylogenetic perspective. *J. Exp. Biol.* **208**, 4495–507.
- Matthews, P. G. D. and White, C. R.** (2010). Discontinuous gas exchange in insects: Is it all in their heads? *Am. Nat.* **177**, 130–4.
- Matthews, P. G. D. and White, C. R.** (2013). Reversible brain inactivation induces discontinuous gas exchange in cockroaches. *J. Exp. Biol.* **216**, 2012–6.
- McCullough, E. L., Ledger, K. J., O'Brien, D. M. and Emlen, D. J.** (2015). Variation in the allometry of exaggerated rhinoceros beetle horns. *Anim. Behav.* **109**, 133–140.
- McGuigan, K., Nishimura, N., Currey, M., Hurwit, D. and Cresko, W. A.** (2010). Quantitative genetic variation in static allometry in the threespine stickleback. *Integr. Comp. Biol.* **50**, 1067–80.
- McLellan, W. A., Koopman, H. N., Rommel, S. A., Read, A. J., Potter, C. W., Nicolas, J. R., Westgate, A. J. and Pabst, D. A.** (2002). Ontogenetic allometry and body composition of harbour porpoises (*Phocoena phocoena*, L.) from the western North Atlantic. *J. Zool.* **257**, 457–471.
- McNab, B. K.** (1980). Food habits, energetics, and the population biology of mammals. *Am. Nat.* **116**, 106–124.
- Merry, J. W., Morehouse, N. I., Yturralde, K. and Rutowski, R. L.** (2006). The eyes of a patrolling butterfly: Visual field and eye structure in the Orange Sulphur, *Colias eurytheme* (Lepidoptera, Pieridae). *J. Insect Physiol.* **52**, 240–248.
- Merry, J. W., Kemp, D. J. and Rutowski, R. L.** (2011). Variation in compound eye structure: Effects of diet and family. *Evolution*. **65**, 2098–2110.
- Michener, C. D.** (2001). Comments on minute Meliponini and the male of the genus *Pariotrigona* (Hymenoptera: Apidae). *J. Kansas Entomol. Soc.* **74**, 231–236.
- Mirth, C. K. and Riddiford, L. M.** (2007). Size assessment and growth control: How adult size is determined in insects. *BioEssays* **29**, 344–355.
- Mirth, C. K., Frankino, W. A. and Shingleton, A. W.** (2016). Allometry and size control: What can studies of body size regulation teach us about the evolution of morphological scaling relationships? *Curr. Opin. Insect Sci.* **13**, 93–98.
- Miyatake, T.** (1993). Male-male aggressive behavior is changed by body size difference in the leaf-footed plant bug, *Leptoglossus australis*, Fabricius (Heteroptera: Coreidae). *J. Ethol.* **11**, 63–65.
- Moczek, A. P. and Emlen, D. J.** (2000). Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: Do alternative reproductive tactics favour alternative phenotypes? *Anim. Behav.* **59**, 459–466.
- Moczek, A. P. and Nijhout, H. F.** (2002). Developmental mechanisms of threshold evolution in a polyphenic beetle. *Evol. Dev.* **4**, 252–264.

- Moerbitz, C. and Hetz, S. K.** (2010). Tradeoffs between metabolic rate and spiracular conductance in discontinuous gas exchange of *Samia cynthia* (Lepidoptera, Saturniidae). *J. Insect Physiol.* **56**, 536–542.
- Montagne, J., Stewart, M. J., Stocker, H., Hafen, E., Kozma, S. C. and Thomas, G.** (1999). Drosophila S6 Kinase: A regulator of cell size. *Science*. **285**, 2126–212.
- Morata, G. and Lawrence, P. A.** (1979). Development of the eye-antenna imaginal disc of *Drosophila*. *Dev. Biol.* **70**, 355–71.
- Moreira, G., Silva, D. and Gonçalves, G.** (2017). Comparative morphology of the prothoracic leg in heliconian butterflies: Tracing size allometry, podite fusions and losses in ontogeny and phylogeny. *Arthropod Struct. Dev.* **46**, 462–471.
- Mori, A., Grasso, D. A. and Moli, F. Le** (2000). Raiding and Foraging Behavior of the Blood-Red Ant, *Formica sanguinea* Latr. (Hymenoptera, Formicidae). *J. Insect Behav.* **13**, 421–438.
- Mortola, J. P.** (1993). Hypoxic hypometabolism in mammals. *Physiology* **8**, 79–82.
- Moser, J. C., Reeve, J. D., Bento, J. M. S., Della Lucia, T. M. C., Cameron, R. S. and Heck, N. M.** (2004). Eye size and behaviour of day- and night-flying leafcutting ant alates. *J. Zool.* **264**, 69–75.
- Nicholls, E., Fowler, R., Niven, J. E., Gilbert, J. D. and Goulson, D.** (2017). Larval exposure to field-realistic concentrations of clothianidin has no effect on development rate, over-winter survival or adult metabolic rate in a solitary bee, *Osmia bicornis*. *PeerJ* **5**, e3417.
- Nicholson, D. J., Judd, S. P. D., Cartwright, B. A. and Collett, T. S.** (1999). Learning walks and landmark guidance in wood ants (*Formica rufa*). *J. Exp. Biol.* **202**, 1831–1838.
- Nijhout, H. F. and Callier, V.** (2015). Developmental mechanisms of body size and wing-body scaling in insects. *Annu. Rev. Entomol.* **60**, 141–156.
- Nijhout, H. F. and Grunert, L. W.** (2010). The cellular and physiological mechanism of wing-body scaling in *Manduca sexta*. *Science*. **330**, 1693–1695.
- Nijhout, H. F., Riddiford, L. M., Mirth, C., Shingleton, A. W., Suzuki, Y. and Callier, V.** (2014). The developmental control of size in insects. *Wiley Interdiscip Rev Dev Biol.* **3**, 113–34.
- Nilsson, D.-E.** (1990). From cornea to retinal image in invertebrate eyes. *Trends Neurosci.* **13**, 55–64.
- Niven, J. E. and Laughlin, S. B.** (2008). Energy limitation as a selective pressure on the evolution of sensory systems. *J. Exp. Biol.* **211**, 1792–804.
- Niven, J. E. and Scharlemann, J. P. .** (2005). Do insect metabolic rates at rest and during flight scale with body mass? *Biol. Lett.* **1**, 346–349.
- Niven, J. E., Graham, C. M. and Burrows, M.** (2008). Diversity and evolution of the insect ventral nerve cord. *Annu. Rev. Entomol.* **53**, 253–271.
- Norberg, U. M. L. and Norberg, R. Å.** (2012). Scaling of wingbeat frequency with body mass in bats and limits to maximum bat size. *J. Exp. Biol.* **215**, 711–22.
- Nudds, R. L.** (2007). Wing-bone length allometry in birds. *J. Avian Biol.* **38**, 515–519.
- Oliver, G. and Gruss, P.** (1997). Current views on eye development. *Trends Neurosci.* **20**, 415–21.
- Orr, W. C. and Sohal, R. S.** (1994). Extension of life-span by overexpression of superoxide

- dismutase and catalase in *Drosophila melanogaster*. *Science*. **263**, 1128–30.
- Pélabon, C., Bolstad, G. H., Egset, C. K., Cheverud, J. M., Pavlicev, M. and Rosenqvist, G.** (2013). On the relationship between ontogenetic and static allometry. *Am. Nat.* **181**, 195–212.
- Pélabon, C., Firmat, C., Bolstad, G. H., Voje, K. L., Houle, D., Cassara, J., Rouzic, A. Le and Hansen, T. F.** (2014). Evolution of morphological allometry. *Ann. N. Y. Acad. Sci.* **1320**, 58–75.
- Perl, C. D. and Niven, J. E.** (2016a). Differential scaling within an insect compound eye. *Biol. Lett.* **12**, 20160042.
- Perl, C. D. and Niven, J. E.** (2016b). Colony-level differences in the scaling rules governing wood ant compound eye structure. *Sci. Rep.* **6**, 24204.
- Petrie, M.** (1992). Are all secondary sexual display structures positively allometric and, if so, why? *Anim. Behav.* **43**, 173–175.
- Pinheiro, J., Bates, D., DebRob, S., Sarkar, D. and R-core** (2016). Linear and nonlinear mixed effects models [R package nlme version 3.1-128].
- Pontzer, H.** (2007). Effective limb length and the scaling of locomotor cost in terrestrial animals. *J. Exp. Biol.* **210**, 1752–61.
- Prange, H. D., Anderson, J. F. and Rahn, H.** (1979). Scaling of skeletal mass to body mass in birds and mammals. *Am. Nat.* **113**, 103–122.
- Quinlan, M. C. and Gibbs, A. G.** (2006). Discontinuous gas exchange in insects. *Respir. Physiol. Neurobiol.* **154**, 18–29.
- Quinlan, M. C. and Hadley, N. F.** (1993). Gas exchange, ventilatory patterns, and water loss in two lubber grasshoppers: Quantifying cuticular and respiratory transpiration. *Physiol. Zool.* **66**, 628–642.
- Quinlan, M. C. and Lighton, J. R. B.** (1999). Respiratory physiology and water relations of three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). *Physiol. Entomol.* **24**, 293–302.
- R Core Team** (2016). R: A Language and Environment for Statistical Computing.
- Rubner, M.** (1883) Über die Einfluss der Körpergrösse auf Stoff und Kraftwechsel. *Z. Biol.*, **19**, 535–562.
- Ready, D. F., Hanson, S. E. and Benzer, T.** (1976). Development of the *Drosophila* retina, a neurocrystalline lattice. *Dev. Biol.* **53**, 217–240.
- Reinhold, K.** (1999). Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Funct. Ecol.* **13**, 217–224.
- Ribi, W. A., Engels, E. and Engels, W.** (1989). Sex and caste specific eye structures in stingless bees and honey bees (Hymenoptera: Trigonidae, Apidae). *Entomol. Gen.* **14**, 233–242.
- Riveros, A. J. and Gronenberg, W.** (2010). Sensory allometry, foraging task specialization and resource exploitation in honeybees. *Behav. Ecol. Sociobiol.* **64**, 955–966.
- Rosenberg, M. S.** (2002). Fiddler crab claw shape variation: A geometric morphometric analysis across the genus *Uca* (Crustacea: Brachyura: Ocypodidae). *Biol. J. Linn. Soc.* **75**, 147–162.

- Rosengren, R.** (1977). Foraging strategy of wood ants (*Formica rufa* group). I. Age polyethism and topographic traditions. *Acta Zool. Fenn.* **149**, 1–30.
- Rosengren, R. and Fortelius, W.** (1986). Ortstreue in foraging ants of the *Formica rufa* group — Hierarchy of orienting cues and long-term memory. *Insectes Soc.* **33**, 306–337.
- Rosengren, R., Fortelius, W., Lindström, K. and Luther, A.** (1987). Phenology and causation of nest heating and thermoregulation in red wood ants of the *Formica rufa* group studied in coniferous forest habitats in southern Finland. *Ann. Zool. Fennici* **24**, 147–155.
- Rutowski, R. L.** (2001). Variation of eye size in butterflies: Inter- and intraspecific patterns. *J. Zool.* **252**, 187–195.
- Rutowski, R. L., Gislén, L. and Warrant, E. J.** (2009). Visual acuity and sensitivity increase allometrically with body size in butterflies. *Arthropod Struct. Dev.* **38**, 91–100.
- Scales, J. A. and Butler, M. A.** (2016). The relationship between microhabitat use, allometry and functional variation in the eyes of Hawaiian *Megalagrion* damselflies. *Funct. Ecol.* **30**, 356–368.
- Schilman, P. E., Lighton, J. R. B. and Holway, D. A.** (2005). Respiratory and cuticular water loss in insects with continuous gas exchange: Comparison across five ant species. *J. Insect Physiol.* **51**, 1295–1305.
- Schimpf, N. G., Matthews, P. G. D., Wilson, R. S. and White, C. R.** (2009). Cockroaches breathe discontinuously to reduce respiratory water loss. *J. Exp. Biol.* **212**, 2773–2780.
- Schimpf, N. G., Matthews, P. G. D. and White, C. R.** (2012). Standard metabolic rate is associated with gestation duration, but not clutch size, in speckled cockroaches *Nauphoeta cinerea*. *Biol. Open* **1**, 1185–1191.
- Schneider, C. A., Rasband, W. S. and Eliceiri, K. W.** (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671–675.
- Schwarz, S., Narendra, A. and Zeil, J.** (2011). Arthropod Structure & Development The properties of the visual system in the Australian desert ant *Melophorus bagoti*. *Arthropod Struct. Dev.* **40**, 128–134.
- Sears, K. E., Kerkhoff, A. J., Messerman, A. and Itagaki, H.** (2012). Ontogenetic scaling of metabolism, growth, and assimilation: testing metabolic scaling theory with *Manduca sexta* larvae. *Physiol. Biochem. Zool.* **85**, 159–173.
- Shingleton, A. W., Das, J., Vinicius, L. and Stern, D. L.** (2005). The temporal requirements for insulin signaling during development in *Drosophila*. *PLoS Biol.* **3**, e289.
- Shingleton, A. W., Frankino, W. A., Flatt, T., Nijhout, H. F. and Emlen, D. J.** (2007). Size and shape : the developmental regulation of static allometry in insects. 536–548.
- Shingleton, A. W., Mirth, C. K. and Bates, P. W.** (2008). Developmental model of static allometry in holometabolous insects. *Proc. R. Soc. B Biol. Sci.* **275**, 1875–1885.
- Shingleton, A. W., Estep, C. M., Driscoll, M. V and Dworkin, I.** (2009). Many ways to be small: Different environmental regulators of size generate distinct scaling relationships in *Drosophila melanogaster*. *Proc. Biol. Sci.* **276**, 2625–33.
- Simmons, L. W. and Tomkins, J. L.** (1996). Sexual selection and the allometry of earwig forceps. *Evol. Ecol.* **10**, 97–104.
- Simmons, L., Beveridge, M. and Krauss, S.** (2004). Genetic analysis of parentage within experimental populations of a male dimorphic beetle, *Onthophagus taurus*, using amplified fragment length polymorphism. *Behav Ecol Sociobiol* **57**, 164–173.

- Skinner, G. J.** (1980). The feeding habits of the wood-ant, *Formica rufa* (Hymenoptera: Formicidae), in limestone woodland in north-west England. *J. Anim. Ecol.* **49**, 417–433.
- Skinner, G. J. and Whittaker, J. B.** (1981). An experimental investigation of inter-relationships between the wood-ant (*Formica rufa*) and some tree-canopy herbivores. *J. Anim. Ecol.* **50**, 313–326.
- Snelling, E. P., Seymour, R. S., Matthews, P. G. D., Runciman, S. and White, C. R.** (2011). Scaling of resting and maximum hopping metabolic rate throughout the life cycle of the locust *Locusta migratoria*. *J. Exp. Biol.* **214**, 3218–24.
- Sommerer, S. and Wehner, R.** (2012). Leg allometry in ants: Extreme long-leggedness in thermophilic species. *Arthropod Struct. Dev.* **41**, 71–77.
- Spaethe, J. and Chittka, L.** (2003). Interindividual variation of eye optics and single object resolution in bumblebees. *J. Exp. Biol.* **206**, 3447–3453.
- Spaethe, J., Brockmann, A., Halbig, C. and Tautz, J.** (2007). Size determines antennal sensitivity and behavioral threshold to odors in bumblebee workers. *Naturwissenschaften* **94**, 733–739.
- Speakman, J. R.** (2005). Body size, energy metabolism and lifespan. *J. Exp. Biol.* **208**, 1717–1730.
- Srinivasan, M. V.** (2010). Honey bees as a model for vision, perception, and cognition. *Annu. Rev. Entomol.* **55**, 267–284.
- Stang, M., Klinkhamer, P. G. L., Waser, N. M., Stang, I. and van der Meijden, E.** (2009). Size-specific interaction patterns and size matching in a plant-pollinator interaction web. *Ann. Bot.* **103**, 1459–69.
- Stevenson, R. D., Hill, M. F. and Bryant, P. J.** (1995). Organ and cell allometry in Hawaiian *Drosophila*: How to make a big fly. *Proc. R. Soc. B* **259**, 105–10.
- Stillwell, R. C., Shingleton, A. W., Dworkin, I. and Frankino, W. A.** (2016). Tipping the scales: Evolution of the allometric slope independent of average trait size. *Evolution* **70**, 433–44.
- Streinzer, M., Brockmann, A., Nagaraja, N. and Spaethe, J.** (2013). Sex and caste-specific variation in compound eye morphology of five honeybee species. *PLoS One* **8**, e57702.
- Streinzer, M., Huber, W. and Spaethe, J.** (2016). Body size limits dim-light foraging activity in stingless bees (Apidae: Meliponini). *J. Comp. Physiol. A* **202**, 643–655.
- Sturm, R.** (2016). Relationship between body size and reproductive capacity in females of the black field cricket (Orthoptera, Gryllidae). *Linzer Biol. Beiträge* **48**, 1823–1834.
- Sundström, L.** (1993). Genetic population structure and sociogenetic organisation in *Formica truncorum* (Hymenoptera; Formicidae). *Behav. Ecol. Sociobiol.* **33**, 345–354.
- Sundström, L., Seppä, P. and Pamilo, P.** (2005). Genetic population structure and dispersal patterns in *Formica* ants — a review. *Ann. Zool. Fennici* **42**, 163–177.
- Tammaru, T. and Esperk, T.** (2007). Growth allometry of immature insects: Larvae do not grow exponentially. *Funct. Ecol.* **21**, 1099–1105.
- Tavares, W. C., Pessôa, L. M. and Seuánez, H. N.** (2016). Phylogenetic and size constraints on cranial ontogenetic allometry of spiny rats (Echimyidae, Rodentia). *J. Evol. Biol.* **29**, 1752–1765.

- Terblanche, J. S., Janion, C. and Chown, S. L.** (2007). Variation in scorpion metabolic rate and rate-temperature relationships: Implications for the fundamental equation of the metabolic theory of ecology. *J. Evol. Biol.* **20**, 1602–1612.
- Terblanche, J. S., White, C. R., Blackburn, T. M., Marais, E. and Chown, S. L.** (2008). Scaling of gas exchange cycle frequency in insects. *Biol. Lett.* **4**, 127–9.
- Therneau, T. M.** (2015). A Package for Survival Analysis in S. version 2.38.
- Thomas, R. H.** (1993). Ecology of body size in *Drosophila buzzatii*: untangling the effects of temperature and nutrition. *Ecol. Entomol.* **18**, 84–90.
- Thompson, D.** (1992). *On Growth and Form*. (J. Bonner, Ed.). Cambridge: Cambridge University Press.
- Tobler, A. and Nijhout, H. F.** (2010). Developmental constraints on the evolution of wing-body allometry in *Manduca sexta*. *Evol. Dev.* **12**, 592–600.
- Toju, H. and Sota, T.** (2006). Adaptive divergence of scaling relationships mediates the arms race between a weevil and its host plant. *Biol. Lett.* **2**, 539–42.
- Tokeshi, M., Ota, N. and Kawai T.** (2000). A comparative study of morphometry in shell-bearing molluscs. *J. Zool.* **251**, 31–38.
- Tomkins, J. L.** (1999). Environmental and genetic determinants of the male forceps length dimorphism in the European earwig *Forficula auricularia* L. *Behav. Ecol. Sociobiol.* **47**, 1–8.
- Tribe, M. A. and Bowler, K.** (1968). Temperature dependence of “standard metabolic rate” in a poikilotherm. *Comp. Biochem. Physiol.* **25**, 427–436.
- Vogt, J. T. and Appel, A. G.** (1999). Standard metabolic rate of the fire ant, *Solenopsis invicta* Buren: effects of temperature, mass, and caste. *J. Insect Physiol.* **45**, 655–666.
- Voje, K. L.** (2016). Scaling of morphological characters across trait type, sex, and environment. *Am. Nat.* **187**, 89–98.
- Voje, K. L., Hansen, T. F., Egset, C. K., Bolstad, G. H. and Pélabon, C.** (2014). Allometric constraints and the evolution of allometry. *Evolution*. **68**, 866–885.
- Wallis, D. I.** (1964). The foraging behaviour of the ant, *Formica fusca*. *Behaviour* **23**, 149–176.
- Wang, Y., Campbell, J. B., Kaftanoglu, O., Page, R. E., Amdam, G. V and Harrison, J. F.** (2016). Larval starvation improves metabolic response to adult starvation in honey bees (*Apis mellifera* L.). *J. Exp. Biol.* **219**, 960–968.
- Wang, J., Zamar, R., Rocke, D., Martin, D. and Maechler, M.** (2017). Package “robust.”
- Warnes, G. R., Bolker, B., Lumley, T. T. and Johnson, R. C.** (2015). Various R programming tools for model fitting.
- Warrant, E. J.** (2008). Seeing in the dark: Vision and visual behaviour in nocturnal bees and wasps. *J. Exp. Biol.* **211**, 1737–46.
- Warrant, E. J. and McIntyre, P. D.** (1993). Arthropod eye design and the physical limits to spatial resolving power. *Prog. Neurobiol.* **40**, 413–61.
- Warton, D. I., Wright, I. J., Falster, D. S. and Westoby, M.** (2006). Bivariate line-fitting methods for allometry. *Biol. Rev. Camb. Philos. Soc.* **81**, 259–91.
- Wcislo, W. T. and Eberhard, W. G.** (1989). Club Fights in the Weevil *Macromerus bicinctus* (Coleoptera: Curculionidae). *J. Kansas Entomol. Soc.* **62**, 421–429.

- Weber, K. E.** (1990). Selection on wing allometry in *Drosophila melanogaster*. *Genetics* **126**, 975–89.
- Weibel, E. R. and Hoppeler, H.** (2005). Exercise-induced maximal metabolic rate scales with muscle aerobic capacity. *J. Exp. Biol.* **208**, 1635–44.
- West, G. B., Brown, J. H. and Enquist, B. J.** (1997). A general model for the origin of allometric scaling laws in biology. *Science*. **276**, 122–126.
- West, G. B., Brown, J. H. and Enquist, B. J.** (1999). The fourth dimension of life: fractal geometry and allometric scaling of organisms. *Science*. **284**, 1677–1679.
- White, C. R., Seymour, R. S. and Thompson, M. B.** (2005). Allometric scaling of mammalian metabolism. *J. Exp. Biol.* **208**, 1611–1619.
- White, C. R., Phillips, N. F. and Seymour, R. S.** (2006). The scaling and temperature dependence of vertebrate metabolism. *Biol. Lett.* **2**, 125–127.
- White, C. R., Blackburn, T. M., Terblanche, J. S., Marais, E., Gibernau, M., Chown, S. L. and Bradley, T. J.** (2007a). Evolutionary responses of discontinuous gas exchange in insects. *Proc. Natl. Acad. Sci.* **104**, 8357–8361.
- White, C. R., Blackburn, T. M., Martin, G. R. and Butler, P. J.** (2007b). Basal metabolic rate of birds is associated with habitat temperature and precipitation, not primary productivity. *Proceedings R. Soc. B* **274**, 287–93.
- Whitford, W. G.** (1973). The effects of temperature on respiration in the Amphibia. *Am. Zool.* **13**, 505–512.
- Whitman, D. W.** (2008). The significance of body size in the Orthoptera: A review. *J. Orthoptera Res.* **17**, 117–134.
- Whittemore, S. B., Hoglin, B., Green, M. A. and Medler, S.** (2015). Stride frequency in relation to allometric growth in ghost crabs. *J. Zool.* **296**, 286–294.
- Wilson, R. S., Franklin, C. E. and James, R. S.** (2000). Allometric scaling relationships of jumping performance in the striped marsh frog *Limnodynastes peronii*. *J. Exp. Biol.* **203**, 1937–1946.
- Winter, Y.** (1999). Flight speed and body mass of nectar-feeding bats (Glossophaginae) during foraging. *J. Exp. Biol.* **202**, 1917–1930.
- Wolff, T. and Ready, D. F.** (1991). Cell death in normal and rough eye mutants of *Drosophila*. **839**, 825–839.
- Woodland, D. J., Hall, B. K. and Calder, J.** (1968). Gross bioenergetics of *Blattella germanica*. *Physiol. Zool.* **41**, 424–431.
- Wright, P. J., Bonser, R. and Chukwu, U. O.** (2000). The size-distance relationship in the wood ant *Formica rufa*. *Ecol. Entomol.* **25**, 226–233.
- Wu, Q. and Brown, M. R.** (2006). Signaling and function of insulin-like peptides in insects. *Annu. Rev. Entomol.* **51**, 1–24.
- Yang, P. J., Pham, J., Choo, J. and Hu, D. L.** (2014). Duration of urination does not change with body size. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 11932–7.
- Zagorski, E. R. and Merry, J. W.** (2014). How do eye size and facet lens size vary by age and sex in *Acheta domesticus*? *Bios* **85**, 151–159.
- Zeil, J.** (1983). Sexual dimorphism in the visual system of flies : The compound eyes and neural

superposition in Bibionidae (Diptera). *J. Comp. Physiol.* **150**, 379–393.

Ziemba, K. S. and Rutowski, R. L. (2000). Sexual dimorphism in eye morphology in a butterfly (*Asterocampa leilia*; Lepidoptera, Nymphalidae). *Psyche (Stuttg)*. **103**, 25–36.

Zollikofer, C. P. E., Wehner, R., Fukushi, T., Institut, Z., Zürich-irchel, U. and Zürich, C.- (1995). Optical scaling in conspecific *Cataglyphis* ants. **1646**, 1637–1646.