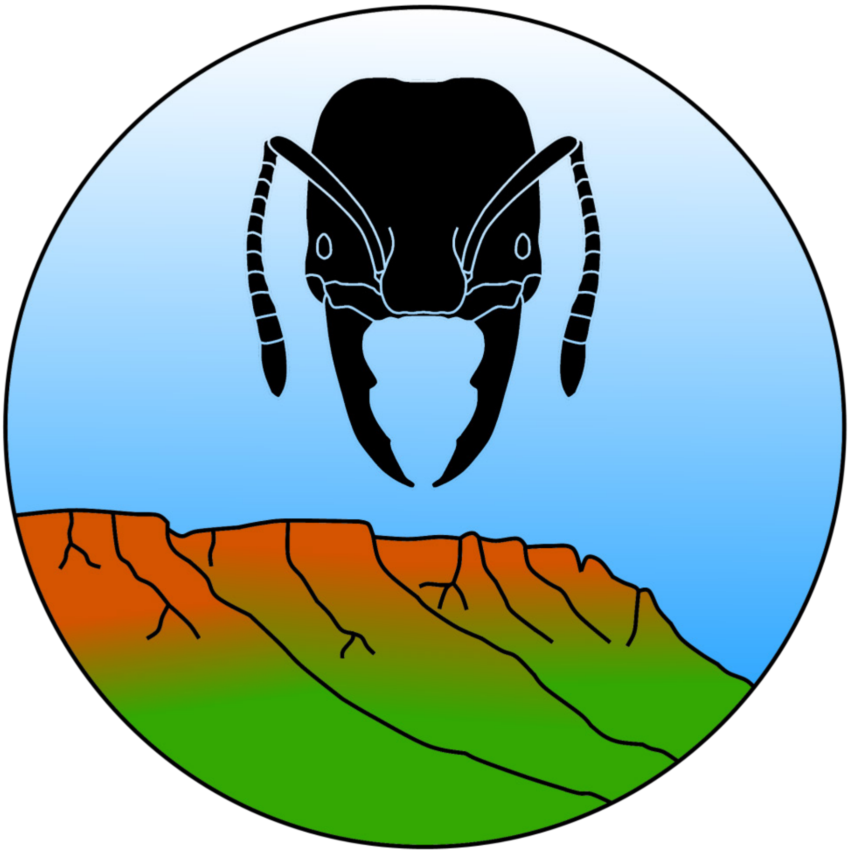
Taxonomic and functional ecology of montane ants



Tom Rhys Bishop

University of Liverpool

March 2016

[Intentionally left blank]

Taxonomic and functional ecology of montane ants

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Tom Rhys Bishop

Liverpool, UK

March 2016

Supervisors

Dr Kate Parr (University of Liverpool)

Professor Mark Robertson (University of Pretoria)

Dr Berndt Janse van Rensburg (University of Queensland)

Professor Rob Marrs (University of Liverpool)

[Intentionally left blank]

Declaration

I hereby declare that this work has been originally produced by myself for this thesis and it has not been submitted for the award of a higher degree to any other institution. Inputs from co-authors are acknowledged throughout.

T. Bishop

Tom Rhys Bishop, Liverpool, March 2016

Acknowledgements

First and foremost, I am super grateful to my supervisors Kate Parr, Mark Robertson, Berndt Janse van Rensburg and Rob Marrs.

Kate has been the most supportive primary supervisor that I could have asked for. She has never failed to offer advice, criticism, encouragement and a good laugh whenever needed - and I've needed them all in spades. I have developed hugely under Kate's guidance and for that I will always be grateful. She has inspired and challenged me throughout this PhD and I'm sure that she will keep doing so for years to come. Thanks for everything KP, you're a legend.

I am thankful to Mark for all of his help and support. His never-ending enthusiasm for mountains, ecology, a cold beer (though perhaps just whisky now, hey?) and living life to the max is infectious and he made my visits to South Africa really productive and fun. He has always been on hand to give me a dose of *MTFUP* - nice one champ.

I thank Berndt for the time and thought he has given me. His combination of fine grain natural history knowledge and macroecological know-how is something I greatly admire and I hope some of it has rubbed off. Rob has been a fantastic source of advice and knowledge on all things academia, publishing and general life strategy.

A huge thank you to Chantal Ferreira. She is so amazingly organised and on-point and without her this whole endeavour wouldn't have been possible. She is never short on some sharp words of advice or Afrikaans lessons. *Dankie , Chantal , vir die bekendstelling van my om die kat met die klokkie.*

Thank you Rudi, Jamie and Gabriel of Team Rutland for all the times we drank whisky, went road-trippin', watched too much weird anime, danced to John Langan Band, cycled around in a gang, went to Bold St Coffee, spoke in tongues, played guitar and generally behaved as irresponsibly as we liked. You've made the last 3½ years a barrel of fun and I'm looking forward to our next Adventure Time. A big shout out to all the major players in my life from Cardiff, Oxford, Liverpool, Silwood and Pretoria. I'd also like to extend my thanks to Vicky Warwick-Evans for being my PhD sister and sharing this journey - it's been fun, sugar baby, well done!

Of course, I owe everything to the support of my family. Mum, Dad and Jess, you have always been on the end of the phone to hear a rant. Thanks for always being there, believing in me and pushing me to do what I loved. Also, thanks a lot for helping out with the lasers and the wheels, both have radically improved my life.

Finally, a word to Ashley Bray: come back with my f*****' skinny latte, you mug.

Abstract

Why is biological diversity distributed in the way that it is? This question has been central to ecology for centuries and is of great importance for pure and applied reasons. Recently, the field of functional trait ecology promises to provide more detailed and general answers to this question be addressing the characteristics of organisms that may actually control their distributions. This is in contrast to the traditional view which described biodiversity simply as a collection of spatially and temporally explicit species lists. This thesis takes a functional trait view of ecology to better understand the distribution of ant (Hymenoptera: Formicidae) diversity across mountain ecosystems. Mountains are useful ecological tools as they compact a large amount of environmental variation into a small space. Ants are a dominant and functionally critical terrestrial animal group. Patterns and drivers of alpha and beta diversity are analysed as well as the distribution of a number of important ant traits. The findings suggest that temperature and the thermoregulatory abilities of individual ant species are critical in determining their diversity patterns.

Contents

List of Figures

List of Tables

Chapter 1

General introduction

Images: Tom Rhys Bishop and Mark Robertson

1.1 THE DISTRIBUTION OF BIODIVERSITY

A central question in ecology is to ask what processes underlie the distribution of biodiversity:

Why do species occur in the places and numbers that they do?

The diversity of life on Earth is vast and not distributed evenly. Not all species are found living together at the same time and in the same place. There are various reasons for this. At the broadest of spatial scales the arrangement of the continents and oceans prevents many species from co-occurring together. Polar bears are unlikely to meet penguins, for example. Similarly, *Azteca*, some of the dominant tree dwelling ants of the Americas, are completely unware of the existence of *Oecophylla*, their ecological equivalents, inhabiting the forests of Africa, Asia and Australia (Hölldobler & Wilson, 1990).

At smaller spatial scales many species' ranges overlap. In these cases it is differences in a range of abiotic and biotic factors that determine which species, and how many of them, can be found at a particular point in space and time. In the United Kingdom, the large blue butterfly, *Maculinea arion,* can only exist where its primary host species, the ant *Myrmica sabuleti*, is also present. Thus, it is limited by its biotic parastii association with another species. In turn, *M. sabuleti* is restricted to grasslands with turf heights that offer favourable thermal conditions for nesting (Thomas *et al.*, 2009). The ant is restricted by abiotic conditions.

Describing the patterns of diversity distribution across the globe and understanding how biotic and abiotic factors combine to cause them falls under the remit of biogeography, macroecology and their related fields. Biogeography concerns itself with describing and elucidating the historical underpinnings of modern distributions (Darwin, 1859; Brown & Lomolino, 1998). Macroecology is a younger field whose aim has been to find general principles that determine the statistical patterns of abundance, distribution and diversity (Brown, 1995; Keith *et al.*, 2012).

The goals of biogeography and macroecology are important for two reasons. First, distributions are a fundamental component of organismal biology and are inherently interesting. Geographic distributions simultaneously tell us about the evolutionary past, ecological present and potential future of species and ecological communities. Second, the rapid rate of global change due to climate warming and habitat loss is placing an unprecedented pressure on the global ecosystem (Cardinale *et al.*, 2012). In order to manage and protect species, and the crucial ecosystem functions that they provide, we need to understand their current distributions, how these stack to generate patterns of diversity and learn how they will change in the face of anthropogenic threats. To this end, biogeography and macroecology have an important role to play in documenting patterns and uncovering general processes that maintain biodiversity.

In short, understanding the causes of species distributions and patterns of biological diversity unites theoretical and applied research programs in ecology, evolutionary biology and environmental science; and is of great importance, not only for our fundamental understanding of the biosphere, but also for the ways in which we may be able to secure it for future generations.

This thesis uses the principles of biogeography and macroecology to describe and understand the taxonomic and functional diversity of ants (Hymenoptera: Formicidae) using elevational gradients as a model.

1.2 FUNCTIONAL TRAITS

Recently, it has become clear that in order to understand the distribution of biodiversity we need to investigate the functional traits of organisms as well as describing their taxonomic identities (McGill *et al.*, 2006; Violle *et al.*, 2014). Functional traits are measurable aspects of an individual organism's morphology, physiology or behaviour that relate to its performance in a given environment. Species differ in their abilities to cope with biotic and abiotic stressors and this non-equivalence is captured by, and is central to, the functional trait approach. It is the traits and evolutionary history of a species, not its taxonomic name, which cause it to be present in a given location and at a particular abundance (McGill *et al.*, 2006).

Functional trait ecology complements the traditional, taxonomic view in a number of ways. First, it offers generality. Species and communities in completely different places can be compared using a common currency. A purely taxonomic approach categorises species *X* and species *Y* as different. A functional trait approach can either reveal the extent of this difference or, conversely, can show that these different species are in fact functionally equivalent as they have near identical trait values. This allows easier comparison between studies and facilitates the discovery of general rules that may be constraining organisms and diversity (McGill *et al.*, 2006; Violle *et al.*, 2014).

Second, functional traits may go some way to overcoming the "Linnaean shortfall" that still exists for many taxa across the globe (Brown & Lomolino, 1998; Whittaker *et al.*, 2005). Species in many taxonomic groups are still not formally described, a problem which is particularly acute for invertebrates (Cardoso *et al.*, 2011). These species can still be sampled from natural habitats, however, and their functional traits can still be quantified. Consequently, an understanding of the processes driving their distributions can be gained without waiting on crucial, but often time consuming and sometimes non-existent, taxonomic reviews.

Third, functional traits allow us to mechanistically understand patterns of biodiversity. Abiotic and biotic factors that may restrict the distributions of species tend to operate on the functional traits of that species, not on its identity *per se* (Weiher & Keddy, 2001; Fukami *et al.*, 2005). Revealing the mechanistic underpinnings of observed patterns is highly attractive and can provide us with a much greater insight into why biodiversity is where it is, and how we should manage it.

Indeed, the linking together of traditional biogeographical and macroecological approaches with functional trait information has been heralded as the way forward for the study of species and diversity distributions (Violle *et al.*, 2014). This work in thesis sits firmly within the fields of macrophysiology (Chown *et al.*, 2004; Chown & Gaston, 2015) and functional biogeography (Violle *et al.*, 2014) by describing patterns of functional trait distribution, and using them to try and explain overall patterns of diversity.

1.3 ECOLOGICAL VS. EVOLUTIONARY FORCES

The factors driving the distribution and organisation of biodiversity may differ when looking across a range of spatial and temporal scales. For example, at large spatial grains and over time scales of thousands of years it is evolutionary forces that are likely to be the most important in dictating diversity patterns. At these scales, it is ultimately differences in the rates of speciation and extinction that are shaping the distribution of biodiversity. Two key evolutionary ideas that have been applied to explain diversity gradients are the time and area hypothesis and the diversification rate hypothesis (Mittelbach *et al.*, 2007). The time and area hypothesis suggests that tropical regions are older and larger, and have consequently had more diversification occurring within them. The diversification rate hypothesis, on the other hand, posits that the rate of speciation itself differs between tropical and temperate regions. Under this idea, more speciation occurs in the tropics than in the temperate zone.

At smaller spatial grains and shorter time scales of ten to a hundred years, on the other hand, it is expected that ecological forces will dominate. It is unlikely that speciation, extinction, or changes in their rates will occur over such small spatio-temporal scales. Instead, it is the ecological interactions that occur between species, their environment and each other that should be determining the composition of local assemblages (Graham *et al.*, 2014).

The work in this thesis is focussed on addressing these smaller spatial and temporal scales. Consequently, the work is centred on local communities and the ecological interactions that occur between species and their environment. The evolutionary question of how these species pools of ants were generated in Africa, Australia and South America over longer time periods is beyond the scope of this thesis.

1.4 ANTS

The work presented here investigates the distribution of taxonomic and functional diversity of ants (Hymenoptera: Formicidae). This is for three key reasons. They are interesting, globally important and have a strong foundation of taxonomic and methodological literature from which to work.

The ants represent a single family of insects (Formicidae) within the order Hymenoptera (the bees, wasps and ants). Ants are abundant on all continents except Antarctica (Fisher, 2010) and there are more than 25,000 species of them worldwide from 21 extant subfamilies (Ward, 2010). The biomass of living ants has been estimated to rival, or perhaps exceed, that of humans (Hölldobler & Wilson, 1990).

Ants are social insects, living in colonies, and have evolved a diversity of functional traits and life strategies. Some are hunter-gatherers whilst others farm fungi. A few species construct huge, permanent colonies while some are nomadic (Hölldobler & Wilson, 1990). They are also involved in a wide range of ecosystem functions. They aerate and enrich soil (Evans et al., 2011), promote decomposition (McGlynn & Poirson, 2012), disperse seeds (Lengyel et al., 2010; Stuble et al., 2014) and form an important link in interaction networks and food chains (Wardle et al., 2011; Zelikova et al., 2011). Given their ubiquity, abundance and importance in maintaining functioning ecosystems investigators have studied their ecology for more than a hundred years. Only recently, however, has the functional trait ecology of ants begun to be explored (Weiser & Kaspari, 2006; Wiescher et al., 2012; Gibb & Parr, 2013; Gibb et al., 2014).

In addition to their charisma, abundance and importance for terrestrial ecosystems, ants are taxonomically and phylogenetically well understood (Ward, 2010), and can be sampled using reliable methods which makes them ideal for biodiversity studies (Ellison *et al.*, 2011).

1.5 ELEVATIONAL GRADIENTS

One of the hardest aspects of studying patterns of biodiversity is accessing a large enough range of environments and conditions on a limited budget and whilst also controlling for a range of confounding factors.

In light of this, elevational gradients provide useful natural experiments (Körner, 2007; Sundqvist *et al.*, 2013). First, they condense a large amount of environmental variation into a small geographical space (Körner, 2007). This allows us to more easily test patterns and processes that occur on larger scales (Sanders *et al.*, 2007; Sundqvist *et al.*, 2013). Second, elevational gradients do not encounter the same confounding factors associated with geographic distance that larger gradients may suffer from. Across latitude, for example, species pools can turnover, geology can change and seasonality will markedly vary. All of these factors may influence biodiversity patterns, making inference of underlying processes more difficult. Along elevational gradients, which are geographically shorter, such issues are less of a problem and are more easily recognised. Finally, elevational gradients are of interest in their own right. Mountain regions are regarded as global hotspots of biodiversity and they provide a range of ecosystem functions (Körner, 2004). The Maloti-Drakensberg in South Africa, for example, provides 25% of the country's surface water run-off yet occupies only 5% of the land surface area (Blignaut *et al.*, 2010). Furthermore, species distributions are expected to shift into higher elevations as well as higher latitudes following global climate change (Wilson *et al.*, 2005; Parmesan, 2006; Colwell *et al.*, 2008). Consequently, understanding life on mountains is important for predicting future changes to the distribution of biodiversity and ecosystem processes.

1.6 STUDY SITE

The majority of data used in this thesis was collected from the Sani Pass in the Maloti-Drakensberg Mountains (Fig. 1.1). The Sani Pass is the only road linking the eastern border of Lesotho to the South African province of KwaZulu-Natal. The Pass itself ranges from 1500 to 2874 m a.s.l. but sampling sites extend beyond this to 900 and 3000 m a.s.l.. The Maloti-

Drakensberg Mountains are part of the grassland biome of southern Africa (Cowling *et al.*, 1997) and are the highest part of the Great Escarpment which encircles the southern African central plateau (Clark *et al.*, 2011) and are a centre of biological endemism within the region (Carbutt & Edwards, 2003, 2006; Kuhlmann, 2009). Unlike many other elevational gradients, there is no strong vegetation turnover in the Sani Pass. It is grassland from 900 to 3000 m a.s.l.. Often, elevational gradients are characterised by different vegetation belts (Körner, 2004) and this may strongly influence the elevational response of animals. This confounding of vegetation type and elevation does not occur in the Sani Pass.

Data from a number of other elevational gradients are used in chapter 3, details of which are given there.

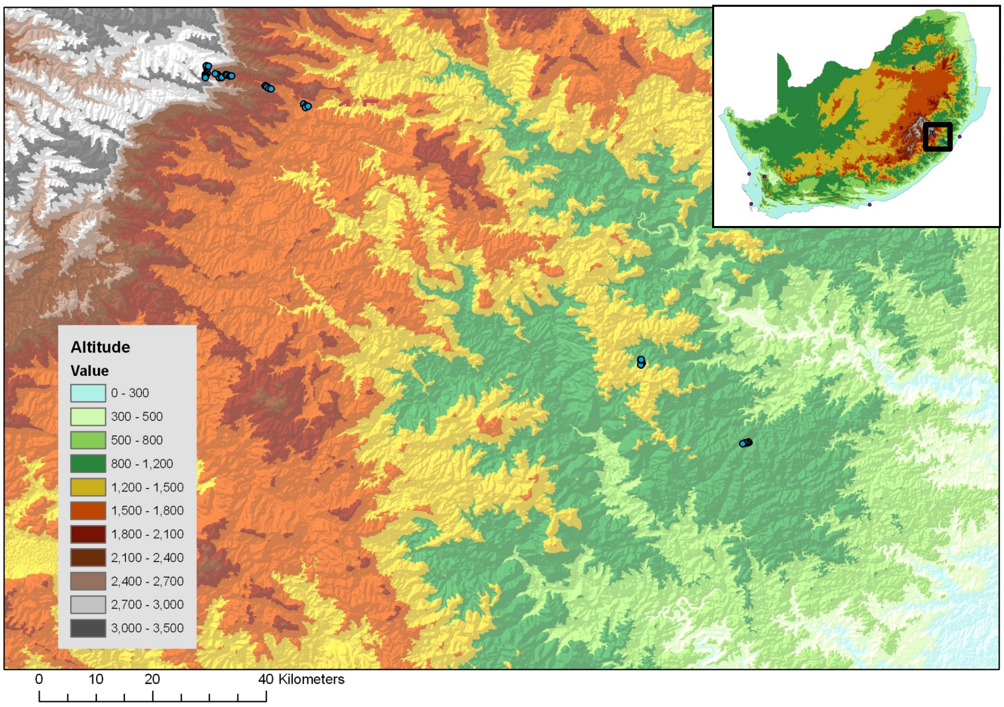


Figure 1.1 Map of the primary field site, the Sani Pass within the Maloti-Drakensberg. Inset - location of expanded region within southern Africa. Credit: Mark Robertson.

1.7 THESIS STRUCTURE

Each of the data chapters of this thesis have been written for publication. Chapters 2 and 3 have been published in the *Journal of Biogeography*. Chapter 4 has been submitted to *Global Ecology and Biogeography* and chapter 5 has been submitted to *Ecological Entomology*. For consistency, all chapters have been formatted according to the style guide for *Journal of Biogeography*. Consequently, this thesis is made up of stand-alone chapters which are linked by a common theme: the taxonomic and functional ecology of montane ants.

Chapters 2 and 3 investigate patterns of alpha and beta diversity within the Sani Pass through space and time. Chapter 4 incorporates data from a large number of elevational gradients from across the southern hemisphere to test a number of hypotheses relating ant cuticle colour to the environment. Chapter 5 analyses changes in the thermal tolerances of ants within the Sani Pass and relates these to their foraging abundances through time. Chapter 6 summarises the key findings from each data chapter and suggests potential avenues of future research.

1.7 REFERENCES

Blignaut, J., Mander, M., Schulze, R., Horan, M., Dickens, C., Pringle, C., Mavundla, K., Mahlangu, I., Wilson, A. & McKenzie, M. (2010) Restoring and managing natural capital towards fostering economic development: Evidence from the Drakensberg, South Africa. *Ecological Economics*, **69**, 1313-1323.

Brown, J.H. (1995) *Macroecology*. University of Chicago Press, Chicago.

Brown, J.H. & Lomolino, M.V. (1998) *Biogeography*. Sinauer Associates, Sunderland, Massachuesetts.

Carbutt, C. & Edwards, T.J. (2003) The flora of the Drakensberg Alpine Centre. *Edinburgh Journal of Botany*, **60**, 581-607.

Carbutt, C. & Edwards, T.J. (2006) The endemic and near-endemic angiosperms of the Drakensberg Alpine Centre. *South African Journal of Botany*, **72**, 105-132.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D. & Wardle, D.A. (2012) Biodiversity loss and its impact on humanity. *Nature*, **486**, 59-67.

Cardoso, P., Erwin, T.L., Borges, P.A. & New, T.R. (2011) The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation*, **144**, 2647-2655.

Chown, S., Gaston, K. & Robinson, D. (2004) Macrophysiology: large-scale patterns in physiological traits and their ecological implications. *Functional Ecology*, **18**, 159-167.

Chown, S.L. & Gaston, K.J. (2015) Macrophysiology-progress and prospects. *Functional Ecology*, **30**, 330-344

Clark, V.R., Barker, N.P. & Mucina, L. (2011) The Great Escarpment of southern Africa: a new frontier for biodiversity exploration. *Biodiversity and Conservation*, **20**, 2543-2561.

Colwell, R.K., Brehm, G., Cardelus, C.L., Gilman, A.C. & Longino, J.T. (2008) Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science*, **322**, 258-261.

Cowling, R.M., Richardson, D.M. & Pierce, S.M. (1997) *Vegetation of Southern Africa*. Cambridge University Press, Cambridge.

Darwin, C. (1859) *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. John Murray, London.

Ellison, A.M., Gotelli, N.J., Dunn, R.R. & Sanders, N.J. (2011) Counting ants (Hymenoptera: Formicidae): biodiversity sampling and statistical analysis for myrmecologists. *Myrmecological News*, **15**, 13-19.

Evans, T.A., Dawes, T.Z., Ward, P.R. & Lo, N. (2011) Ants and termites increase crop yield in a dry climate. *Nature Communications*, **2**, 262.

Fisher, B. (2010) Biogeography. *Ant Ecology* (ed. by L. Lach, C.L. Parr and K.L. Abbott). Oxford University Press, Oxford.

Fukami, T., Bezemer, T.M., Mortimer, S.R. & van der Putten, W.H. (2005) Species divergence and trait convergence in experimental plant community assembly. *Ecology Letters*, **8**, 1283-1290.

Gibb, H. & Parr, C.L. (2013) Does structural complexity determine the morphology of assemblages? An experimental test on three continents. *Plos One*, **8**, e64005.

Gibb, H., Stoklosa, J., Warton, D., Brown, A., Andrew, N. & Cunningham, S. (2014) Does morphology predict trophic position and habitat use of ant species and assemblages? *Oecologia*, **177**, 1-13.

Graham, C.H., Carnaval, A.C., Cadena, C.D., Zamudio, K.R., Roberts, T.E., Parra, J.L., McCain, C.M., Bowie, R.C., Moritz, C. & Baines, S.B. (2014) The origin and maintenance of montane diversity: integrating evolutionary and ecological processes. *Ecography*, **37**, 711-719.

Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Springer-Verlag, Berlin.

Keith, S.A., Webb, T.J., Böhning-Gaese, K., Connolly, S.R., Dulvy, N.K., Eigenbrod, F., Jones, K.E., Price, T., Redding, D.W. & Owens, I.P. (2012) What is macroecology? *Biology Letters*, rsbl20120672.

Körner, C. (2004) Mountain biodiversity, its causes and function. *Ambio*, **13**, 11-17.

Körner, C. (2007) The use of 'altitude' in ecological research. *Trends in Ecology & Evolution*, **22**, 569-574.

Kuhlmann, M. (2009) Patterns of diversity, endemism and distribution of bees (Insecta: Hymenoptera: Anthophila) in southern Africa. *South African Journal of Botany*, **75**, 726-738.

Lengyel, S., Gove, A.D., Latimer, A.M., Majer, J.D. & Dunn, R.R. (2010) Convergent evolution of seed dispersal by ants, and phylogeny and biogeography in flowering plants: a global survey. *Perspectives in Plant Ecology, Evolution and Systematics*, **12**, 43-55.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006) Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, **21**, 178-185.

McGlynn, T.P. & Poirson, E.K. (2012) Ants accelerate litter decomposition in a Costa Rican lowland tropical rain forest. *Journal of Tropical Ecology*, **28**, 437-443.

Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Brown, J.M., Bush, M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N. & Lessios, H.A. (2007) Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology Letters*, **10**, 315-331.

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics*, **37**, 637-669.

Sanders, N.J., Lessard, J.-P., Fitzpatrick, M.C. & Dunn, R.R. (2007) Temperature, but not productivity or geometry, predicts elevational diversity gradients in ants across spatial grains. *Global Ecology and Biogeography*, **16**, 640-649.

Stuble, K.L., Patterson, C.M., Rodriguez-Cabal, M.A., Ribbons, R.R., Dunn, R.R. & Sanders, N.J. (2014) Ant-mediated seed dispersal in a warmed world. *PeerJ*, **2**, e286.

Sundqvist, M.K., Sanders, N.J. & Wardle, D.A. (2013) Community and ecosystem responses to elevational Gradients: processes, mechanisms, and insights for global change. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 261-280.

Thomas, J.A., Simcox, D.J. & Clarke, R.T. (2009) Successful Conservation of a Threatened Maculinea Butterfly. *Science*, **325**, 80-83.

Violle, C., Reich, P.B., Pacala, S.W., Enquist, B.J. & Kattge, J. (2014) The emergence and promise of functional biogeography. *Proceedings of the National Academy of Sciences*, **111**, 13690-13696.

Ward, P.S. (2010) Taxonomy, Phylogenetics and Evolution. *Ant Ecology* (ed. by L. Lach, C.L. Parr and K.L. Abbott). Oxford University Press, Oxford.

Wardle, D.A., Hyodo, F., Bardgett, R.D., Yeates, G.W. & Nilsson, M.-C. (2011) Long-term aboveground and belowground consequences of red wood ant exclusion in boreal forest. *Ecology*, **92**, 645-656.

Weiher, E. & Keddy, P. (2001) *Ecological Assembly Rules: Perspectives, Advances, Retreats*. Cambridge University Press, Cambridge.

Weiser, M.D. & Kaspari, M. (2006) Ecological morphospace of New World ants. *Ecological Entomology*, **31**, 131-142.

Whittaker, R.J., Araújo, M.B., Jepson, P., Ladle, R.J., Watson, J.E.M. & Willis, K.J. (2005) Conservation Biogeography: assessment and prospect. *Diversity and Distributions*, **11**, 3-23.

Wiescher, P.T., Pearce-Duvet, J.M.C. & Feener, D.H. (2012) Assembling an ant community: species functional traits reflect environmental filtering. *Oecologia*, **169**, 1063-1074.

Wilson, R.J., Gutierrez, D., Gutierrez, J., Martinez, D., Agudo, R. & Monserrat, V.J. (2005) Changes to the elevational limits and extent of species ranges associated with climate change. *Ecology Letters*, **8**, 1138-1146.

Zelikova, T.J., Sanders, N.J. & Dunn, R.R. (2011) The mixed effects of experimental ant removal on seedling distribution, belowground invertebrates, and soil nutrients. *Ecosphere*, **2**, art63.

Chapter 2

Elevation-diversity patterns through space and time: ant communities of the Maloti-Drakensberg Mountains of southern Africa

Images: Jesse Kalwij, Glen McLean and Kate Parr

Elevation-diversity patterns through space and time: ant communities of the Maloti-Drakensberg Mountains of southern Africa

Tom R. Bishop[1,2]*, Mark P. Robertson[2], Berndt J. van Rensburg[2,3]

and Catherine L. Parr[1]

[1]Department of Earth, Ocean and Ecological Sciences, University of Liverpool, Liverpool, L69 3GP, UK,

[2]Centre for Invasion Biology, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa,

[3]School of Biological Sciences, University of Queensland, St. Lucia, Queensland 4072, Australia

**Correspondence: tbish@liv.ac.uk / thomasrhys.bishop@gmail.com*

Published in *Journal of Biogeography* (2014), volume 41 (12), pages 2256-2268

2.1 ABSTRACT

Aim Patterns of biological diversity are often investigated across space but little work has attempted to explore the consistency of such observations through time. Here, our aim was to understand the patterns of diversity for a functionally critical taxon, the ants (Hymenoptera: Formicidae) through space and time using an extensive dataset collected across an elevational gradient. In addition, we evaluated the importance of two key postulated drivers of elevational diversity patterns: temperature and available area.

Location The Maloti-Drakensberg Mountains of southern Africa.

Methods We sampled epigaeic ant communities biannually for 7 years (2006-2012) at eight different elevational sites. We then used an information theoretic approach combined with generalized linear mixed models to: (1) describe diversity patterns through space and time; (2) assess the importance of different abiotic drivers; and (3) understand how much spatio-temporal variation can be explained by these drivers. Simple regression approaches were also used to test for differences in seasonal variation along the elevational gradient.

Results We found clear mid-elevational peaks of species density and evenness measures. Abundance patterns were complex. The spatial distributions of all three metrics changed across seasons and years. Temperature variables had important roles in explaining both species density and abundance patterns, whilst species density was also influenced by available area. In conjunction, we found much greater seasonal variability in species density at low elevations. This variation was independent of differences in species pool size.

Main conclusions We found patterns of ant diversity that are strongly modulated by temporal change. There was a consistent and strong signature of seasonality on the elevation-diversity patterns of the ants, whilst annual changes throughout the study period had a weaker influence. We conclude that both spatial and temporal patterns are driven primarily by temperature, with only a weak influence of available elevational area. This study is the first to describe the spatio-temporal distribution of a suite of community-level metrics along an elevational gradient and implies that temporal variation should be considered more carefully in studies of invertebrate diversity, particularly with respect to elevation and the mechanisms that may be maintaining diversity patterns.

Keywords: Ant, elevational gradient, evenness, Formicidae, mid-domain peak, seasonality, southern Africa, spatio-temporal variability, species diversity, species richness.

Author contributions: M.P.R. and B.J.V.R. designed the sampling protocol and oversaw all data collection. T.R.B. and C.L.P. developed the research questions. T.R.B. analysed the data and led the writing of the paper. M.P.R., B.J.V.R., and C.L.P. all contributed to the final draft of the paper.

2.2 INTRODUCTION

Describing patterns of biodiversity and revealing the factors that are driving them has long been a central research theme in ecology (Pianka, 1966; Rohde, 1992; Gaston, 2000). Such a goal not only allows ecologists to gain a better understanding of the diversity of life on Earth, but also provides information typically needed for decision-making in conservation science (Myers *et al.*, 2000; Samways, 2005). Elevational gradients provide useful natural experiments when investigating the distribution of biodiversity (Körner, 2007; Sundqvist *et al.*, 2013). They condense a large amount of environmental variation into a small geographical space (Körner, 2007), allowing us to more easily test patterns and processes that occur on larger scales (Sanders *et al.*, 2007; Sundqvist *et al.*, 2013). Elevational gradients are also of interest in their own right, particularly given that species distributions are expected to shift into higher elevations as well as higher latitudes following global climate change (Wilson *et al.*, 2005; Parmesan, 2006; Colwell *et al.*, 2008). An understanding of the patterns of biodiversity across elevation can therefore contribute towards basic and applied ecological goals.

Whilst much emphasis has been placed on describing and explaining spatial patterns, few studies have investigated how elevational diversity and its explanatory factors may vary through time. This is despite the potential importance of a temporal component in allowing us to fully understand diversity patterns and tease apart the mechanisms generating them (Willis & Whittaker, 2002; White *et al.*, 2010). Can the patterns and drivers of diversity across space be generalized through inter- and intra-annual time-scales?

With respect to space, unimodal peaks and monotonic declines of species density (the number of species per unit area, i.e. Gotelli & Colwell, 2001) with elevation are the most commonly reported patterns (Rahbek, 1995, 2005). Many proposed explanations for these diversity patterns concern elevational clines in variables such as temperature, precipitation and available area. Here, species density is predicted to peak at elevations that offer an optimal set of environmental conditions. The role of area in this context is analogous to a species-area effect: elevational bands with the most available area will have a larger number of individuals, and therefore likelihood of a larger species pool with which to supply local communities (Lomolino, 2001; Romdal & Grytnes, 2007). Other explanations for elevational diversity patterns rely on the geometric constraints of mountain topology. For example, the mid-domain effect model predicts peaks in diversity at mid-elevations as a result of the random overlap of species ranges placed between the hard bounds of the base (i.e. sea level) and peak of an elevational gradient (Colwell & Lees, 2000; Dunn *et al.*, 2007).

Few studies have investigated potential temporal changes in these diversity measures and their putative drivers. Beck *et al.* (2010) found a linear decline in moth species number with elevation during spring and autumn, which changed to a unimodal pattern in the summer. McCain (2004) reported that the mid-elevational peak in diversity of non-volant Neotropical mammals shifted to higher elevations between the dry and wet seasons. These results suggest that there is more to learn about the distribution and drivers of elevational diversity through time. Many studies do sample elevational gradients over multiple seasons or years, yet this temporal variation is often pooled or averaged away for analysis (Axmacher *et al.*, 2004; Naniwadekar & Vasudevan, 2007; Wu *et al.*, 2013). The lack of interest in, or availability of, time-series data means that we are likely to be underestimating the true range of elevational diversity patterns and the extent to which these patterns are related to potential explanatory variables. Here, we address this significant gap by presenting the first results from a long-term monitoring scheme of ants

(Hymenoptera: Formicidae) along an elevational gradient in southern Africa.

Ants are a globally important and abundant taxonomic group (Hölldobler & Wilson, 1990; Ness *et al.*, 2010; Del Toro *et al.*, 2012). Linear declines (Brühl *et al.*, 1999; Robertson, 2002; Yusah *et al.*, 2012) and mid-elevational peaks (Fisher, 1999; Sanders, 2002; Sanders *et al.*, 2003; Bharti *et al.*, 2013) of ant species density have been reported. Reports of ant abundance patterns are also mixed, with examples of declines with elevation, mid-elevation peaks, and some examples of no significant change in abundance with elevation (Sabu *et al.*, 2008; Munyai & Foord, 2012; Yusah *et al.*, 2012). Temperature and available land area have been cited as being important factors governing the distribution of ant diversity across elevation (Sanders, 2002; Botes *et al.*, 2006; Sanders *et al.*, 2007; Machac *et al.*, 2011; Chaladze, 2012; Munyai & Foord, 2012).

No studies have investigated temporal variation in ant elevational diversity. Ant species density and abundance, however, are known to shift with season in a range of habitats (Andersen, 1983; Kaspari & Weiser, 2000; Deblauwe & Dekoninck, 2007). Typically, greater species density and abundance are observed at the time of year which has greater humidity, temperatures or forage availability (Whitford, 1978; Andersen, 1983; Kaspari & Weiser, 2000). In addition, this seasonal flux of diversity may be modulated by other environmental factors. For example, the response of ant communities to different seasons can depend on the vegetation type sampled (Deblauwe & Dekoninck, 2007; Barrow & Parr, 2008).

Here, we tested previously reported relationships of ant diversity with elevation, temperature and available area. Crucially, we incorporated a temporal component into our analyses in order to assess the inter- and intra-annual generality of both the patterns and the potential drivers of ant diversity. We also investigated the relatively unknown relationship of species evenness with elevation. Evenness provides information of the equitability of abundances of species in a given community and is likely to be important in fully understanding the structure and function of biological communities. We investigated these relationships using an extensive dataset from a region previously unexplored in detail for ants: the Sani Pass of the Maloti-Drakensberg Mountains, southern Africa. Our dataset ranges from 900 to 3000 m a.s.l. and encompasses seven consecutive years of biannual sampling, making it one of the largest standardized, spatio-temporal invertebrate community datasets available. We did not directly investigate hypotheses of geometric constraints because we lacked a hard lower elevational boundary (Colwell & Lees, 2000; Sanders *et al.*, 2003) and were more interested in effects driving relatively small and local-scale diversity, for which mid-domain effect predictions have been shown to be inappropriate (Dunn *et al.*, 2007).

We asked the following questions: (1) How does ant diversity vary in space and time within the Sani Pass? (2) Does ant diversity respond positively to the drivers of temperature and available area? (3) How much of the spatio-temporal variation in ant diversity can be explained by these drivers? In line with previous work, we predicted that species density, the abundance of individuals and evenness would decline with increasing elevation. In addition, we expected that departures from a monotonic decline in these metrics across elevation would be explained by variation in temperature or available area (Sanders, 2002; Sanders *et al.*, 2007). We expected no systematic trend in any of our diversity metrics across years but anticipated reduced species density, abundance and evenness in the harsher dry season. Finally, we expected that the form of the diversity-elevation relationship would change between the seasons, in line with previous work (McCain, 2004; Beck *et al.*, 2010).

2.3 MATERIALS AND METHODS

2.3.1 Study site

We sampled ants in the Maloti-Drakensberg Transfrontier Conservation Area of South Africa and Lesotho. This area forms part of the grassland biome of southern Africa (Cowling *et al.*, 1997) and is recognized as a centre of endemism within southern Africa (Carbutt & Edwards, 2006; Kuhlmann, 2009). We sampled along an elevational transect located in the southern region of the Maloti-Drakensberg which runs from near the village of Ixopo at 900m a.s.l. (30°09' S 30°03' E) to a point above the top of the Sani Pass at 3000 m a.s.l. (29°35' S 29°17' E). Eight sampling sites were placed at increasing elevations of 300 m. The transect is 104 km long; however, the majority of the sampling sites were located in the final 7 km of the Sani Pass (1800-3000 m a.s.l.). All sampling sites were placed in natural vegetation. Mean annual rainfall between 2002 and 2005 along the transect ranged from 793 to 861mm (Nel & Sumner, 2008). Annual rainfall increases with elevation with the exception of the very highest elevations, where rainfall is reduced (Nel & Sumner, 2008). The majority of the rainfall occurs during the summer (October-March) with as much as 19% occurring in January. In contrast, only 5% of the annual rainfall occurs in September (Nel & Sumner, 2006). Mean ground temperatures vary strongly between seasons and tend to decline with increasing elevation. Mean ground temperatures range from 13.1 to 22.1 °C in January, and from 9.2 to 15.2 °C in September (M.P.R., unpublished data). Snowfall is common during the winter, particularly at high elevations.

2.3.2 Ant sampling

Epigaeic (ground-dwelling) ant sampling took place during January (wet season) and September (dry season) for the years 2006 to 2012 inclusive (7 years in total). We established four replicate blocks, spaced at least 300 m apart, at each elevational site. We consider each block to be an independent ecological community. Within each block, 10 pitfall traps were dug in two parallel lines, with 10 m spacing between traps. This design mirrors that used in sister sampling schemes throughout South Africa (Botes *et al.*, 2006; Munyai & Foord, 2012). Pitfall traps were 150 ml in volume with a diameter of 55 mm and a depth of 70 mm. Rain guards of 220 mm diameter, supported on wire legs 100 mm above the traps, were used to prevent flooding. Traps contained a 50% ethylene glycol solution to preserve ant specimens. Traps were left out for five trapping nights in total but were replaced after 2 or 3 days to prevent overfilling. Ants were later transferred to 70% alcohol in the laboratory and were identified to morphospecies, and species level where possible.

2.3.3 Environmental variables

Thermocron iButtons (DS1921G, Semiconductor Corporation, Dallas/Maxim, TX, USA) were buried 10 mm below the soil surface at two replicates at each elevational site. These iButtons were replaced each sampling period. Temperature was recorded every 1.5 hours. Hourly readings were taken from January 2010 as higher capacity iButtons (DS1922L) were phased into use. Mean, minimum and maximum temperatures were calculated for January and September for each year of the study for each elevational band. Plots of temperature through time were inspected for each iButton and instances where recording had malfunctioned or where the iButton had clearly been exposed were excluded from analysis.

Available area was calculated for each elevational site. Vertical bands ± 150 m of the focal elevations were set. Then, the available area of each of these elevational bands in a 40-km buffer around the sampling sites was determined using ARCMAP GIS 9.3.1 (ESRI, Redlands, CA, USA).

2.3.4 Statistical methods

For each of the 440 ant communities in the dataset, i.e. replicated blocks (4 blocks per sampling site x 8 sites x 7 years x 2 seasons = 440 following the removal of those eight communities without valid temperature data), species density (Gotelli & Colwell, 2001), total abundance and evenness were calculated. Species density and abundance are counts of the number of species and the number of individuals, respectively, in each standardized replicate block. The probability of interspecific encounter (PIE) was used as a measure of evenness and was calculated as:

$$\mathrm{PIE} = 1 - \textstyle\sum_{i=1}^{S} p_i^2 \ ,$$

where S is the number of species and p is their relative abundance within a community. This represents the probability of interspecific encounter within a community (Hurlbert, 1971). Strictly, this is the probability that two individuals drawn at random from a given community will represent different species. To assess spatial independence between communities, Moran's I was used to test for spatial autocorrelation of species density, abundance and evenness values at each elevational band for each year and season.

Three sets of generalized linear mixed models (GLMMs) and an information theoretic approach based on the bias-corrected Akaike's information criterion ($\mathrm{AIC_c}$) were used to examine the extent to which ant species density, abundance and evenness is related to temperature and available area across a spatio-temporal scale. The first set of models aimed only to describe the pattern of the spatio-temporal distribution of the response variables, namely ant species density, abundance and evenness. Elevation, elevation^2, season, year and various two-way and three-way interactions were used as explanatory variables in these models. A polynomial term of elevation was included in order to detect potential hump shapes in the relationships between elevation and the response variables. Sixteen plausible models combining these variables were constructed for this first set of models. The second set of models examined the extent to which ant diversity is related to temperature and available area. Area, the mean and standard deviation in temperature and their two-way interactions were used as explanatory variables. These variables were chosen because of their lack of collinearity. The third set investigated the spatio-temporal patterns of the residuals of the second set. This procedure would reveal whether any spatio-temporal patterning remained after removing the effect of the most important drivers. For example, a model of the residuals that included only the intercept would indicate that the best model of the drivers could account for all previously observed spatio-temporal patterns.

A Poisson error structure was used for models of species density and abundance. For models of evenness, a logit transformation on the response variable, to meet model assumptions, and Gaussian errors were used. Models of residuals used a Gaussian error structure. All numeric explanatory variables were centred around the mean and standardized to allow for greater interpretability of coefficients (Schielzeth, 2010). Season was coded as a binary variable. Replicate was included as a random effect to account for temporal pseudoreplication. Maximum likelihood estimation was used and AIC$_c$ values compared to choose the best model for each response variable in each set of models. Modelling was performed using the LME4 package in R (Bates *et al.*, 2013; R Core Team, 2013). Marginal R^2 (R^2_m, due to fixed effects only) and conditional R^2 (R^2_c, due to fixed effects and random effects) were calculated for each model (Nakagawa & Schielzeth, 2013). Conditional R^2 cannot yet be computed for models using a Poisson error structure (Bartoń, 2013).

Following the results of the modelling, we tested the hypothesis that communities at lower elevations had greater seasonal fluctuations in species density than communities at high elevations. For each replicate, we calculated the seasonal component of variation in species density values. This was performed by treating individual replicates as time series with 14 data points (seven years and two seasons). From these time series, the seasonal component was extracted by classical decomposition using moving averages (Kendall & Stuart, 1976). This quantifies the seasonal variation in species density values, for each of the 32 replicates, across the entire 7 years of sampling. These values were then regressed against elevation. A negative relationship would indicate that the seasonal variation is greater at lower elevations. To account for species pool effects, the same analysis was performed twice again on species density values that had been corrected for differences in species pool sizes. This was performed by expressing species density values as a proportion of (1) the total number of species seen at that given replicate across the 7 years, and (2) the total number of species seen at all four replicates within that elevational band across the 7 years.

2.4 RESULTS

We collected 60,236 individuals from 92 ant species in 28 genera and seven subfamilies throughout the study period. Our assumption that the communities were spatially independent was confirmed by non-significant results of the Moran *I* tests for each response variable in each year and season. All coefficients are reported on the standardised scale as phenotypic standard deviations with their associated standard errors and consequently do not have units (Schielzeth, 2010).

2.4.1 Species density

Spatio-temporal patterns

Species density was best modelled by elevation, elevation^2, season, year and interactions between both elevational terms and both temporal terms, as well as an interaction between season and year (Table 2.1). The form of this model describes a hump-shaped relationship of species density with elevation that changes depending on the season (Fig. 2.1, Table 2.2). The hump shape is more pronounced, and peaks at a higher elevations, in the dry season. Overall, species density also tends to be lower in the dry season. The interaction between the elevational terms and year describes a situation where the hump shape becomes increasingly linear through time. Fewer species are found in later years and this effect is greatest in the wet season due to the inclusion of the season + year interaction. The fixed effects explained a large amount of variation in species density ($R^2_m = 0.68$).

Drivers

The best model of the drivers of species density included mean temperature and area. Positive estimates were found for both of these variables, indicating that species density increases with increasing mean temperature (0.32 ± 0.02) and area (0.08 ± 0.05). The influence of area, however, was weak, with the next best model including only mean temperature ($\Delta AIC_c = 0.53$). The best model explained a relatively large amount of variation with an R^2_m of 0.43. The relationship of species density with mean temperature is displayed in Fig. 2.2a.

Residual analysis

The best model of the residuals obtained from the drivers model (immediately above) included the same explanatory variables as that for the spatio-temporal patterns, namely elevation, elevation^2, season, year and their two-way interactions. This model explained very little variation with an R^2_{m} of 0.08. This indicates that the residuals are modelled very poorly by the spatio-temporal variables. There is little to no interpretable pattern across space or time; in other words, the drivers of available area and temperature explain the patterns of species density.

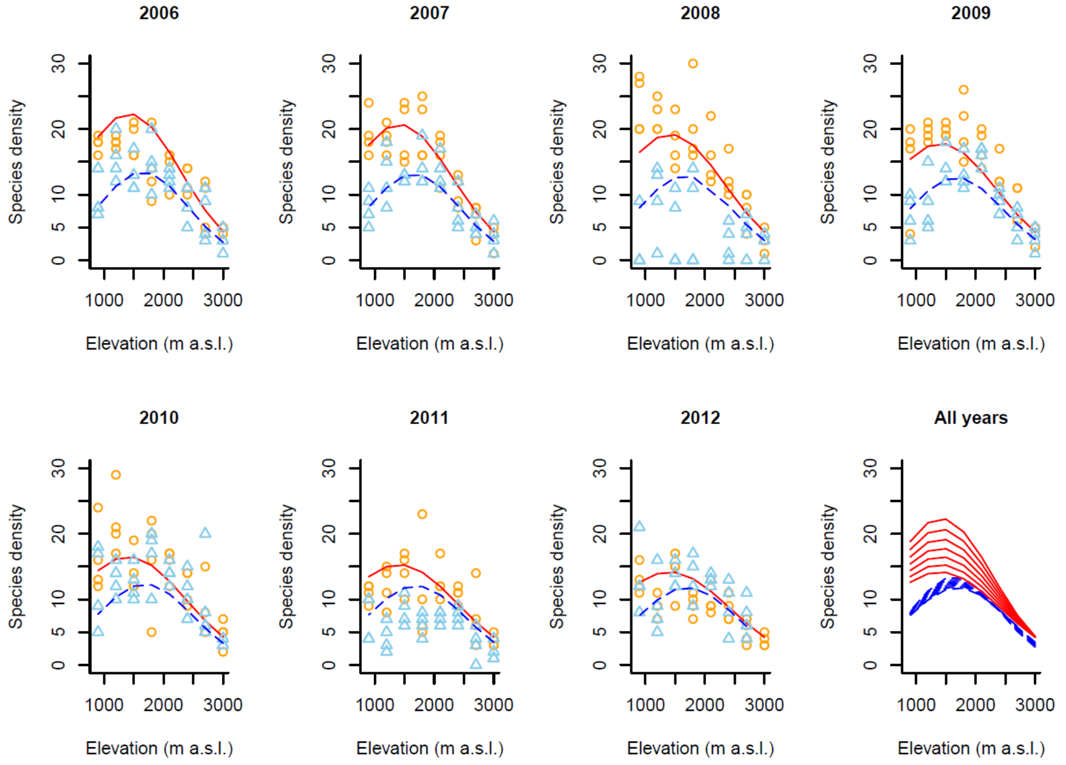


Figure 2.1 Plots of ant species density against elevation in the Sani Pass, southern Africa, for consecutive years and for all years combined. Red solid lines indicate predicted values from generalized linear mixed modelling for January (wet season; circles) and blue dashed lines for September (dry season; triangles). Each point represents a sampled community. Marginal R^2 (due to fixed effects only) = 0.68.

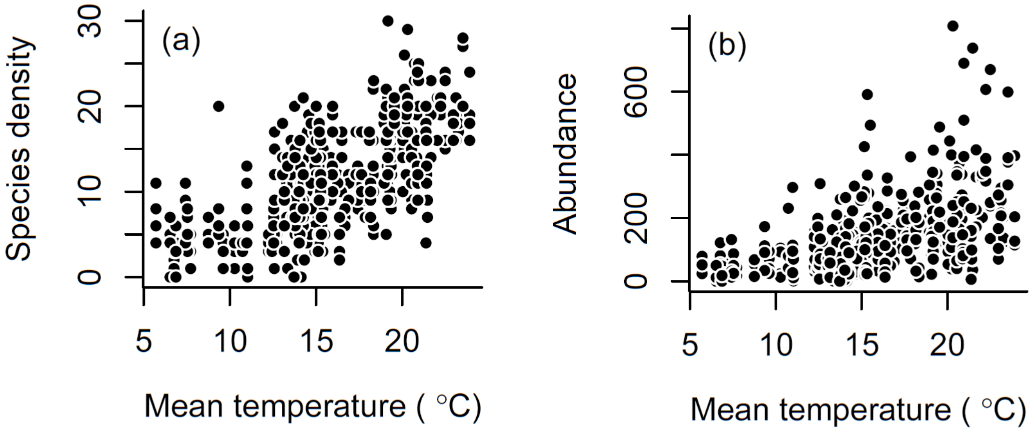


Figure 2.2 Plots of (a) ant species density and (b) ant abundance against mean temperature within the Sani Pass, southern Africa. All data across the elevational gradient and time series is plotted.

2.4.2 Abundance

Spatio-temporal patterns

The best model for abundance was the most complicated one included in the candidate list. Elevation, elevation^2, season, year, their two-way interactions and the three-way interactions between season, year and each elevational term were included (Table 2.1). Abundance displays a mild hump-shaped relationship with elevation, which differs with the season (Fig. 2.3, Table 2.2). The hump is more pronounced in the dry season. Essentially, however, this hump is nearly flat as the estimates for elevation (0.45 ± 0.44) and elevation^2 (-0.7 ± 0.44) are close to 0. Abundance is lower in the dry season and also decreases through time, but more so in the wet season and at lower elevations. This is indicated by the three-way interactions between elevation:season:year and elevation^2:season:year. The interactions of the elevation terms with year show that the abundance-elevation relationship has become less pronounced through time. This model of abundance has an R^2_m of 0.67.

Drivers

Mean temperature (0.63 ± 0.01) and the standard deviation in temperature (0.08 ± 0.01) were both included in the best model of abundance. Both had positive estimates, indicating that abundance increases with increasing mean (Fig. 2.2b) and standard deviation of temperature. This model has an R^2_m of 0.7.

Residual analysis

The model including only the intercept was the best (Table 2.1). Consequently, patterns of abundance are entirely explained by variation in the mean and standard deviation in temperature.

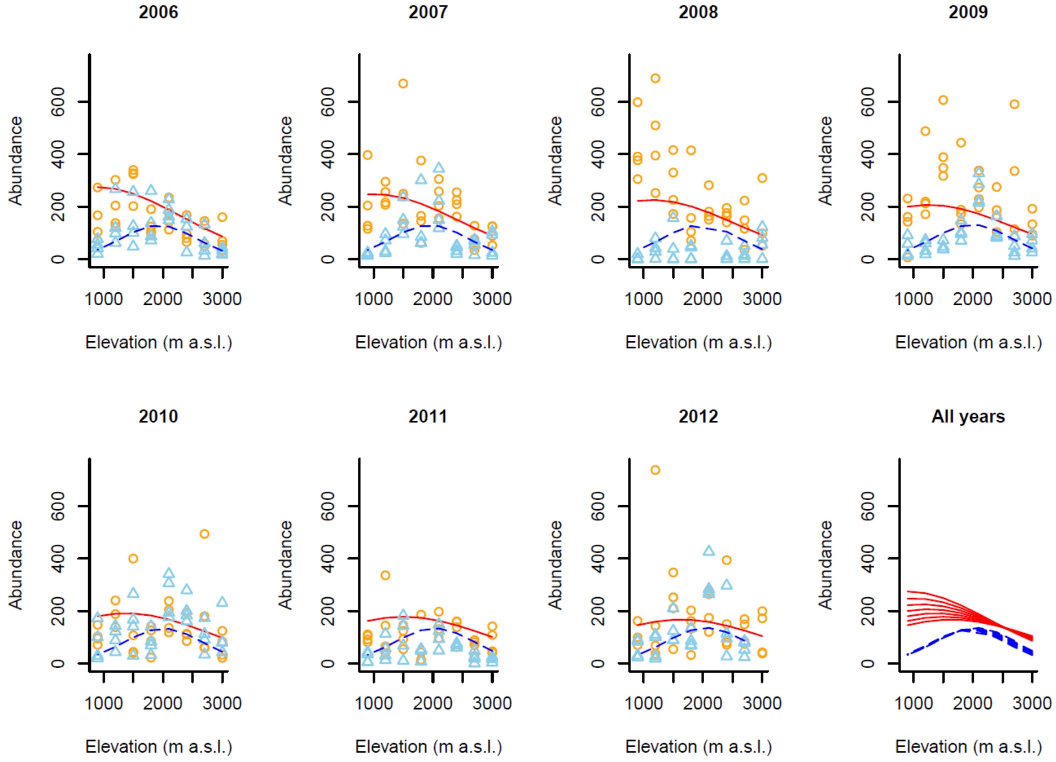


Figure 2.3 Plots of ant abundance against elevation in the Sani Pass, southern Africa, for consecutive years and for all years combined. Red solid lines indicate predicted values from generalized linear mixed modelling for January (wet season; circles) and blue dashed lines for September (dry season; triangles). Each point represents a sampled community. Marginal R^2 (due to fixed effects only) = 0.67.

2.4.3 Evenness

Spatio-temporal patterns

The best model for evenness included the main effects of elevation, elevation^2, season and year. This model describes a hump-shaped relationship between evenness and elevation (Table 2.2). Evenness is lower in the dry season and also decreases across years (Fig. 2.4). The ant communities are more even in the wet season and in early years. This is the poorest fitting of the spatio-temporal models with an R^2_{m} of 0.32.

Drivers

PIE was best modelled by mean temperature only (0.09 ± 0.05). Mean temperature had a positive effect on evenness. However, this model actually explained very little variation ($R^2_{\mathrm{m}} = 0.006$, $R^2_{\mathrm{c}} = 0.43$). The next best model included the mean and standard deviation of temperature ($\Delta\mathrm{AIC_c} = 0.19$).

Residual analysis

The best model for the residuals obtained from the drivers model (immediately above) included elevation and year as main effects (Table 2.1). Both had negative estimates, indicating that the residuals decrease with elevation and across time. The result from this model indicates that the curvature in the pattern of evenness distribution (elevation^2) and the seasonal effect are explained by differences in mean temperature. This model explains very little variation ($R^2_{\mathrm{m}} = 0.03$, $R^2_{\mathrm{c}} = 0.03$).

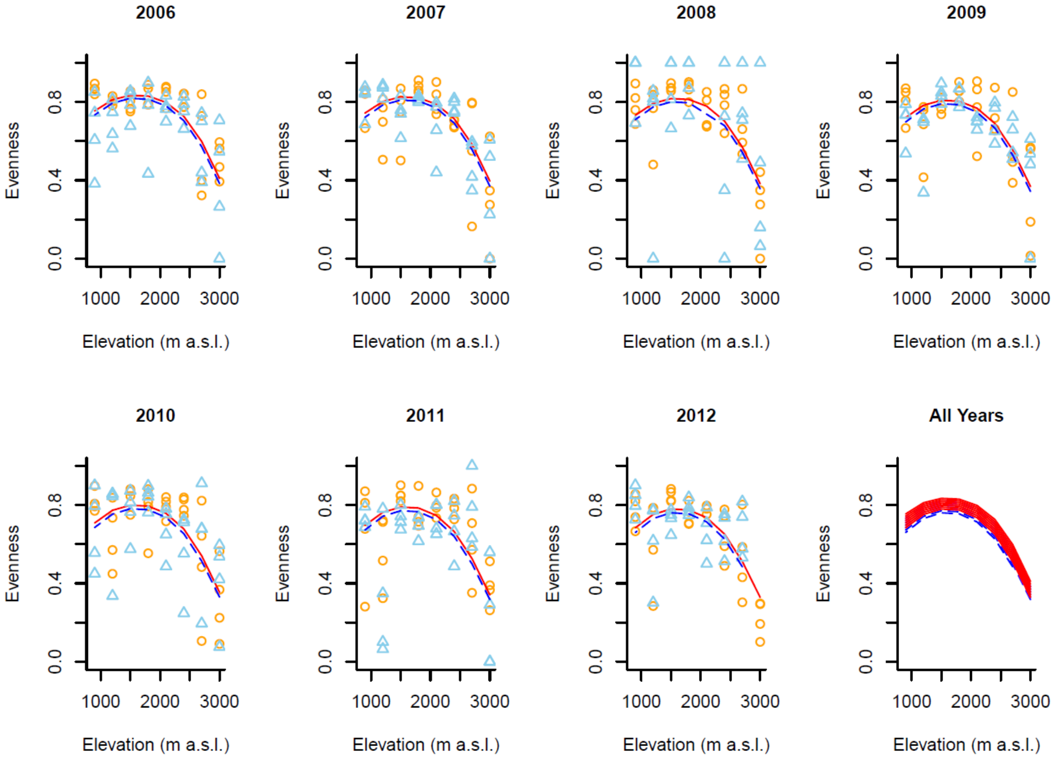


Figure 2.4 Plots of ant community evenness against elevation in the Sani Pass, southern Africa, for consecutive years and for all years combined. Red solid lines indicate predicted values from generalized linear mixed modelling for January (wet season; circles) and blue dashed lines for September (dry season; triangles). Predicted values have been back transformed onto the original scale. Each point represents a sampled community. Marginal R^2 (due to fixed effects only) = 0.32.

2.4.4 Seasonal fluctuation

There was a significant decline in the seasonal component with elevation for all three levels of analysis: raw species density ($b = -0.002$, d.f. = 30, $P < 0.01$, Fig. 2.5a), controlling for the replicate species pool ($b = -2.98^{-5}$, d.f. = 30, $P < 0.01$, Fig. 2.5b) and controlling for the elevational species pool ($b = -2.33^{-5}$, d.f. = 30, $P < 0.01$, Fig. 2.5c). The seasonal variation of species density is greater at lower elevations than at higher elevations and is independent of the differing sizes of the available species pools at different elevations.

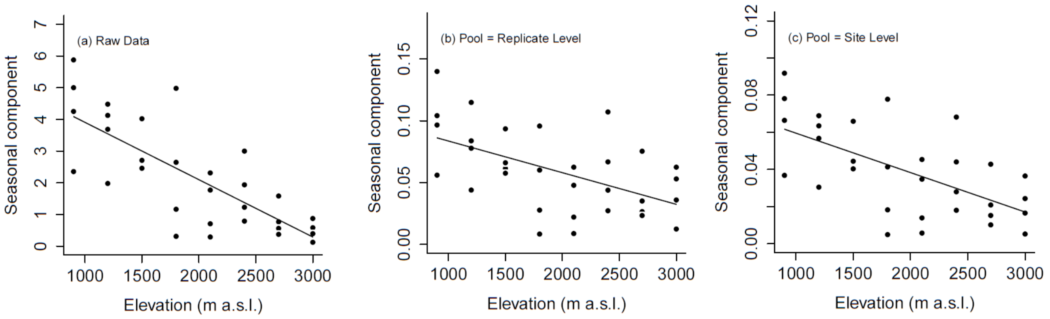


Figure 2.5 Plots of the seasonal component of temporal variation in ant species density within the Sani Pass, southern Africa, against elevation for (a) raw species density values (seasonal component = 6.23-0.002 × elevation), (b) species density controlled by the replicate level species pool (seasonal component = $0.124\text{-}2.98^{-5}$ × elevation), and (c) species density controlled by the site level species pool (seasonal component = $0.89\text{-}2.33^{-5}$ × elevation).

Table 2.1 Summaries of generalized linear mixed models linking ant species density, abundance and evenness to (1) spatio-temporal explanatory variables, (2) environmental drivers and (3) residual spatio-temporal variables with the effect of the drivers removed, along an elevational gradient within the Sani Pass, southern Africa. The best models, according to the bias-corrected Akaike information criterion (AIC_c) are reported. The change in AIC_c between the best model and the next best and worst are also given. Marginal R^2 (R^2_m), measuring variation explained by fixed effects only, and conditional R^2 (R^2_c), measuring variation explained by both fixed and random effects, are given.

Response	Model	AIC_c	ΔAIC_c (next best)	ΔAIC_c (worst)	R^2_m	R^2_c
Ant species density						
Spatio-temporal patterns	~ elevation + elevation^2 + season + year + elevation:season + elevation:year + elevation^2:season + elevation^2:year + year:season	683.96	3.07	356.29	0.68	NA
Drivers	~ mean temperature + area	774.85	0.53	262.45	0.43	NA
Residual analysis	~ elevation + elevation^2 + season + year + elevation:season + elevation:year + elevation^2:season + elevation^2:year + year:season	1348.77	4.13	23.89	0.08	0.08
Ant abundance						
Spatio-temporal patterns	~ elevation + elevation^2 + season + year + elevation:season + elevation:year + elevation^2:season + elevation^2:year + year:season + season:year:elevation + season:year:elevation^2	20599.09	5.75	9889.83	0.67	NA
Drivers	~ mean temperature + SD temperature	19551.81	224.52	10938.31	0.7	NA
Residual analysis	~ Intercept	2939.08	1.84	7.27	0	0
Ant community evenness						
Spatio-temporal patterns	~ elevation + elevation2 + season + year	1174.77	0.53	37.12	0.32	0.46
Drivers	~ mean temperature	1211.43	0.19	1.47	0.006	0.43
Residual analysis	~ elevation + year	1094.38	1.02	14.19	0.03	0.03

Table 2.2 Parameter estimates from generalized linear mixed models linking ant species density, abundance and evenness to spatio-temporal explanatory variables, along an elevational gradient within the Sani Pass, southern Africa. Estimates are on the standardized scale ± standard error. Empty cells indicate terms not included in the best model for a given response variable.

	Estimates		
Term	Species density	Abundance	Evenness
Elevation	2.42 ± 0.03	0.45 ± 0.44	2.24 ± 0.59
Elevation^2	−1.46 ± 0.22	−0.70 ± 0.44	−2.77 ± 0.59
Season	−0.39 ± 0.03	−0.75 ± 0.01	−0.13 ± 0.08
Year	−0.13 ± 0.02	−0.08 ± 0.01	−0.14 ± 0.04
Elevation:Season	0.76 ±0.22	2.71 ± 0.07	
Elevation^2:Season	−0.63 ± 0.23	−2.43 ± 0.07	
Elevation:Year	−0.17 ± 0.10	0.08 ± 0.03	
Elevation^2:Year	0.20 ± 0.11	0.01 ± 0.04	
Season:Year	0.09 ± 0.03	0.11 ± 0.01	
Season:Year:Elevation		−0.15 ± 0.07	
Season:Year:Elevation^2		0.12 ± 0.07	

2.5 DISCUSSION

Whilst many patterns of elevational diversity have been documented from various taxa and regions, the time dependence of these relationships has rarely been investigated (but see McCain, 2004; Beck *et al.*, 2010). Here, we found that the spatial distribution of ant species density, abundance and evenness depends on both inter- and intra-annual time. The drivers of this variation, however, appear to be consistent across time as they are able to account for the spatio-temporal patterns observed. These results suggest that environmental factors, rather than geometric constraints, control the diversity of ants within the Maloti-Drakensberg.

We found a mid-elevational peak of species density that differs in both size and shape depending on the season and year (Fig. 2.1). A number of studies have found mid-elevational peaks of ant species density (e.g. Fisher, 1999; Sanders, 2002; Bharti *et al.*, 2013). This pattern is the most common across a range of taxa and regions (Rahbek, 1995, 2005). Whereas Beck *et al.* (2010) found that the elevational diversity pattern of European moths changed from a monotonic decline to a mid-elevational peak with the onset of summer, we found a mid-elevational peak throughout our study period. This peak was found to change in magnitude and position depending on the season. Fewer species and a more pronounced hump shape were observed in the dry season than in the wet season (Fig. 2.1). This is comparable to the findings of McCain (2004) where the mid-elevational peak of small mammal density shifts between two of the seasonal replicates sampled. Our dataset extends this result as we found consistent seasonal shifts in each year against a backdrop of annual change. We recorded an annual decline in species density, although this was a relatively weak effect (−0.13 ± 0.02, Table 2.2).

In our dataset, the seasonal change in the elevation-species density relationship is due to greater seasonal fluctuation at low elevations. As the species pool is larger at lower elevations there is inherently more opportunity for variation at these sites. Consequently, the greater variation at low elevations could be a mathematical artefact. This is not the case, however, as we controlled for the size of the available species pools (Fig. 2.5). This distinction is important as it confirms the dependence of the seasonal effect on elevation, as found in our spatio-temporal model (Table 2.2). Species density fluctuates more at lower elevations.

These patterns of species density appear to be entirely explained by the effects of mean temperature and available area. The residual model of species density had poor explanatory power, indicating that after removing the effects of these two variables there is no spatio-temporal patterning remaining (R^2_{m} = 0.08, Table 2.1). Many studies have stressed the importance of temperature in promoting ant diversity at both local and global scales (Botes *et al.*, 2006; Sanders *et al.*, 2007; Jenkins *et al.*, 2011). The effect of temperature has also been cited to be responsible for seasonal changes in ant diversity (Andersen, 1983). Low temperatures are likely to limit ant foraging, resulting in fewer species being active at particular elevations or time periods. The ubiquitous role of temperature across a range of habitats and scales implies that it is a general mechanism controlling ant diversity (Kaspari *et al.*, 2004; Sanders *et al.*, 2007; Jenkins *et al.*, 2011). By describing ant species density patterns across consecutive seasons and years, we are able to further strengthen this view. We found cooler temperatures in both the dry season and in later years (Appendices S2.1 & S2.2).

Area has also previously been linked to ant diversity across elevation. Sanders (2002) found mid-elevational peaks in ant species richness that were largely explained by the amount of available area within elevational bands. This effect is variable, however, as area had no role in a separate mountain range (Sanders *et al.*, 2003). In our case, a weak indirect area effect (Romdal & Grytnes, 2007) may be in operation: the number of species found in a local community was correlated with the available area within that elevational band. The larger available area could result in a larger species pool which feeds into local communities. This is the indirect elevational area effect (Romdal & Grytnes, 2007). We emphasize the weakness of the area effect in this instance as the next best model included only temperature (Table 2.1).

Sanders (2002) also found that geometric constraints, in the form of the mid-domain effect model, explained large portions of variation in species richness values. Although we did not directly test for such an effect it is unlikely to be responsible for generating the mid-elevational peaks we observe here. This is because the shape of the species density-elevation relationship changes both between seasons and across years. As highlighted by Beck *et al.* (2010), mechanisms that do not accommodate a temporal component cannot be primary drivers of time dependent elevational diversity patterns. There are no temporal predictions in the mid-domain effect model. It must also be noted that the same reasoning could be applied to the effect of available area. Consequently, we interpret the relatively weak influence of area in our models of species density (0.08 ± 0.05, Table 2.2) as driving spatial patterns only. Mean temperature (0.32 ± 0.02) was the primary observed driver of species density through both space and time.

In general, a weak linear decline in abundance across elevation was seen in the wet season and a bell-shaped curve in the dry season (Fig. 2.3). These patterns are largely consistent through time, with the exception of a gradual decline in abundance at low elevations in the wet season across years. Mean temperature and the standard deviation in temperature were completely able to explain these patterns of abundance (Table 2.1). The relationship between mean temperature and abundance has previously been described (Kaspari *et al.*, 2000; Munyai & Foord, 2012): ants are thermophilic and consequently more individuals are found where temperatures are higher.

The role of the standard deviation in temperature in driving abundance is less clear. Higher abundances are seen with greater variation in temperature. One explanation for this may be the thermal partitioning of the day by the ants. Ant species can partition foraging times during the day based on temperature. This can be a mechanism of avoiding competition (Cerda *et al.*, 1997; Stuble *et al.*, 2013). If in operation, we would have expected to see greater abundances of ants at sites where there is a greater variation in temperature. A wider range of temperatures could allow species with high abundance to be active at all time of the day. The same prediction applies to species density but the standard deviation in temperature is not important in explaining this variable. This disparity may be caused by the weak effect of standard deviation in temperature on abundance (0.08 ± 0.01). Such a small effect may not be strong enough to also influence species density.

Evenness displayed a mid-elevational peak. Season and year were included in the spatio-temporal model of evenness, yet both of these had relatively weak effects (Table 2.2). Evenness was marginally lower in the dry season and decreased across years. Consequently, evenness displays a strong elevational pattern, which is largely unaffected by time. To our knowledge, this is the first time that mid-elevational peaks in evenness have been found. Previously, evenness measures have been reported to decline with elevation in northern temperate litter-dwelling arthropods (Lessard *et al.*, 2011) and to have shown no systematic variation with elevation in the Costa Rican herpetofauna (Fauth *et al.*, 1989). Whilst mean temperature was found to be the single best driver of evenness in our study, it explained only a tiny proportion of the existing variation (R^2_{m} = 0.006, Table 2.1). Therefore, it is not clear which variables are driving changes in evenness. Broadly speaking, evenness appears to correlate with species density (Pearsons $r = 0.5$). This is compatible with an assemblage level dominance-impoverishment rule (Parr, 2008). This rule predicts lower species density with low evenness or high dominance - the conceptual inverse of evenness. Two mechanisms may account for this pattern. First, the superior competitive and resource monopolization abilities of dominant ant species could reduce species density (Andersen, 1992; Parr *et al.*, 2005). Dominant ant species may not be present at mid-elevational sites, leading to the pattern we observe here. Second, it may be that only a small number of species are able to thrive, and dominate, in the harsh conditions (cool temperatures) at high elevations. Further studies investigating the functional traits and ecologies of high-elevation ants may resolve which of these mechanisms is responsible for generating the pattern of evenness across elevation.

This is the first analysis of ant diversity within an extensive, combined spatial and temporal context and one of only a few studies to explicitly investigate the temporal variation that is associated with elevational diversity patterns. We found that species density, abundance and evenness vary through time, although to different extents. Time strongly influences patterns of species density whilst evenness is only mildly affected. For species density and abundance, elevational patterns show a strong seasonal signature. This temporal variation is largely explained by variation in mean temperature. More ant individuals and species are found in the hotter parts of the elevational gradient and at warmer time periods. These results suggest a strong environmental, rather than geometrical, control on ant diversity patterns with the Maloti-Drakensberg. Such a conclusion was only possible with the availability of time-series data. This highlights the importance of monitoring ecological communities at multiple points in time if we are to properly understand the factors generating them.

2.6 ACKNOWLEDGEMENTS

We are grateful to the DST-NRF Centre of Excellence for Invasion Biology and the University of Pretoria for supporting the Sani Pass transect and to Natural Environment Research Council for supporting T.R.B. We thank Chantal Ferreira for her work curating the Sani ants, all the volunteers and University of Pretoria students who have helped with fieldwork, the Mazda Wildlife Fund for providing logistical support, and Ezemvelo KZN Wildlife and the Lesotho Ministry of Tourism, Environment and Culture for permission to sample. Hannah Buckley and one other anonymous referee provided comments that greatly improved the manuscript.

2.7 REFERENCES

Andersen, A.N. (1983) Species diversity and temporal distribution of ants in the semi-arid mallee region of northwestern Victoria. *Australian Journal of Ecology*, **8**, 127-137.

Andersen, A.N. (1992) Regulation of momentary diversity by dominant species in exceptionally rich ant communities of the Australian seasonal tropics. *The American Naturalist*, **140**, 401-420.

Axmacher, J.C., Holtmann, G., Scheuermann, L., Brehm, G., Muller-Hohenstein, K. & Fiedler, K. (2004) Diversity of geometrid moths (Lepidoptera : Geometridae) along an Afrotropical elevational rainforest transect. *Diversity and Distributions*, **10**, 293-302.

Barrow, L. & Parr, C.L. (2008) A preliminary investigation of temporal patterns in semiarid ant communities: variation with habitat type. *Austral Ecology*, **33**, 653-662.

Bartoń, K. (2013) *MuMIn: multi-model inference*. Available at: http://www.cran.r-project.org/.

Bates, D., Maechler, M. & Bolker, B. (2013) *lme4: linear mixed-effects models using S4 classes*. Available at: http://www.cran.r-project.org/.

Beck, J., Altermatt, F., Hagmann, R. & Lang, S. (2010) Seasonality in the altitude-diversity pattern of Alpine moths. *Basic and Applied Ecology*, **11**, 714-722.

Bharti, H., Sharma, Y.P., Bharti, M. & Pfeiffer, M. (2013) Ant species richness, endemicity and functional groups, along an elevational gradient in the Himalayas. *Asian Myrmecology*, **5**, 79-101.

Botes, A., McGeoch, M.A., Robertson, H.G., van Niekerk, A., Davids, H.P. & Chown, S.L. (2006) Ants, altitude and change in the northern Cape Floristic Region. *Journal of Biogeography*, **33**, 71-90.

Brühl, C.A., Mohamed, V. & Linsenmair, K.E. (1999) Altitudinal distribution of leaf litter ants along a transect in primary forests on Mount Kinabalu, Sabah, Malaysia. *Journal of Tropical Ecology*, **15**, 265-277.

Carbutt, C. & Edwards, T.J. (2006) The endemic and near-endemic angiosperms of the Drakensberg Alpine Centre. *South African Journal of Botany*, **72**, 105-132.

Cerda, X., Retana, J. & Cros, S. (1997) Thermal disruption of transitive hierarchies in Mediterranean ant communities. *Journal of Animal Ecology*, **66**, 363-374.

Chaladze, G. (2012) Climate-based model of spatial pattern of the species richness of ants in Georgia. *Journal of Insect Conservation*, **16**, 791-800.

Colwell, R.K. & Lees, D.C. (2000) The mid-domain effect: geometric constraints on the geography of species richness. *Trends in Ecology and Evolution*, **15**, 70-76.

Colwell, R.K., Brehm, G., Cardelus, C.L., Gilman, A.C. & Longino, J.T. (2008) Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science*, **322**, 258-261.

Cowling, R.M., Richardson, D.M. & Pierce, S.M. (eds) (1997) *Vegetation of southern Africa*. Cambridge University Press, Cambridge, UK.

Deblauwe, I. & Dekoninck, W. (2007) Spatio-temporal patterns of ground-dwelling ant assemblages in a lowland rainforest in southeast Cameroon. *Insectes Sociaux*, **54**, 343-350.

Del Toro, I., Ribbons, R.R. & Pelini, S.L. (2012) The little things that run the world revisited: a review of ant-mediated ecosystem services and disservices (Hymenoptera: Formicidae). *Myrmecological News*, **17**, 133-146.

Dunn, R.R., McCain, C.M. & Sanders, N.J. (2007) When does diversity fit null model predictions? Scale and range size mediate the mid-domain effect. *Global Ecology and Biogeography*, **16**, 305-312.

Fauth, J.E., Crother, B.I. & Slowinski, J.B. (1989) Elevational patterns of species richness, evenness, and abundance of the Costa Rican leaf-litter herpetofauna. *Biotropica*, **21**, 178-185.

Fisher, B.L. (1999) Ant diversity patterns along an elevational gradient in the Reserve Naturelle Integrale d'Andohahela, Madagascar. *Fieldiana Zoology*, **85**, 93-108.

Gaston, K.J. (2000) Global patterns in biodiversity. *Nature*, **405**, 220-227.

Gotelli, N.J. & Colwell, R.K. (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379-391.

Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Springer-Verlag, Berlin.

Hurlbert, S.H. (1971) Nonconcept of species diversity: critique and alternative parameters. *Ecology*, **52**, 577-585.

Jenkins, C.N., Sanders, N.J., Andersen, A.N. *et al.* (2011) Global diversity in light of climate change: the case of ants. *Diversity and Distributions*, **17**, 652-662.

Kaspari, M. & Weiser, M.D. (2000) Ant activity along moisture gradients in a neotropical forest. *Biotropica*, **32**, 703-711.

Kaspari, M., Alonso, L. & O'Donnell, S. (2000) Three energy variables predict ant abundance at a geographical scale. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 485-489.

Kaspari, M., Ward, P.S. & Yuan, M. (2004) Energy gradients and the geographic distribution of local ant diversity. *Oecologia*, **140**, 407-413.

Kendall, M.G. & Stuart, A. (1976) *The advanced theory of statistics*. Griffin, London.

Körner, C. (2007) The use of 'altitude' in ecological research. *Trends in Ecology and Evolution*, **22**, 569-574.

Kuhlmann, M. (2009) Patterns of diversity, endemism and distribution of bees (Insecta: Hymenoptera: Anthophila) in southern Africa. *South African Journal of Botany*, **75**, 726-738.

Lessard, J.-P., Sackett, T.E., Reynolds, W.N., Fowler, D.A. & Sanders, N.J. (2011) Determinants of the detrital arthropod community structure: the effects of temperature and resources along an environmental gradient. *Oikos*, **120**, 333-343.

Lomolino, M.V. (2001) Elevation gradients of species-density: historical and prospective views. *Global Ecology and Biogeography*, **10**, 3-13.

Machac, A., Janda, M., Dunn, R.R. & Sanders, N.J. (2011) Elevational gradients in phylogenetic structure of ant communities reveal the interplay of biotic and abiotic constraints on diversity. *Ecography*, **34**, 364-371.

McCain, C.M. (2004) The mid-domain effect applied to elevational gradients: species richness of small mammals in Costa Rica. *Journal of Biogeography*, **31**, 19-31.

Munyai, T.C. & Foord, S.H. (2012) Ants on a mountain: spatial, environmental and habitat associations along an altitudinal transect in a centre of endemism. *Journal of Insect Conservation*, **16**, 677-695.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853-858.

Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, **4**, 133-142.

Naniwadekar, R. & Vasudevan, K. (2007) Patterns in diversity of anurans along an elevational gradient in the Western Ghats, South India. *Journal of Biogeography*, **34**, 842-853.

Nel, W. & Sumner, P.D. (2006) Trends in rainfall total and variability (1970-2000) along the KwaZulu-Natal Drakensberg foothills. *South African Geographic Journal*, **88**, 130-137.

Nel, W. & Sumner, P. (2008) Rainfall and temperature attributes on the Lesotho-Drakensberg escarpment edge, southern Africa. *Geografiska Annaler: Series A, Physical Geography*, **90**, 97-108.

Ness, J., Mooney, K. & Lach, L. (2010) Ants as mutualists. *Ant Ecology* (ed. by L. Lach, C.L. Parr and K.L. Abbott), pp. 97-114. Oxford University Press, Oxford.

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 637-669.

Parr, C.L. (2008) Dominant ants can control assemblage species richness in a South African savanna. *Journal of Animal Ecology*, **77**, 1191-1198.

Parr, C.L., Sinclair, B.J., Andersen, A.N., Gaston, K.J. & Chown, S.L. (2005) Constraint and competition in assemblages: a cross-continental and modeling approach for ants. *The American Naturalist*, **165**, 481-494.

Pianka, E.R. (1966) Latitudinal gradients in species diversity: a review of the concepts. *The American Naturalist*, **100**, 33-46.

R Core Team (2013) *R: a language and environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. Available at: http://www.r-project.org/.

Rahbek, C. (1995) The elevational gradient of species richness: a uniform pattern? *Ecography*, **18**, 200-205.

Rahbek, C. (2005) The role of spatial scale and the perception of large-scale species-richness patterns. *Ecology Letters*, **8**, 224-239.

Robertson, H.G. (2002) Comparison of leaf litter ant communities in woodlands, lowland forests and montane forests of north-eastern Tanzania. *Biodiversity and Conservation*, **11**, 1637-1652.

Rohde, K. (1992) Latitudinal gradients in species diversity: the search for the primary cause. *Oikos*, **65**, 514-527.

Romdal, T.S. & Grytnes, J.-A. (2007) An indirect area effect on elevational species richness patterns. *Ecography*, **30**, 440-448.

Sabu, T.K., Vineesh, P.J. & Vinod, K.V. (2008) Diversity of forest litter-inhabiting ants along elevations in the Wayanad region of the Western Ghats. *Journal of Insect Science*, **8**, Article 69.

Samways, M.J. (2005) *Insect diversity conservation*. Cambridge University Press, Cambridge.

Sanders, N.J. (2002) Elevational gradients in ant species richness: area, geometry, and Rapoport's rule. *Ecography*, **25**, 25-32.

Sanders, N.J., Moss, J. & Wagner, D. (2003) Patterns of ant species richness along elevational gradients in an arid ecosystem. *Global Ecology and Biogeography*, **12**, 93-102.

Sanders, N.J., Lessard, J.-P., Fitzpatrick, M.C. & Dunn, R.R. (2007) Temperature, but not productivity or geometry, predicts elevational diversity gradients in ants across spatial grains. *Global Ecology and Biogeography*, **16**, 640-649.

Schielzeth, H. (2010) Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, **1**, 103-113.

Stuble, K.L., Rodriguez-Cabal, M.A., McCormick, G.L., Jurić, I., Dunn, R.R. & Sanders, N.J. (2013) Tradeoffs, competition, and coexistence in eastern deciduous forest ant communities. *Oecologia*, **171**, 981-992.

Sundqvist, M.K., Sanders, N.J. & Wardle, D.A. (2013) Community and ecosystem responses to elevational gradients: processes, mechanisms, and insights for global change. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 261-280.

White, E.P., Ernest, S.K.M., Adler, P.B., Hurlbert, A.H. & Lyons, S.K. (2010) Integrating spatial and temporal approaches to understanding species richness. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 3633-3643.

Whitford, W.G. (1978) Structure and seasonal activity of Chihuahuan desert ant communities. *Insectes Sociaux*, **25**, 79-88.

Willis, K.J. & Whittaker, R.J. (2002) Species diversity - scale matters. *Science*, **295**, 1245-1248.

Wilson, R.J., Gutiérrez, D., Gutiérrez, J., Martínez, D., Agudo, R. & Monserrat, V.J. (2005) Changes to the elevational limits and extent of species ranges associated with climate change. *Ecology Letters*, **8**, 1138-1146.

Wu, Y., Yang, Q., Wen, Z., Xia, L., Zhang, Q. & Zhou, H. (2013) What drives the species richness patterns of non-volant small mammals along a subtropical elevational gradient? *Ecography*, **36**, 185-196.

Yusah, K.M., Turner, E.C., Yahya, B.E. & Fayle, T.M. (2012) An elevational gradient in litter-dwelling ant communities in Imbak Canyon, Sabah, Malaysia. *Journal of Tropical Biology and Conservation*, **9**, 192-199.

2.8 APPENDICES

Appendix S2.1 Generalized linear mixed models (GLMM) of spatio-temporal patterns of mean temperature.

A GLMM of the spatio-temporal patterns of mean temperature was constructed in the same way as described for the three response variables in the main text. Gaussian errors were used.

Table S2.1 Parameter estimates and model summary of a generalised linear mixed model linking mean temperature recorded within the Sani Pass to spatio-temporal explanatory variables. The best model, according to the bias corrected Akaike information criterion (AIC_c) is reported. The change in AIC_c between the best model and the next best and worst are also given. Marginal R^2 (R^2_m), measuring variation explained by fixed effects only, and conditional R^2 (R^2_c), measuring variation explained by both fixed and random effects, are given. Estimates are on the standardized scale ± standard error.

AIC_c	416.47
ΔAIC_c (next best)	3.38
ΔAIC_c (worst)	181.72
R^2_m	0.86
R^2_c	0.9

Term	Estimates
Elevation	1.2 ± 2.3
$Elevation^2$	-4.24 ±2.3
Season	-5.52 ±0.26
Year	-0.37 ± 0.13
Elevation:Season	7.17 ± 1.74
$Elevation^2$:Season	-6.33 ± 1.74

Appendix S2.2

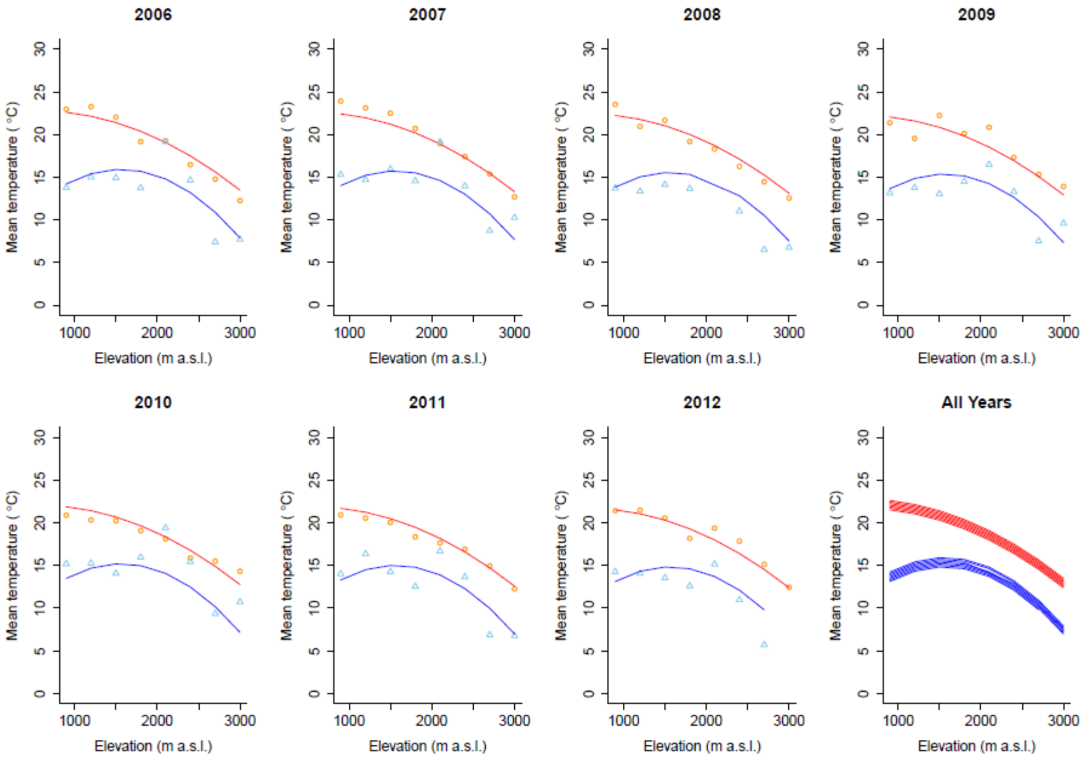


Figure S2.1 Plots of mean temperature in the Sani Pass against elevation for consecutive years and for all years combined. Red lines indicate predicted values from a generalised linear mixed model for January (wet season; circles) and blue lines for September (dry season; triangles).

Chapter 3

Contrasting species and functional beta diversity in montane ant assemblages

Images: Mark Robertson, Tom Rhys Bishop, Berndt Janse van Resnburg and Kate Parr

Contrasting species and functional beta diversity in montane ant assemblages

Tom R. Bishop[1,2*], Mark P. Robertson[2], Berndt J. van Rensburg[2,3]

and Catherine L. Parr[1]

[1]Department of Earth, Ocean and Ecological Sciences, University of Liverpool, Liverpool, L69 3GP, UK,

[2]Centre for Invasion Biology, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa,

[3]School of Biological Sciences, University of Queensland, St. Lucia, Queensland 4072, Australia

**Correspondence: tbish@liv.ac.uk / thomasrhys.bishop@gmail.com*

Published in *Journal of Biogeography* (2015), volume 42 (9), pages 1776-1786

3.1 ABSTRACT

Aim Beta diversity describes the variation in species composition between sites and can be used to infer why different species occupy different parts of the globe. It can be viewed in a number of ways. First, it can be partitioned into two distinct patterns: turnover and nestedness. Second, it can be investigated from either a species identity or a functional-trait point of view. We aim to document for the first time how these two aspects of beta diversity vary in response to a large environmental gradient.

Location Maloti-Drakensberg Mountains, southern Africa.

Methods We sampled ant assemblages along an extensive elevational gradient (900-3000 m a.s.l.) twice yearly for 7 years, and collected functional-trait information related to the species' dietary and habitat-structure preferences. We used recently developed methods to partition species and functional beta diversity into their turnover and nestedness components. A series of null models were used to test whether the observed beta diversity patterns differed from random expectations.

Results Species beta diversity was driven by turnover, but functional beta diversity was composed of both turnover and nestedness patterns at different parts of the gradient. Null models revealed that deterministic processes were likely to be responsible for the species patterns but that the functional changes were indistinguishable from stochasticity.

Main conclusions Different ant species are found with increasing elevation, but they tend to represent an increasingly nested subset of the available functional strategies. This finding is unique and narrows down the list of possible factors that control ant existence across elevation. We conclude that diet and habitat preferences have little role in structuring ant assemblages in montane environments and that some other factor must be driving the non-random patterns of species turnover. This finding also highlights the importance of distinguishing between different kinds of beta diversity.

Keywords: Ants, beta diversity, biodiversity, elevational gradient, Formicidae, functional beta diversity, functional traits, nestedness, southern Africa, turnover

Author contributions: T.R.B. and C.L.P. conceived the research questions. M.P.R. and B.J.v.R. designed and oversaw all historical data collection. T.R.B. collected morphological measurements. T.R.B. analysed the data and wrote the manuscript. All authors contributed to the final draft.

3.2 INTRODUCTION

The concept of beta diversity has a long history in ecology and can be broadly understood as a measure of the variation in species composition between sites. Beta diversity was originally conceived in order to bridge the gap between local (alpha) and regional (gamma) measures of diversity (Whittaker, 1960) and has since become a multifaceted concept with a large number of verbal and mathematical definitions (Tuomisto, 2010; Anderson et al., 2011). Studies of beta diversity describe the extent of compositional differences between sites and also attempt to reveal the assembly mechanisms that drive these differences. Understanding how communities are assembled and structured in space and time, and the variation therein, has basic and applied relevance in ecology (Kraft et al., 2011; Beaudrot et al., 2013) and conservation science (Paknia & Pfeiffer, 2011; Olivier & van Aarde, 2014). Typically, data on species occurrences at sites across a landscape are used to calculate some estimate of beta diversity, but a number of conceptual advances indicate that this approach may only give us a limited insight into the patterns and drivers of beta diversity.

The first of these advances is the partitioning of beta diversity into separate, antithetical components: turnover and nestedness patterns. Although the distinction between these two components is not new (Harrison et al., 1992; Wright & Reeves, 1992; Williams et al., 1999), frameworks in which to study them explicitly have only recently been developed (Baselga, 2010; Schmera & Podani, 2011; Carvalho et al., 2012). Turnover occurs when existing species are replaced by different ones at new sites, whereas nestedness patterns result when species loss or gain causes species-poor sites to resemble a strict subset of species-rich sites (Gaston & Blackburn, 2000). Crucially, these two phenomena imply the operation of different ecological processes. Patterns of turnover are expected to be produced by factors that promote endemism at various spatial scales (Bond et al., 2001; Baselga, 2010), whereas nestedness is a result of ordered extinctions or colonizations along gradients (Ulrich et al., 2009). Given the variety of underlying mechanisms that can produce turnover and nestedness, it is important that we are able to distinguish between these patterns if we are to fully understand and apply our knowledge of beta diversity (Williams et al., 1999; Baselga, 2010; Marini et al., 2013).

The second advance is the continued development and implementation of functional-trait-based ecology. Species identities alone do not provide information on their ecology and so analyses that only use taxonomic data are inherently limited (McGill et al., 2006). By incorporating data on functional traits (measurable aspects of organisms that influence their ecology and performance; McGill et al., 2006), we can gain a more detailed insight into biodiversity patterns and processes (Fukami et al., 2005; Swenson et al., 2012; Villéger et al., 2012). In addition, a functional-trait approach allows comparisons to be made between geographical regions that possess different faunas. Indices of functional alpha diversity are already widely used in the ecological literature (Mouchet et al., 2010). More recently, measures of functional beta diversity (Ricotta & Burrascano, 2008; Swenson et al., 2011) - and their decomposition into turnover and nestedness components - have been developed (Villéger et al., 2013; Cardoso et al., 2014).

Here, we explored how the turnover and nestedness components of ant (Hymenoptera: Formicidae) species and functional beta diversity are influenced by elevation. This is the first such investigation of animal beta diversity over an extensive gradient. We characterized functional diversity using a number of morphological measures that relate to the feeding and foraging ecology of the ant species. We hypothesized that these functional traits represent key spectra of ant ecology and could thus drive compositional change across elevations. It must be noted that other behavioural traits that may influence the ecology of species (e.g. foraging time preference or dominance) are not used here: such traits are notoriously difficult to quantify for diverse and little-studied faunas. In addition, purely morphological approaches have previously been shown to capture a wide range of ecological strategies employed by ants (Weiser & Kaspari, 2006; Bihn et al., 2010; Silva & Brandão, 2010).

In conjunction with the morphological trait data, we used an assemblage dataset - sampled twice yearly, representing the two main seasons (wet and dry) - collected over 7 years and ranging in elevation from 900 to 3000 m above sea level (a.s.l.). We asked the following questions: (1) How do species and functional beta diversities relate to changes in elevation? (2) Do these relationships depend on the beta diversity component being analysed or on the season? (3) What can we infer about the ecological processes that drive these patterns? In this case, we are interested in whether deterministic or stochastic processes are in operation. Deterministic processes highlight the role of the niche (e.g. habitat filtering or competitive interactions) in determining the composition of local communities. Stochastic effects, on the other hand, emphasize how random chance generates observed patterns of diversity through sampling and priority effects (Chase & Myers, 2011).

We predicted a distance decay in similarity (increasing beta diversity) with increasing elevational distance. We expected species beta diversity to be driven largely by turnover, because ants (Brühl et al., 1999; Longino & Colwell, 2011) and other organisms (Wang et al., 2012) typically display elevational turnover patterns (although nestedness is not unknown, e.g. Lessard et al., 2007; Bernadou et al., 2015). No previous work has looked at the partitioning of functional beta diversity across elevation, and communities can become phylogenetically clustered at high elevations (Machac et al., 2011; Hoiss et al., 2012; Smith et al., 2014). This suggests that functional diversity could also shrink in size and become restricted to particular phenotypes. In addition, functional diversity is known to shrink at higher latitudes and in harsher climates (Stevens et al., 2003; Lamanna et al., 2014). Consequently, we predict nestedness to underlie our functional beta diversity patterns. We also expected to see strong seasonal effects, based on previous work (Bishop et al., 2014) which found that alpha diversity was dependent on season. We predicted that greater beta diversity will be found during the dry season, when conditions become unfavourable for ants, potentially limiting the elevational range of individual species; i.e. elevations will be more dissimilar from each other in the dry season than they are in the wet season.

Our finding that species and functional beta diversity are actually the products of contrasting patterns and processes highlights the need to distinguish between different views of biodiversity and sheds further light on the elevational ecology of ants.

3.3 MATERIALS AND METHODS

3.3.1 Study site

Sampling took place throughout the Sani Pass, which forms part of the Maloti-Drakensberg Transfrontier Conservation Area and is classified as part of the grassland biome (Mucina & Rutherford, 2006). Sampling locations were located along an elevational transect ranging from 900 m a.s.l. near the village of Ixopo (30°09′ S; 30°03′ E) to 3000 m a.s.l. at a point above the top of the Sani Pass (29°35′ S; 29°17′ E). Eight sampling locations were established in natural vegetation at 300-m vertical intervals. For further details, see Bishop et al. (2014).

3.3.2 Data collection

Ant sampling

Pitfall traps were used to sample the epigaeic (ground-dwelling) ant fauna in the wet season (January) and the dry season (September) from 2006 to 2012. Four replicate sampling blocks were established at each elevational site. Blocks were spaced at least 300 m apart. Each block consisted of 10 pitfall traps arranged in two parallel lines with traps 10 m apart. Each trap had a volume of 150 mL, a diameter of 55 mm and a depth of 70 mm. Rain guards were used to prevent flooding. A 50% solution of ethylene glycol was used to preserve the ant specimens that were caught in the traps. Trapping took place over 5 nights in total, but traps were serviced every two or three days to avoid overfilling. Specimens were transferred into 70% ethanol in the laboratory and identified to morphospecies and species level where possible. These sites and sampling design are the same as those used in Bishop et al. (2014).

Functional traits

Six morphological traits were measured for each species. These were used in various combinations to produce four indices of ecological importance. The resulting indices are expected to capture ecological variation in the feeding and foraging strategies of the different ant species.

1. Weber's length is a measure of body size taken from the anterodorsal margin of the pronotum to the posteroventral margin of the propodeum (Brown, 1953). Body size can influence prey size selection during solitary foraging (Traniello, 1987). Body size can also influence the microhabitats in which different species forage. Large-bodied ants are likely to forage in open conditions on the soil surface, whereas smaller species may occupy finer 'grains' in closed habitats in the leaf litter and soil (Weiser & Kaspari, 2006; Gibb & Parr, 2013).

2. Eye position is calculated as the interocular distance subtracted from the total head width across the eyes. This measure is divided by Weber's length to control for body size. Large values of eye position indicate dorsally positioned eyes (favoured in open habitats; Gibb & Parr, 2013), whereas small values relate to eyes positioned on the side of the head. This distinction is expected to relate both to habitat complexity and to predatory behaviour. Predatory species tend to have small eyes and this trait is correlated with our measure of eye position (Weiser & Kaspari, 2006).

3. Relative leg length is calculated as the sum of the hind femur length and the hind tibia length, divided by Weber's length. Short relative leg lengths correlate with predatory behaviour (Weiser & Kaspari, 2006). Relative leg length may also relate to the complexity of the habitat occupied. Longer legs can be selected for in simple, planar environments (Gibb & Parr, 2013).

4. Relative mandible length is calculated by dividing the length of the mandible from insertion to tip by the head width across the eyes. This measure expresses the size of the mandible as a proportion of head width. Longer mandibles are expected to relate to specialization in a predatory role (Hölldobler & Wilson, 1990; Gronenberg et al., 1997).

Traits were measured to the nearest 0.01 mm using an ocular micrometer attached to a Stemi 2000 stereomicroscope (Carl Zeiss Microscopy, Jena, Germany). Species without eyes were assigned a value of zero for all eye measurements. We used the highest magnification that allowed the structure under measurement to be fitted within the range of the ocular micrometer. Only workers of the minor caste were included in the analyses. Six individuals from each species were measured where possible; 92 species were caught and measured across the entire time series. On average, 5.52 individuals were measured per species.

3.3.3 Analysis

Beta diversity

The species beta-diversity partition proposed by Baselga (2010) and the analogous partition for functional beta diversity developed by Villéger et al. (2013) were used. We chose to use these rather than the alternative developed by Carvalho et al. (2012) and Cardoso et al. (2014) because we were interested in compositional differences strictly due to nestedness, rather than those due to the more general case of richness differences (Carvalho et al., 2012). Differences in richness between elevations have already been investigated at this site (Bishop et al., 2014).

For species and functional compositions, three pairwise beta-diversity metrics were calculated. First, βsor accounts for the total compositional variation between assemblages - including both turnover and nestedness patterns. This is the Sørensen dissimilarity index. Second, βsim captures only compositional changes due to species turnover. This is the Simpson dissimilarity index and is invariant to richness differences (Baselga, 2010). Third, βsne represents the nestedness-resultant dissimilarity and is calculated as the difference between βsor and βsim. For species composition, these pairwise metrics use information on the number of species shared between two sites and the number of species unique to each site. Only species occurrence data were used. For functional composition, the volumes of multivariate trait space shared by two sites and unique to each were used as inputs in the dissimilarity equations (Villéger et al., 2013). To generate this multivariate space, a principal coordinates analysis (PCoA) was used to summarize the trait data. The PCoA allows us to break correlations between traits, creating orthogonal "traits". We calculated a species-by-species Euclidean distance matrix from scaled and centred trait data. The PCoA was run on this distance matrix and the resulting axes were used as four independent, synthetic traits representing different spectra of ant ecological strategies. The ecological meaning of these axes was interpreted based on the loadings of the raw trait values. Assemblages of ants were projected onto this space as a convex hull, with the synthetic trait values of the present species defining the vertices of the hull (Villéger et al., 2008). Species and functional pairwise beta-diversity measures were calculated using the BETAPART package in R (Baselga & Orme, 2012; R Core Team, 2013).

Observed patterns

For each year and season, the four ant assemblages sampled within each elevational band were pooled in order to create assemblages at the elevational site level. This produced a total of 111 assemblages for analysis (8 elevational sites × 2 seasons × 7 years = 111 assemblages, after one assemblage was removed for having too few species to be projected as a convex hull). The three beta-diversity metrics (βsor, βsim and βsne) were then calculated between the lowest-elevation site (900 m a.s.l.) and the seven higher-elevation sites. This was carried out for both taxonomic and functional assemblage composition. We limited this analysis to comparisons against the lowest-elevation site for simplicity and clarity. We present the analyses of all pairwise comparisons in Appendices S1 & S2 in the Supporting Information; the overall finding did not differ. We used generalized linear mixed models (GLMMs) to describe the relationship of each beta-diversity metric to changes in elevation, and to test whether this depended on the season and type of assemblage composition being used (species or functional composition). A polynomial term of elevation was also included to detect nonlinear patterns. Year was used as a random effect to control for temporal pseudoreplication. The LME4 package in R was used to perform the GLMMs (Bates et al., 2014). The numerical variable of change in elevation was centred and standardized to improve the interpretability of the resulting model coefficients (Schielzeth, 2010). An information-theoretic approach was taken to compare models with different combinations of explanatory variables. Bias-corrected Akaike information criterion (AICc) values were compared in order to select the best descriptive model for each beta-diversity metric. Marginal R2 (due to fixed effects only) and conditional R2 (due to both fixed and random effects) were calculated for each model (Nakagawa & Schielzeth, 2013) using the MUMIN package in R (Bartoń, 2013). Model predictions were averaged across years for clarity when plotting. This modelling approach allowed us to simultaneously ask (1) if beta diversity was related to changes in elevation, and (2) whether this relationship differed between species and functional compositions and the seasons.

Standardized patterns

To investigate what processes were driving the patterns of beta diversity and to answer our third question, we used a null modelling approach. This tested whether our observed beta-diversity values were larger or smaller than expected under a stochastic model of community assembly. A separate null modelling procedure was performed for species and functional compositions. For each season and year, a null distribution of beta-diversity values was generated for each of the three metrics. For species composition, this was carried out by generating 1000 random assemblage matrices using the independent swap algorithm (Gotelli, 2000) and recalculating the beta-diversity metrics. This algorithm maintains species occurrence frequency and sample species richness while shuffling species co-occurrence across sites. For functional composition, the assemblage data matrix was kept constant but the synthetic traits associated with each species were randomized 1000 times by randomly shuffling the names of the species in a species-by-traits matrix and recalculating the functional beta-diversity metrics. This procedure retains the structure of the overall trait space, but randomly assigns which species has which phenotype (Swenson, 2014). Standardized effect sizes (SES) were calculated using the observed beta-diversity values and the mean and standard deviation of the null distributions for species and functional compositions in every year and season:

$$\mathrm{SES} = \frac{\mathrm{observed} - \overline{\mathrm{mean(null)}}}{\mathrm{SD\ (null)}}$$

SES values can serve as a measure of departure from a pure null expectation. Values greater than zero are larger than expected whereas those smaller than zero are less than expected. Essentially, departures from 0 indicate non-randomness: values greater than 1.96 or less than −1.96 are significantly greater or less than expected, at $\alpha = 0.05$. As well as the magnitude of departure from our null expectation, we were also interested in any directional trends in the SES values across the elevational gradient. For this, we used GLMMs as described for the observed beta-diversity values.

3.4 RESULTS

3.4.1 Functional trait space

The first two PCoA axes captured c. 80% of the variation present in the morphological structure of the ant traits (Table 3.1). This variation was split nearly evenly between the two axes. Given the loadings of the original traits in the PCoA, we interpret axis 1 as a gradient in predatory specialization (Table 3.1, Fig. 3.1): species with traits associated with being predatory specialists (relatively large mandibles, laterally positioned eyes and relatively short legs) had negative scores on axis 1, whereas species with more generalized traits (relatively short mandibles, dorsally positioned eyes and relatively long legs) had positive scores on axis 1. We interpret axis 2 as representing preference for different habitat complexities (Table 3.1, Fig. 3.1). Species with traits indicating that they occupy dense, complex habitats (small bodies, relatively short mandibles and short legs) had positive scores on axis 2; species with traits indicating that they occupy open, simple habitats (large bodies, relatively long mandibles and relatively long legs) had negative scores on axis 2. As the first two axes contain most of the variation in the morphological traits, we only use these axes for the interpretation of our results.

Table 3.1 Eigenvalues and trait loadings of a principal coordinates analysis (PCoA) describing the morphological structure of the ant fauna of the Sani Pass, southern Africa. Eigenvalues describe the importance of each PCoA axis in explaining variation in ant traits. Trait loadings indicate how strongly each trait is correlated with each axis and in which direction.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalue	154.92	135.82	41.77	31.49
Relative eigenvalue	0.43	0.37	0.11	0.09
Cumulative eigenvalue	0.43	0.80	0.91	1
Trait loadings				
Weber's length	−0.24	−0.69	−0.47	−0.49
Relative leg length	0.63	−0.28	−0.47	0.55
Relative mandible length	−0.54	−0.46	0.29	0.64
Eye position	0.5	−0.48	0.69	−0.23

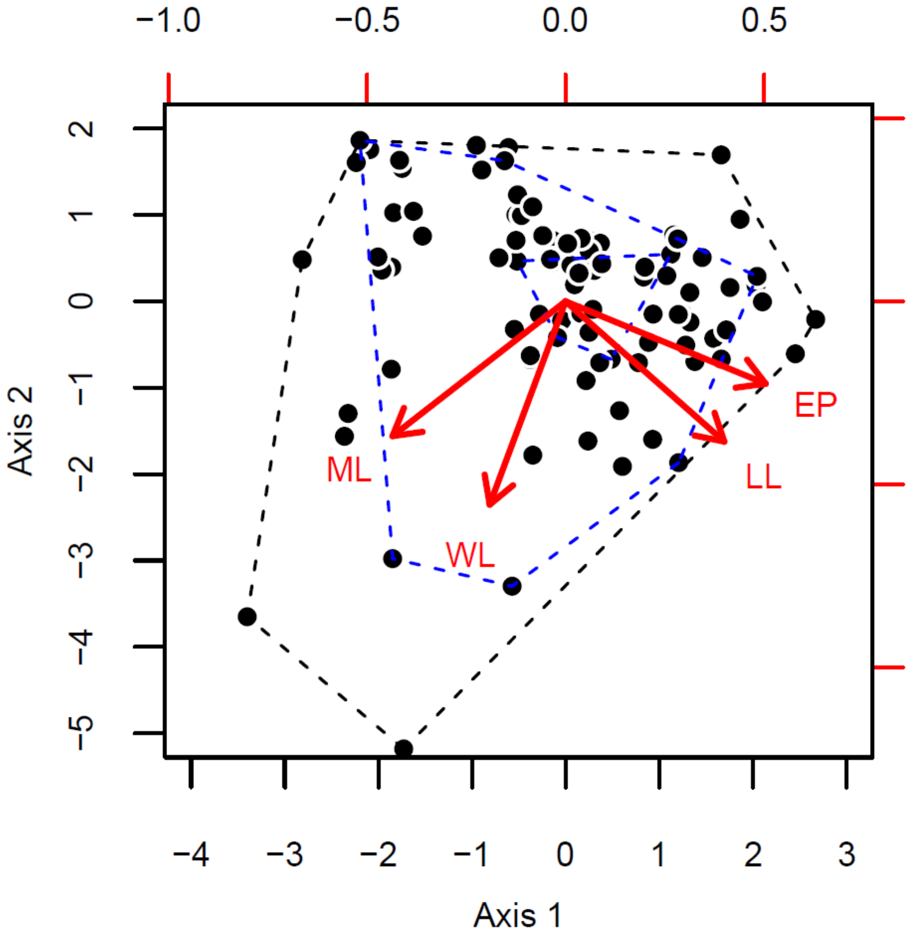


Figure 3.1 Biplot displaying the structure of the morphological space on the first two principal coordinate axes occupied by the ant fauna of the Sani Pass, southern Africa. Each data point is a species. Lower and left hand axes describe the axis scores (synthetic traits) for each species. Upper and right hand axes describe the loadings of each original trait on the principal coordinate axes. The loadings of each original trait are visualized with red labels and arrows (WL, Weber's length; ML, relative mandible length; LL, relative leg length; EP, eye position). For illustration, black dashed lines represent the convex hull of the entire ant fauna. Inner and outer blue dashed lines represent the convex hull of the assemblages at 3000 and 900 m a.s.l., respectively, for the wet season of 2009. These two assemblages display functional nestedness.

3.4.2 Observed patterns

Total species and functional beta diversity (βsor) increase with increasing elevational distance (Fig. 3.2a, Table 3.2). Total species beta diversity is typically higher than functional beta diversity and is higher during the dry season than during the wet season.

Species and functional turnover (βsim) patterns differ in their response to changing elevation (Fig. 3.2b, Table 3.2). Species turnover increases almost linearly with increasing elevational distance, whereas functional turnover peaks at intermediate elevational distances before declining, producing a hump-shaped relationship (Fig. 3.2b). Both species and functional turnover are higher during the dry season than in the wet season.

Species nestedness effectively shows no change with elevational distance and is very low (typically less than βsne = 0.2, Fig. 2c). Functional nestedness displays a U-shaped relationship with elevational distance. It marginally declines from low to intermediate distances and then rapidly increases as elevational distance becomes greater than 1500 m. Both compositions display a small seasonal difference: during the dry season, nestedness is greater at small elevational distances whereas, during the wet season, it is greater at large elevational distances.

Models of total beta diversity and turnover explain a large proportion of the variation in the data (R2m = 0.65-0.66; Table 2), whereas the model for nestedness explains less (R2m = 0.4). None of these three metrics have qualitatively different results when all pairwise comparisons are included (Appendix S3.2).

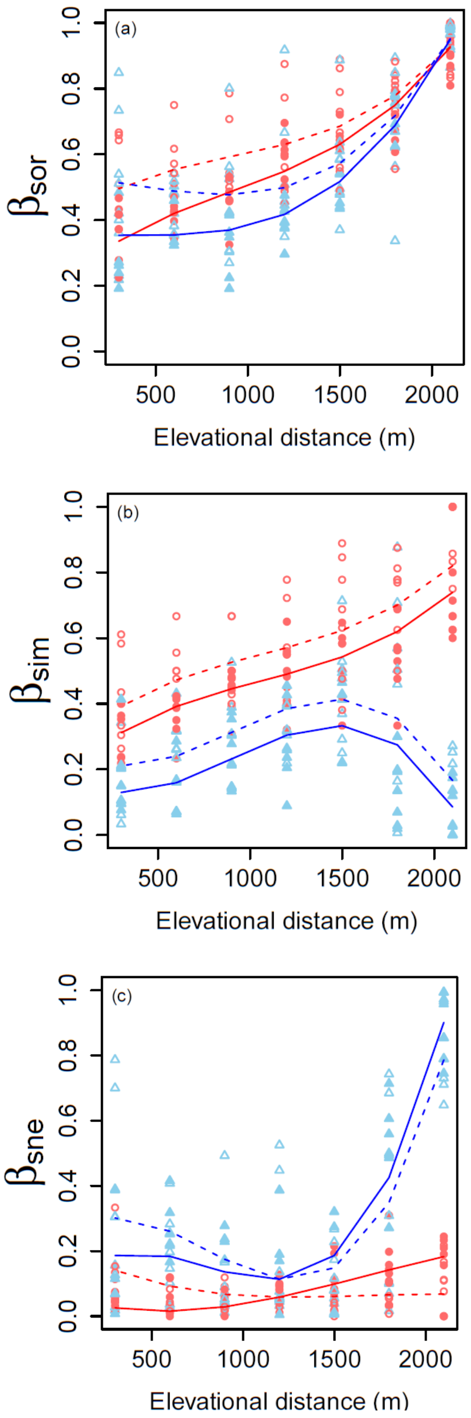


Figure 3.2 Plots showing the relationship between ant species and functional (a) β_{sor} (total beta diversity), (b) β_{sim} (turnover component), and (c) β_{sne} (nestedness-resultant component) and elevational distance in the Sani Pass, southern Africa. Red lines and circles indicate species beta diversity. Blue lines and triangles indicate functional beta diversity. Filled shapes and solid lines indicate data and mixed model predictions for the wet season. Empty shapes and dotted lines indicate those for the dry season. Each data point represents a comparison between the lowest elevation (900 m) and the subsequent higher elevations. Data from all years in the dataset is modelled and plotted.

Table 3.2 Model summaries and parameter estimates for generalized linear mixed models explaining variation in observed and standardized beta diversity of ant assemblages within the Sani Pass, southern Africa. The best model, according to the bias-corrected Akaike information criterion (AIC_c) is reported. Each column reports results from each metric. β_{sor} is total beta diversity, β_{sim} is turnover and β_{sne} is nestedness. The SES prefix indicates beta diversity values standardized by a null model. Marginal R^2 (R^2_m), measuring variation explained by fixed effects only, and conditional R^2 (R^2_c), measuring variation explained by both fixed and random effects, are given. Estimates are on the standardized scale ± standard error. Blank cells indicate variables not included in the best model for that metric.

Model summaries	β_{sor}	β_{sim}	β_{sne}	SES β_{sor}	SES β_{sim}	SES β_{sne}
AIC_c	−292.42	−224.39	−239.72	492.98	530.79	532.11
R^2_m	0.72	0.67	0.73	0.56	0.51	0.5
R^2_c	0.76	0.7	0.74	0.56	0.56	0.54
Estimates						
Composition	−0.008 ± 0.01	−0.2 ± 0.01	0.2 ± 0.01	0.7 ± 0.2	−0.09 ± 0.2	0.006 ± 0.2
Season	0.06 ± 0.01	−0.03 ± 0.01	0.09 ± 0.009	−0.2 ± 0.2	0.1 ± 0.1	−0.4 ± 0.2
Elevational distance	0.07 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.2 ± 0.1	0.03 ± 0.1	−0.1 ± 0.1
Elevational distance^2	−0.1 ± 0.008	−0.06 ± 0.009	−0.08 ± 0.007	0.3 ± 0.09	0.5 ± 0.1	−0.4 ± 0.1
Composition : season				0.2 ± 0.2		0.3 ± 0.2
Composition : elevational distance	0.07 ± 0.02	0.003 ± 0.02	0.07 ± 0.01	1 ± 0.2	−1 ± 0.1	2 ± 0.2
Composition : elevational distance^2	0.02 ± 0.01	0.03 ± 0.01	−0.02 ± 0.009	−0.4 ± 0.1	−0.4 ± 0.1	0.3 ± 0.1
Elevational distance : season		−0.03 ± 0.01	0.03 ± 0.007	0.05 ± 0.2	0.2 ± 0.1	0.05 ± 0.2
Elevational distance : elevational distance^2	0.08 ± 0.005	0.07 ± 0.006	0.02 ± 0.004			
Elevational distance^2 : season	−0.01 ± 0.007		−0.02 ± 0.006			
Composition : elevational distance : season				−0.6 ± 0.2		−0.6 ± 0.2
Composition : elevational distance : elevational distance^2	−0.03 ± 0.007	−0.06 ± 0.009	0.03 ± 0.006			

3.4.3 Standardized patterns

The standardized values of total species beta diversity (SES βsor) increase with increasing elevational distance (Fig. 3.3a, Table 3.2). This increase describes a gradient in SES values from those that are smaller than expected (less than 0), to those that are greater than expected (greater than 0). This relationship has a shallower slope and a higher intercept in the dry season than in the wet season. The standardized values of total functional beta diversity show no strong relationship with elevational distance in either season and deviate little from the null expectation.

There is a near-identical pattern of results for standardized turnover (SES βsim). Standardized species turnover increases from less to greater than expected with increasing elevational distance (Fig. 3.3b, Table 3.2). The slope is shallower and the intercept higher in the dry season. Standardized functional turnover displays a slightly hump-shaped relationship with elevational distance, but again shows no major departure from the null expectation in either season.

Standardized nestedness displays patterns opposite to those for standardized turnover. Standardized species nestedness (SES βsne) decreases with increasing elevational distance (Fig. 3.3c, Table 3.2) with only a minor change between the seasons. Standardized functional nestedness does not differ from the null expectation but does display a mildly U-shaped relationship with elevational distance.

All three models explain similar proportions of variation (R2m ≈ 0.5; Table 3.2). These results show that there is a clear trend for species turnover to be lower than expected at small elevational distances and higher than expected at large elevational distances. This pattern is reversed for species nestedness. Neither functional turnover nor nestedness displays any meaningful departure from the null model.

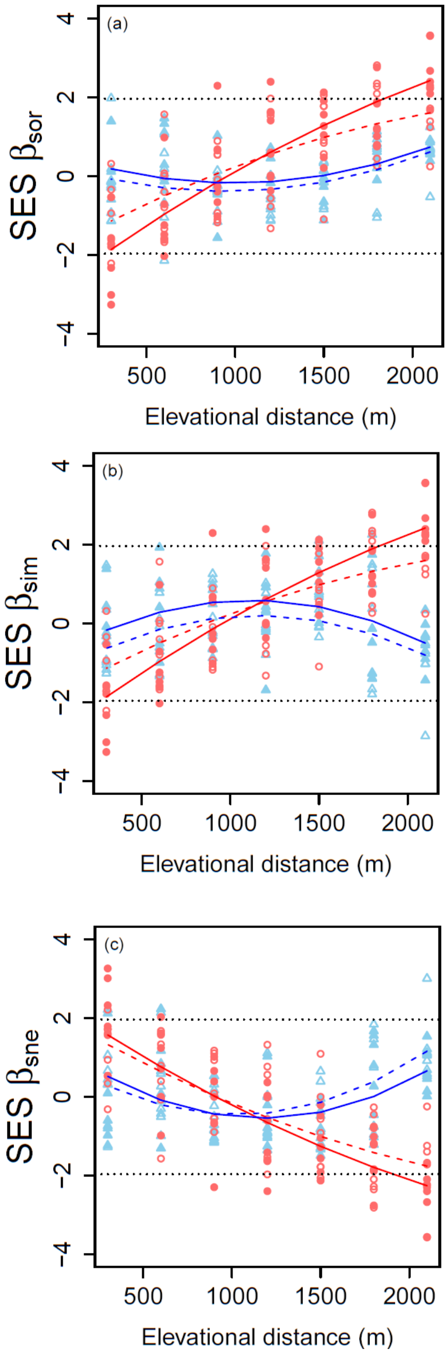


Figure 3.3 Plots showing the relationship between the standardized effect size (SES) of ant species and functional (a) β_{sor} (total beta diversity), (b) β_{sim} (turnover component) and (c) β_{sne} (nestedness-resultant component) and elevational distance in the Sani Pass, southern Africa. Red lines and circles indicate species beta diversity. Blue lines and triangles indicate functional beta diversity. Filled shapes and solid lines indicate data and mixed model predictions for the wet season. Empty shapes and dotted lines indicate those for the dry season. Black dotted lines indicate the $\alpha = 0.05$ threshold of ± 1.96 SES for significantly non-random values. Each data point represents a comparison between the lowest elevation (900 m) and the subsequent higher elevations. Data from all years in the dataset is modelled and plotted.

3.5 DISCUSSION

To our knowledge, this is the first study to partition both species and functional beta diversity for animals along an extensive environmental gradient (but see Villéger et al., 2013, for an example of wide geographical scope). These results give fresh insight into the mechanisms that may control ant elevational diversity. We find that species compositional change is driven by turnover patterns (Fig. 3.2) that cannot be properly explained by stochastic effects (Fig. 3.3). Functional compositional change is more complicated and is produced by a mixture of turnover and nestedness patterns operating between different elevational ranges (Fig. 3.2). These functional changes, however, appear to be completely random with respect to the underlying species beta diversity (Fig. 3.3). Consequently, the deterministic changes in ant assemblages across elevation are not a result of the ecological strategies described by the functional traits that we investigate here.

Our broadest finding is that the further apart two sites are, the more dissimilar they are in terms of both species and functional composition (βsor; Fig. 3.2a). We predicted this classic distance decay of assemblage similarity with elevation, and it has been reported for a range of organisms for species and functional traits (Swenson et al., 2011; Wang et al., 2012). Similar to the results presented by Wang et al. (2012) for macroinvertebrates, we find that species beta diversity is driven by turnover. Species tend to specialize at particular bands of elevation rather than exist across the entire gradient. This is in line with our predictions, and similar patterns have been observed for ants in Malaysia (Brühl et al., 1999) and Tanzania (Robertson, 2002). These comparable patterns across mountains in tropical and subtropical environments imply that there may be a common underlying mechanism generating ant elevational beta diversity. In addition, the species turnover pattern that we report highlights the importance of mountains as reservoirs of unique biodiversity across their entire range. A different interpretation would be reached if species nestedness was observed - under a nestedness scenario, only the lowest elevations would possess unique species.

We also find that beta diversity tends to be higher in the dry season than in the wet season (Fig. 3.2, Table 3.2). We predicted this, because we expected species ranges to shrink into their optimal elevational range during this harsh time of the year, increasing the differences between elevations. This is a relatively small effect, however, and is not consistent across the beta-diversity components. The amount of nestedness in each season depends on the elevational distance, for example (Fig. 3.2).

Contrary to our predictions, we see both functional turnover and nestedness. We expected to observe primarily functional nestedness based on previous work which showed that phylogenetic and functional diversity can shrink in harsher climates (Machac et al., 2011; Lamanna et al., 2014). Our functional turnover patterns show that novel strategies can be favoured in parts of the gradient.

Across our entire 7-year dataset, there is consistency with where changes in the functional structure of assemblages take place. Functional turnover is largely seen through the introduction of species with traits indicating predatory specialization and life in open habitats (negative values on axes 1 and 2, Fig. 3.4). Functional nestedness results in extreme trait combinations being lost from the functional space. This leaves the assemblages at the highest elevations with species that possess generalized traits centred on the origin (0, 0) of the functional trait space. These species tend to be dietary generalists with no strong preference for open or closed environments (Figs 3.1 & 3.4). These patterns could be reflecting deterministic community-assembly processes. Environmental conditions may selectively filter which species are able to successfully establish and survive at each elevation. Such filtering would act on the functional trait values of the species. For this case, it would seem that species with extreme trait values are not able to exist at the highest elevations. This idea is consistent with the clustering and shrinking of phylogenetic diversity at higher elevations (Machac et al., 2011; Hoiss et al., 2012).

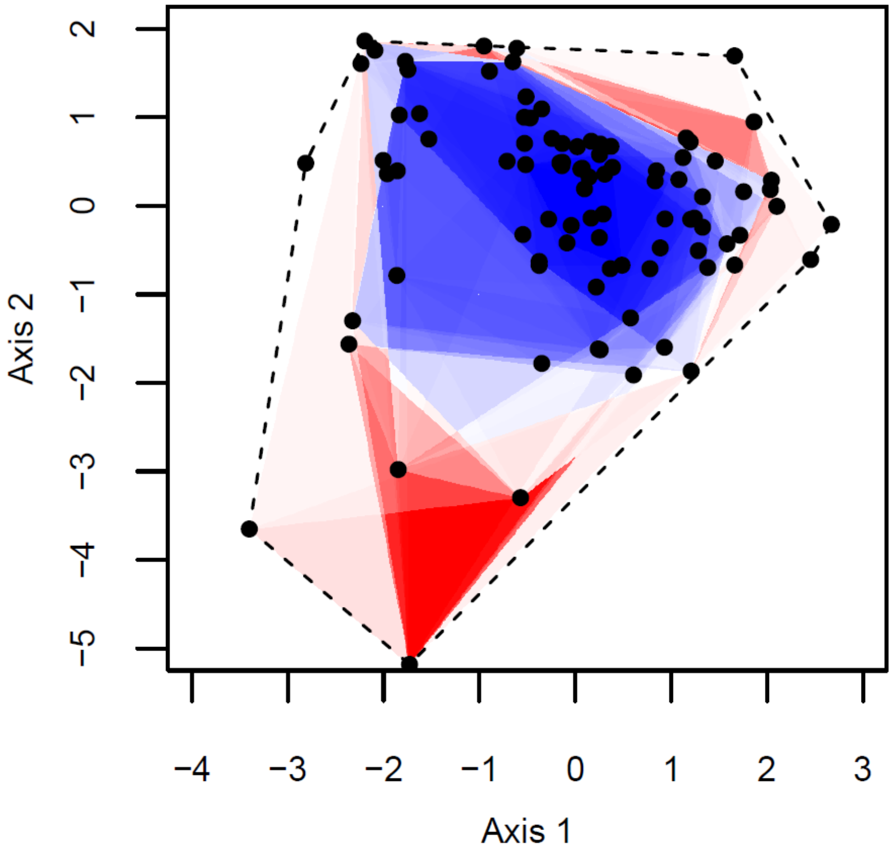


Figure 3.4 Plot of the first two principal coordinate axes of functional trait spaces occupied by the ant fauna of the Sani Pass, southern Africa. Regions where turnover (darker red) and nestedness (darker blue) dominate are highlighted. The density of turnover and nestedness occurrence throughout the space was calculated separately and then combined to produce a single gradient describing the dominance of either pattern. Turnover was defined as areas present in a higher elevations that were not present in the lowest elevation. Nestedness was defined as areas present in higher elevations that were also present in the lowest elevation.

By standardizing our beta diversity measures against appropriate null models, however, we cannot support such a model of trait-based community assembly. Functional beta diversity is random with respect to species beta diversity. In other words, our observed result is indistinguishable from a random assignment of trait values to species. This implies that the traits we investigate have no role in driving the elevational patterns of species beta diversity. We suggest that two factors combine to produce this ‘null’ result. First, there are fewer species at higher elevations than at low elevations (Bishop et al., 2014). Second, the structure of trait space (points in Fig. 3.1) and the species occurrence data (observed species beta diversity) are kept constant during each iteration of the null model. The combination of these factors produces a sampling effect whereby greater functional volumes are achieved with more species - giving rise to our patterns of functional turnover and nestedness. We cannot distinguish the observed patterns of functional beta diversity from this stochastic effect.

If the functional traits we use here are not responsible for the apparent deterministic species turnover, then what is? Factors such as soil structure and the presence of parasitoids are known to influence the structure of ant communities (LeBrun, 2005; Ríos-Casanova et al., 2006). It seems unlikely that these would be able to drive the strong turnover of ant species we observe. There is no directional change in soil composition across the gradient (M. Robertson, pers comm.). In addition, any top-down forces that regulate ant species turnover would presumably have to change with elevation themselves. Instead, we suggest that currently unmeasured physiological traits such as thermal tolerances may be playing an important role in generating species turnover. Such traits have been suggested to be important for other terrestrial insects along elevational gradients (Hodkinson, 2005). Furthermore, the coexistence and distribution of ant species can be influenced by thermal regimes and species tolerances (Wittman et al., 2010; Wiescher et al., 2012).

An investigation into phylogenetic beta diversity (Leprieur et al., 2012) may yield further insights into the relative roles of deterministic and stochastic processes (e.g. Molina-Venegas et al., 2015). This is particularly true if traits are distributed across the phylogeny in interesting ways, such as being clustered or segregated.

This study has begun to reveal in greater detail what kinds of ecological mechanisms may drive ant diversity across broad environmental gradients. We emphasize the importance of beta-diversity partitioning and the functional-trait view of ecology in fully appreciating the distribution and organization of biodiversity. Without using these techniques, we would have been unable to accurately describe how assemblages change with elevation, and we would not have been able to investigate whether a given set of ecologies were able to explain the changes in species distribution.

3.6 ACKNOWLEDGEMENTS

We are grateful to the DST-NRF Centre of Excellence for Invasion Biology and the University of Pretoria for supporting the Sani Pass transect and to the Natural Environment Research Council (NERC)for supporting T.R.B. We thank Chantal Ferreira for her curation work, all volunteer students who helped with fieldwork, the Mazda Wildlife Fund for providing logistical support, Ezemvelo KZN Wildlife and the Lesotho Ministry of Tourism, Environment and Culture for permission to sample, and Ian Smith for advice on high-throughput computing.

3.7 REFERENCES

Anderson, M.J., Crist, T.O., Chase, J.M., Vellend, M., Inouye, B.D., Freestone, A.L., Sanders, N.J., Cornell, H.V., Comita, L.S., Davies, K.F., Harrison, S.P., Kraft, N.J.B., Stegen, J.C. & Swenson, N.G. (2011) Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. *Ecology Letters*, **14**, 19-28.

Bartoń, K. (2013) MuMIn: multi-model inference. R package version 1.9.13. http://cran.r-project.org/package=MuMIn.

Baselga, A. (2010) Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, **19**, 134-143.

Baselga, A. & Orme, C.D.L. (2012) betapart: an R package for the study of beta diversity. *Methods in Ecology and Evolution*, **3**, 808-812.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H. & Dai, B. (2014) lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-6. http://cran.r-project.org/package=lme4.

Beaudrot, L., Rejmánek, M. & Marshall, A.J. (2013) Dispersal modes affect tropical forest assembly across trophic levels. *Ecography*, **36**, 984-993.

Bernadou, A., Espadaler, X., Le Goff, A. & Fourcassié, V. (2015) Ant organization along elevational gradients in a temperate ecosystem. *Insectes Sociaux*, **62**, 59-71.

Bihn, J.H., Gebauer, G. & Brandl, R. (2010) Loss of functional diversity of ant assemblages in secondary tropical forests. *Ecology*, **91**, 782-792.

Bishop, T.R., Robertson, M.P., van Rensburg, B.J. & Parr, C.L. (2014) Elevation-diversity patterns through space and time: ant communities of the Maloti-Drakensberg Mountains of southern Africa. *Journal of Biogeography*, **41**, 2256-2268.

Bond, W.J., Smythe, K.-A. & Balfour, D.A. (2001) Acacia species turnover in space and time in an African savanna. *Journal of Biogeography*, **28**, 117-128.

Brown, W.L. (1953) Revisionary studies in the ant tribe Dacetini. *American Midland Naturalist*, **50**, 1-137.

Brühl, C.A., Mohamed, M. & Linsenmair, K.E. (1999) Altitudinal distribution of leaf litter ants along a transect in primary forests on Mount Kinabalu, Sabah, Malaysia. *Journal of Tropical Ecology*, **15**, 265-277.

Cardoso, P., Rigal, F., Carvalho, J.C., Fortelius, M., Borges, P.A.V., Podani, J. & Schmera, D. (2014) Partitioning taxon, phylogenetic and functional beta diversity into replacement and richness difference components. *Journal of Biogeography*, **41**, 749-761.

Carvalho, J.C., Cardoso, P. & Gomes, P. (2012) Determining the relative roles of species replacement and species richness differences in generating beta-diversity patterns. *Global Ecology and Biogeography*, **21**, 760-771.

Chase, J.M. & Myers, J.A. (2011) Disentangling the importance of ecological niches from stochastic processes across scales. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **366**, 2351-2363.

Fukami, T., Bezemer, T.M., Mortimer, S.R. & van der Putten, W.H. (2005) Species divergence and trait convergence in experimental plant community assembly. *Ecology Letters*, **8**, 1283-1290.

Gaston, K.J. & Blackburn, T.M. (2000) Pattern and process in macroecology. Blackwell Science, Oxford.

Gibb, H. & Parr, C.L. (2013) Does structural complexity determine the morphology of assemblages? An experimental test on three continents. *PLoS ONE*, **8**, e64005.

Gotelli, N.J. (2000) Null model analysis of species co-occurrence patterns. *Ecology*, **81**, 2606-2621.

Gronenberg, W., Paul, J., Just, S. & Hölldobler, B. (1997) Mandible muscle fibers in ants: fast or powerful? *Cell and Tissue Research*, **289**, 347-361.

Harrison, S., Ross, S.J. & Lawton, J.H. (1992) Beta diversity on geographic gradients in Britain. *Journal of Animal Ecology*, **61**, 151-158.

Hodkinson, I.D. (2005) Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews*, **80**, 489-513.

Hoiss, B., Krauss, J., Potts, S.G., Roberts, S. & Steffan-Dewenter, I. (2012) Altitude acts as an environmental filter on phylogenetic composition, traits and diversity in bee communities. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 4447-4456.

Hölldobler, B. & Wilson, E.O. (1990) The Ants. Springer, Berlin.

Kraft, N.J.B., Comita, L.S., Chase, J.M., Sanders, N.J., Swenson, N.G., Crist, T.O., Stegen, J.C., Vellend, M., Boyle, B., Anderson, M.J., Cornell, H.V., Davies, K.F., Freestone, A.L., Inouye, B.D., Harrison, S.P. & Myers, J.A. (2011) Disentangling the drivers of β diversity along latitudinal and elevational gradients. *Science*, **333**, 1755-1758.

Lamanna, C., Blonder, B., Violle, C. et al. (2014) Functional trait space and the latitudinal diversity gradient. *Proceedings of the National Academy of Sciences USA*, **111**, 13745-13750.

LeBrun, E.G. (2005) Who is the top dog in ant communities? Resources, parasitoids, and multiple competitive hierarchies. *Oecologia*, **142**, 643-652.

Leprieur, F., Albouy, C., Bortoli, D.J. & Cowman, P.F. (2012) Quantifying phylogenetic beta diversity: distinguishing between 'true' turnover of lineages and phylogenetic diversity gradients. *PLoS ONE*, **7**, e42760.

Lessard, J.-P., Dunn, R.R., Parker, C.R. & Sanders, N.J. (2007) Rarity and diversity in forest ant assemblages of Great Smoky Mountains National Park. *Southeastern Naturalist*, **6**, 215-228.

Longino, J.T. & Colwell, R.K. (2011) Density compensation, species composition, and richness of ants on a neotropical elevational gradient. *Ecosphere*, **2**, art29.

Machac, A., Janda, M., Dunn, R.R. & Sanders, N.J. (2011) Elevational gradients in phylogenetic structure of ant communities reveal the interplay of biotic and abiotic constraints on diversity. *Ecography*, **34**, 364-371.

Marini, L., Bertolli, A., Bona, E., Federici, G., Martini, F., Prosser, F. & Bommarco, R. (2013) Beta-diversity patterns elucidate mechanisms of alien plant invasion in mountains. *Global Ecology and Biogeography*, **22**, 450-460.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006) Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution*, **21**, 178-185.

Molina-Venegas, R., Aparicio, A., Slingsby, J.A., Lavergne, S. & Arroyo, J. (2015) Investigating the evolutionary assembly of a Mediterranean biodiversity hotspot: deep phylogenetic signal in the distribution of eudicots across elevational belts. *Journal of Biogeography*, **42**, 507-518.

Mouchet, M.A., Villéger, S., Mason, N.W.H. & Mouillot, D. (2010) Functional diversity measures: an overview of their redundancy and their ability to discriminate community assembly rules. *Functional Ecology*, **24**, 867-876.

Mucina, L. & Rutherford, M.C. (2006) The vegetation of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria.

Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, **4**, 133-142.

Olivier, P.I. & van Aarde, R.J. (2014) Multi-scale sampling boosts inferences from beta diversity patterns in coastal forests of South Africa. *Journal of Biogeography*, **41**, 1428-1439.

Paknia, O. & Pfeiffer, M. (2011) Hierarchical partitioning of ant diversity: implications for conservation of biogeographical diversity in arid and semi-arid areas. *Diversity and Distributions*, **17**, 122-131.

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.r-project.org/.

Ricotta, C. & Burrascano, S. (2008) Beta diversity for functional ecology. *Preslia*, **80**, 61-72.

Ríos-Casanova, L., Valiente-Banuet, A. & Rico-Gray, V. (2006) Ant diversity and its relationship with vegetation and soil factors in an alluvial fan of the Tehuacán Valley, Mexico. *Acta Oecologica*, **29**, 316-323.

Robertson, H.G. (2002) Comparison of leaf litter ant communities in woodlands, lowland forests and montane forests of north-eastern Tanzania. *Biodiversity and Conservation*, **11**, 1637-1652.

Schielzeth, H. (2010) Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, **1**, 103-113.

Schmera, D. & Podani, J. (2011) Comments on separating components of beta diversity. *Community Ecology*, **12**, 153-160.

Silva, R.R. & Brandão, C.R.F. (2010) Morphological patterns and community organization in leaf-litter ant assemblages. *Ecological Monographs*, **80**, 107-124.

Smith, M.A., Hallwachs, W. & Janzen, D.H. (2014) Diversity and phylogenetic community structure of ants along a Costa Rican elevational gradient. *Ecography*, **37**, 720-731.

Stevens, R.D., Cox, S.B., Strauss, R.E. & Willig, M.R. (2003) Patterns of functional diversity across an extensive environmental gradient: vertebrate consumers, hidden treatments and latitudinal trends. *Ecology Letters*, **6**, 1099-1108.

Swenson, N.G. (2014) Functional and phylogenetic ecology in R. Springer, New York.

Swenson, N.G., Anglada-Cordero, P. & Barone, J.A. (2011) Deterministic tropical tree community turnover: evidence from patterns of functional beta diversity along an elevational gradient. *Proceedings of the Royal Society B: Biological Sciences*, **278**, 877-884.

Swenson, N.G., Stegen, J.C., Davies, S.J., Erickson, D.L., Forero-Montaña, J., Hurlbert, A.H., Kress, W.J., Thompson, J., Uriarte, M., Wright, S.J. & Zimmerman, J.K. (2012) Temporal turnover in the composition of tropical tree communities: functional determinism and phylogenetic stochasticity. *Ecology*, **93**, 490-499.

Traniello, J.F.A. (1987) Comparative foraging ecology of north temperate ants: the role of worker size and cooperative foraging in prey selection. *Insectes Sociaux*, **34**, 118-130.

Tuomisto, H. (2010) A diversity of beta diversities: straightening up a concept gone awry. Part 1. Defining beta diversity as a function of alpha and gamma diversity. *Ecography*, **33**, 2-22.

Ulrich, W., Almeida-Neto, M. & Gotelli, N.J. (2009) A consumer's guide to nestedness analysis. *Oikos*, **118**, 3-17.

Villéger, S., Mason, N.W.H. & Mouillot, D. (2008) New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, **89**, 2290-2301.

Villéger, S., Ramos Miranda, J., Flores Hernandez, D. & Mouillot, D. (2012) Low functional β-diversity despite high taxonomic β-diversity among tropical estuarine fish communities. *PLoS ONE*, **7**, e40679.

Villéger, S., Grenouillet, G. & Brosse, S. (2013) Decomposing functional β-diversity reveals that low functional β-diversity is driven by low functional turnover in European fish assemblages. *Global Ecology and Biogeography*, **22**, 671-681.

Wang, J., Soininen, J., Zhang, Y., Wang, B., Yang, X. & Shen, J. (2012) Patterns of elevational beta diversity in micro- and macroorganisms. *Global Ecology and Biogeography*, **21**, 743-750.

Weiser, M.D. & Kaspari, M. (2006) Ecological morphospace of New World ants. *Ecological Entomology*, **31**, 131-142.

Whittaker, R.H. (1960) Vegetation of the Siskiyou Mountains, Oregon and California. *Ecological Monographs*, **30**, 279-338.

Wiescher, P.T., Pearce-Duvet, J.M.C. & Feener, D.H. (2012) Assembling an ant community: species functional traits reflect environmental filtering. *Oecologia*, **169**, 1063-1074.

Williams, P.H., de Klerk, H.M. & Crowe, T.M. (1999) Interpreting biogeographical boundaries among Afrotropical birds: spatial patterns in richness gradients and species replacement. *Journal of Biogeography*, **26**, 459-474.

Wittman, S.E., Sanders, N.J., Ellison, A.M., Jules, E.S., Ratchford, J.S. & Gotelli, N.J. (2010) Species interactions and thermal constraints on ant community structure. *Oikos*, **119**, 551-559.

Wright, D.H. & Reeves, J.H. (1992) On the meaning and measurement of nestedness of species assemblages. *Oecologia*, **92**, 416-428.

3.8 APPENDICES

Appendix S3.1 Summary of generalized linear mixed models (GLMMs) explaining variation in observed and standardized beta diversity of ant communities within the Sani Pass, southern Africa. Modelling took place as described in the main text, except that all pairwise comparisons between elevations were included.

Table S3.1 Model summaries for generalized linear mixed models explaining variation in observed and standardized beta diversity of ant communities within the Sani Pass, southern Africa. The best model, according to the bias-corrected Akaike information criterion (AIC_c) is reported. β_{sor} is total beta diversity, β_{sim} is turnover and β_{sne} is nestedness. Marginal R^2 (R^2_m), measuring variation explained by fixed effects only, and conditional R^2 (R^2_c), measuring variation explained by both fixed and random effects, are given.

Model summaries	β_{sor}	β_{sim}	β_{sne}
AIC_c	10462.72	11135.52	10960.81
R^2_m	0.20	0.15	0.15
R^2_c	0.20	0.16	0.15

Appendix S3.2

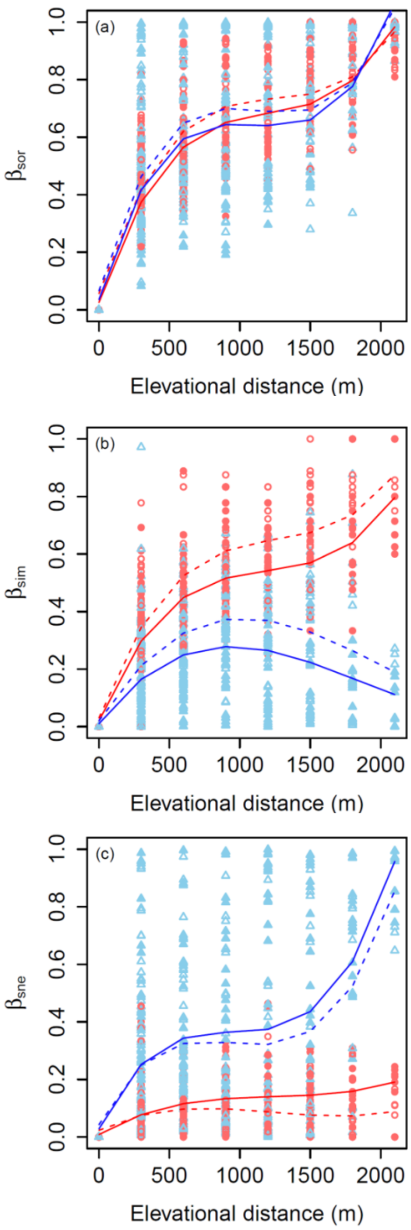


Figure S3.2.1 Plots showing the relationship between ant species and functional (a) β_{sor} (total beta diversity), (b) β_{sim} (turnover component) and (c) β_{sne} (nestedness-resultant component) and elevational distance in the Sani Pass, southern Africa. Red lines and circles indicate species beta diversity. Blue lines and triangles indicate functional beta diversity. Filled shapes and solid lines indicate data and mixed-model predictions for the wet season. Empty shapes and dotted lines indicate those for the dry season. Data points represent all pairwise comparisons between elevations. Data from all years in the dataset are modelled and plotted.

Chapter 4

Ant assemblages have darker and larger members in cold environments

Images: Mark Robertson, Tom Rhys Bishop and Kate Parr

Ant assemblages have darker and larger members in cold environments

Tom R. Bishop[1, 2*], Mark P. Robertson[2], Heloise Gibb[3], Berndt J. van Rensburg[4, 5], Brigitte Braschler[6, 7], Steven L. Chown[8], Stefan H. Foord[9], Caswell T. Munyai[9, 10], Iona Okey[3], Pfarelo G. Tshivhandekano[2], Victoria Werenkraut[11] and Catherine L. Parr[1, 12]

[1] Department of Earth, Ocean and Ecological Sciences, University of Liverpool, Liverpool, L69 3GP, UK; [2] Centre for Invasion Biology, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa; [3] Department of Zoology, La Trobe University, Melbourne, Victoria, 3068, Australia; [4] School of Biological Sciences, University of Queensland, St. Lucia, Queensland 4072, Australia; [5] Centre for Invasion Biology, Department of Zoology, University of Johannesburg, Auckland Park, Johannesburg, 2006, South Africa; [6] Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Matieland, South Africa; [7] Section of Conservation Biology, Department of Environmental Sciences, University of Basel, St. Johanns-Vorstadt 10, 4056 Basel, Switzerland; [8] School of Biological Sciences, Monash University, Victoria 3800, Australia; [9] Centre for Invasion Biology, Department of Zoology, University of Venda, Thohoyandou 0950, South Africa; [10] School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, 3209; [11] Laboratorio Ecotono, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, INIBIOMA-CONICET, Quintral 1250, 8400 Bariloche, Rio Negro, Argentina; [12] School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag X3, Wits 2050, South Africa

**Correspondence: tbish@liv.ac.uk / thomasrhys.bishop@gmail.com*

Submitted to *Global Ecology and Biogeography* 21st March 2016

4.1 ABSTRACT

Aim In ectotherms, the colour of an individual's cuticle may have important consequences for thermoregulation and protection from harmful UV-B radiation. Biophysical theory and individual case studies suggest that cuticle lightness should increase with ambient temperature and decrease with UV-B radiation and body size. We test these predictions in ants (Hymenoptera: Formicidae) across space and through time based on a new, spatially and temporally explicit, global-scale combination of assemblage level and environmental data.

Location Africa, Australia and South America

Methods We sampled ant (Hymenoptera: Formicidae) assemblages (n = 274) along fourteen elevational transects at eight sites on three continents. Individual assemblages ranged from 250 to 3000 m a.s.l. with a minimum to maximum range in summer temperature of 0.5 to 35°C. We used mixed-effects models to explain variation in assemblage cuticle lightness. Explanatory variables were average assemblage body size, temperature and UV-B irradiation. Temporal changes in lightness were examined for a subset of the data.

Results Lightness declined with increasing body size. Lightness increased with increasing temperatures, but declined again at the highest temperatures when there were high levels of UV-B. Through time, temperature and body size explained variation in lightness. Both the spatial and temporal models explained ~50% of the variation in lightness.

Main conclusions Our results provide global support for the thermal melanism hypothesis, and for the importance of considering body size and UV-B radiation exposure in explaining insect cuticle colour. Crucially, this finding is at the assemblage level. Consequently, the relative abundances and identities of ant species that are present in an assemblage can change in accordance with environmental conditions over elevation, latitude and relatively short time-spans of seven years. These findings suggest that there are important constraints on how ectotherm assemblages may be able to respond to rapidly changing environmental conditions.

Keywords: Assemblage structure, colour, elevation, latitude, lightness, temperature, thermal melanism, thermoregulation.

Author contributions: T.R.B. and C.L.P. conceived the research questions. M.P.R. and B.J.v.R. oversaw all historical assemblage data collection for the Maloti-Drakensberg. M.P.R. and P.G.T. collected data from the Mariepskop. S.H.F. and C.T.M. collected from the Soutpansberg. S.L.C. and B.B. oversaw data collection and curation from the Cederberg. H.G. and I.O. collected data from Australia. V.W. collected data from Argentina. T.R.B. collected body size and colour data from the Maloti-Drakensberg and supplemented trait data collection efforts from all other transects except those in Australia. T.R.B. analysed the data and wrote the manuscript. All authors contributed to the final draft.

4.2 INTRODUCTION

Life displays a huge diversity of colour which has captured the imagination of biologists for centuries. Animals use different patterns and hues of colour to disguise or advertise themselves (Ruxton *et al.*, 2004), attract mates (Andersson, 1994) or thermoregulate (Clusella-Trullas *et al.*, 2007). For ectotherms, which make up over 90% of all animal species (Wilson, 1999), thermoregulation is of great importance. Ectotherm metabolism is largely dependent on ambient temperatures and, because of this, their performance and geographic distribution is strongly influenced by temperature gradients (Buckley *et al.*, 2012; Overgaard *et al.*, 2014). Consequently, the ability to thermoregulate in response to these gradients is critical for ectotherm survival (Heinrich, 1996; Chown & Nicolson, 2004).

Ectotherm cuticle colour affects thermoregulation through its reflectivity. A dark coloured or unreflective individual, with high levels of melanin, will heat up faster and achieve higher temperature excesses than a light coloured individual of the same size and shape (Gates, 1980; Willmer & Unwin, 1981). The thermal melanism hypothesis is based on this basic biophysical principle, predicting that darker individuals should predominate in low temperature environments because they will have a higher fitness (Clusella-Trullas *et al.*, 2007). Higher fitness is a consequence of the longer periods of activity available to darker individuals as they are able to warm up and achieve operating temperatures more rapidly (Bogert, 1949; Clusella-Trullas *et al.*, 2007). Indeed clines in melanism along temperature gradients have been reported in several taxa (e.g. butterflies, dragonflies, reptiles, springtails), across a range of spatial scales and at both intra- and interspecific levels (Mani, 1968; Rapoport, 1969; Alho *et al.*, 2010; Zeuss *et al.*, 2014).

A key assumption of the thermal melanism hypothesis is that individuals have the same size and shape, yet, in reality body size and shape varies greatly within and between species. This is important, as body size is a critical factor in determining ectotherm heat budgets. Larger bodies gain and lose heat more slowly than smaller bodies, but also reach higher temperature excesses (Stevenson, 1985; Dzialowski, 2005). This size effect underpins wide-ranging biogeographical predictions such as Bergmann's rule which states that organisms should be larger in cold environments (Blackburn *et al.*, 1999; Olalla-Tárraga & Rodríguez, 2007; Chown & Gaston, 2010).

The effects of colour and body size on ectotherm thermoregulation are expected to interact. Being large in a cold environment may be advantageous in terms of heat conservation, but it also means that the animal in question will heat up relatively slowly. Melanism increases the rate at which heat is gained, so may provide a mechanism by which ectotherms could overcome the limitations of a large body size to operate more effectively in a cold environment (Clusella-Trullas et al., 2007; Moreno Azócar et al., 2015). This melanism-body size interaction is predicted from both theory and experiments (Stevenson, 1985; Shine & Kearney, 2001) and has been shown to operate across large geographic scales in ectotherms (Schweiger & Beierkuhnlein, 2015). We therefore expect both body size and ambient temperature to explain variation in ectotherm colouration - darker forms should be larger and occur more frequently in cold environments.

In addition to these thermoregulatory effects, colour, and specifically melanin, has long been linked with a protective role against harmful ultraviolet-B radiation (e.g. Mani, 1968). UV-B radiation can cause a range of deleterious direct effects on ectotherms. These include genetic and embryonic damage, and indirect effects through changes in host plant morphology and biochemistry (Hodkinson, 2005; Beckmann *et al.*, 2014;

Williamson *et al.*, 2014). Both experiments (Wang *et al.*, 2008) and correlative studies (Bastide *et al.*, 2014) have provided evidence that melanistic individuals or species can be favoured under high UV-B conditions. Gloger's rule (Gaston *et al.*, 2008), that endotherms should be darker at low latitudes, suggests that pigmentation provides protection against a range of factors including UV-B irradiance. Patterns in accordance with Gloger's rule and the influence of UV-B have been observed in a number of endotherms (Burtt, 1981; Caro, 2005) and, more recently, in plants (Koski & Ashman, 2015).

The biophysical principles underlying how temperature, body size and UV-B radiation may affect ectotherm colour are understood and accepted at the level of the individual or the species (e.g. Kingsolver, 1995; Ellers & Boggs, 2004). It is unknown, however, to what extent these effects scale to the assemblage level and how important they are at broad spatial and temporal scales. Understanding assemblage level variation in colour is important as it can reveal how traits influence the performance of species in different environments. In addition, assemblage analyses can generalise across the individualistic responses of each species (McGill *et al.*, 2006; Millien *et al.*, 2006). Assemblage level variation represents changes in the relative abundances of different species - this reflects which trait values appear to be successful under a given set of environmental conditions. In the search for general rules in ecology, rising above the contingencies of extreme behaviours, physiologies or morphologies of individual species is crucial (Chown & Gaston, 2015).

Here, we test if temperature, body size and UV-B can explain variation in ant (Hymenoptera: Formicidae) assemblage cuticle colour - specifically, how light or dark the colour is. The ants are a diverse, numerically dominant and ecologically important group of insects (Hölldobler & Wilson, 1990) with a wide range of body colours (e.g. www.antweb.org). There are also anecdotal reports that ant cuticle colour may change systematically with the environment (Collingwood, 1979; Warren & Chick, 2013). We sampled ant assemblages across replicated elevational gradients on three continents and over multiple years. This design is novel and powerful for two reasons. First, the combined use of assemblage data, elevational gradients and continental variation provides broad ranging yet fine scale insight across a huge range of environmental conditions and geography. This combination of fine grain and large extent is rarely achieved (Beck *et al.*, 2012). Second, our use of time-series data provides greater power to assign mechanistic links between cuticle lightness, temperature, body size and UV-B than spatial data would alone.

If cuticle lightness has a thermoregulatory and protective role then we would expect that average cuticle lightness will be (1) positively related to temperature, (2) negatively related to average body size, and (3) negatively related to UV-B radiation. We test all three predictions across space at a global scale, but only the first two through time.

4.3 MATERIALS AND METHODS

4.3.1 Ant assemblage data

Ant assemblage data were compiled from 14 elevational transects within eight mountain ranges and across three continents (Table 4.1). Ant assemblages were sampled using pitfall traps in almost exactly the same way across all locations. In South Africa and Lesotho, pitfall traps were arranged into a 10 m by 40 m grid. Four grids were placed in each elevational band separated by at least 300 m between grids. Traps were 55 mm in diameter and used a 50% ethylene glycol or propylene glycol solution to preserve caught specimens (Botes *et al.*, 2006; Munyai & Foord, 2012; Bishop *et al.*, 2014). Sampling grids in Australia were the same dimensions, but those within the same elevation were separated by at least 100 m. In Argentina, a sampling grid consisted of nine pitfall traps arranged in a 10 m by 10 m grid, each trap separated from the next by 5 m. A single grid was used at each elevation. Traps had a diameter of 90 mm and used a 40% propylene glycol solution to preserve specimens (Werenkraut *et al.*, 2015). For all sites, specimens were transferred into 70 - 80% ethanol in the laboratory and identified to morphospecies or species level, where possible. Hereafter, all morphospecies and species are collectively referred to as species

All transects were sampled during the austral summer (November - May). Each transect was sampled during a single season, except those in the Maloti-Drakensberg, Cederberg and Soutpansberg of South Africa. These transects were been sampled biannually in two seasons for a number of years. These long-term data are only used in the temporal patterns analysis (see below). For the spatial patterns analysis, only a single summer sampling period was used. For the Maloti-Drakensberg, Cederberg and Soutpansberg a single year was randomly chosen for the spatial patterns analysis. The Argentinian transects were also sampled in two years but only data from 2006 are used here (Werenkraut *et al.*, 2015). Preliminary analyses indicated that both years show the same pattern.

In this study, a sampling grid is considered to be an independent assemblage of ants. We did not pool replicate assemblages within elevational bands. Apart from testing for phylogenetic signal at the genus level, all analyses are performed at the assemblage level. 274 assemblages were available for the main spatial analysis after some assemblages were removed because they did not contain any ants, or environmental data could not be gathered for them.

Table 4.1 Details on the geographical and elevational characteristics of the transects used in this study.

Continent	Mountain range	Approx. lat/long	Transect	Lowest point (m a.s.l.)	Highest point (m a.s.l.)	Number of elevations	Assemblages per elevation	Species richness	References
Africa	Maloti-Drakensberg	-29.73, 29.52	Sani Pass	900	3000	8	4	92	Bishop *et al.* (2014); Bishop *et al.* (2015)
	Soutpansberg	-22.98, 29.42	North Aspect	800	1700	5	4	129	Munyai and Foord (2012); Munyai and Foord (2015)
		-23.04, 29.46	South Aspect	900	1600	5	4 - 8		
	Cederberg	-32.19, 19.1	East Aspect	500	1800	6	4	94	Botes *et al.* (2006)
		-32.23, 19.03	West Aspect	250	1900	10	4		
	Mariepskop	-24.51, 30.87	Mariepskop	700	1900	5	4	92	Tshivhandekano & Robertson. unpublished
Australia	Snowy Mountains	-35.6, 148.51	Back Perisher	400	2000	9	4	109	Gibb et al. unpublished
	Ben Lomond plateau, Tasmania	-41.65, 147.7	Stack's Bluff	400	1400	6	1 - 3	12	Gibb et al. unpublished
	MacDonnell Ranges	-23.36, 132.37	Mt. Zeil	600	1400	5	4	49	Gibb et al. unpublished
South America	Andes, North West Patagonia	-40.75, -71.6	Bayo	900	1700	9	1	15	Werenkraut *et al.* (2015)
		-41.25, -71.3	Chall-Huaco	900	2000	12	1		
		-40.57, -71.69	La Mona	800	1800	11	1		
		-41.09, -71.55	Lopez	800	1800	10	1		
		-40.93, -71.34	Pelado	800	1800	8	1		

4.3.2 Lightness data

The colour of each ant species was classified categorically by eye using a predetermined set of colours (Appendix S4.1). This method allows for a simple and standardised assessment of colour without the need for specialist imaging equipment. The colour of the head, mesosoma and gaster for six individuals of every species in the dataset was recorded. We focussed only on the colour of the cuticle and ignored any colouration offered by hairs. The most common colour across all body parts and individuals was assigned as the dominant colour for each species. Each categorical colour was associated with a set of RGB (red, blue and green) values which were extracted from the original colour wheels using the image editing software paint.NET (v 4.0.3). RGB values were converted into HSV (hue, saturation and value) format using the *rgb2hsv* function in *R.* The HSV model is a common cylindrical-coordinate representation of colour where hue describes the dominant wavelength, saturation indicates the amount of hue present in the colour and the value sets the amount of light in the colour. Only lightness (v, or value, in HSV) is analysed here. A standardised set of 71 photographs from antweb.org was used to assess observer error. Error was low (Appendix S4.1), with the standard error of lightness values estimated from different observers on the same photograph averaging at ~0.04. The five observers in this study tended to assign the same lightness value to the same image.

4.3.3 Body size data

The body size of each species was measured as Weber's length. This is the distance between the anterodorsal margin of the pronotum to the posteroventral margin of the propodeum (Brown, 1953). Weber's length was measured to the nearest 0.01 mm using ocular micrometers attached to stereomicroscopes. The highest level of magnification that allowed the entire mesosoma of the specimen to be fitted under the range of the ocular micrometer was used. Only minor workers were measured. Six specimens for each species were measured where possible. Physical specimens were not available for eight species from the Cederberg transects. For these species Weber's length was measured using high resolution images from AntWeb (http://www.antweb.org) and from existing taxonomic publications (Mbanyana & Robertson, 2008) using the tpsDig2 morphometric software (http://life.bio.sunysb.edu/morph).

Weber's length was not available for the ant species from the MacDonnell Ranges. Instead, it was estimated for these species using the relationship between head width, head length and Weber's length. All three of these traits were available for the Australian Snowy Mountains and Tasmanian ants. Only head width and head length were available for the MacDonnell Ranges ants. Multivariate imputation by chained equations (MICE; Buuren & Groothuis-Oudshoorn, 2011) was performed to estimate the missing Weber's length for these species (Appendix S4.2).

4.3.4 Temperature data

Global environmental data

Estimates of air temperature for all of the assemblages from January to March (peak of the austral summer) were extracted from the *WorldClim* dataset at 30 arc second resolution (Hijmans *et al.*, 2005). Levels of UV-B irradiance for all assemblages were extracted from the *glUV* dataset (Beckmann *et al.*, 2014). Mean UV-B irradiances were calculated using data from January to March.

Data loggers

At all transects in Argentina and at two ranges in southern Africa (Maloti-Drakensberg and Soutpansberg) data loggers were used to record daily temperature. In Argentina, a single HOBO H8 logger (Onset Computer Corporation, MA, USA) was placed at ground level in the centre of each replicate block during the sampling months (Werenkraut *et al.*, 2015). In the two southern African sites Thermocron iButtons (Semiconductor Corporation, Dallas/Maxim, TX, USA) were buried 10 mm below ground level at two replicate blocks (of a possible four) in each elevational band (Munyai & Foord, 2012; Bishop *et al.*, 2014). All temperature data were inspected for cases where the data loggers had been exposed to direct sunlight or had clearly malfunctioned. The mean temperature for each replicate in the sampling month was calculated. These data logger temperatures were used to validate the temperature estimates from *microclim* (Kearney et al., 2014). Furthermore, the data from southern Africa was used to investigate temporal trends (see below).

4.3.5 Statistical methods

All data manipulation and analyses took place in the *R* statistical environment (R Core Team, 2014).

Phylogenetic signal

A genus level, time calibrated ant phylogeny derived from Moreau and Bell (2013) was used to estimate the phylogenetic signal of lightness and body size using Pagel's λ (Pagel, 1999) and Blomberg's K (Blomberg *et al.*, 2003). Lightness and body size traits were averaged at the genus level to test for signal. A likelihood ratio test was used to assess if there was a significant departure of these statistics from 0 (no phylogenetic signal). This was done using the *phytools* package in *R* (Revell, 2012). 77.4% of the genera in this study were present on the phylogeny. Genera missing from the phylogeny were omitted from this analysis.

Assemblage level lightness and body size

Assemblage weighted means (AWM) of lightness and body size were calculated for each assemblage (n = 274) according to:

$$AWM = \sum_{i=1}^{S} p_i x_i$$

Where S is the number of species in an assemblage, p_i is the proportional abundance of each species and x_i is the trait value (lightness or body size) of each species.

Data loggers vs WorldClim

The relationship between the mean temperatures collected through the data loggers and those extracted from *WorldClim* was investigated using type II major axis regression. This was done with the *lmodel2* package in *R* (Legendre, 2008). If the 95% confidence intervals of the intercept and slope encompassed zero and one, respectively, this would indicate that the *WorldClim* temperature data accurately matched that from the data loggers. The significance of the correlation coefficient was assessed using 999 permutations.

Spatial patterns

Linear mixed models (LMMs) were used to assess how much variation in assemblage weighted lightness could be explained by *WorldClim* estimates of temperature, amount of UV-B radiation and assemblage weighted mean body size. Modelling was done using the *lme4* package in *R* (Bates *et al.*, 2014). A term for the temperature-UV-B interaction was also fitted. As temperature correlates positively with UV-B in our dataset

($r = 0.81$, $p < 0.001$), UV-B was regressed on temperature and the residuals of this relationship were used as the UV-B variable. All explanatory variables were scaled and standardised to allow greater interpretability of the regression coefficients (Schielzeth, 2010). Explanatory variables were coded as second order orthogonal polynomials to detect curvature in the relationships between them and assemblage weighted lightness. A nested random effects structure of transect within mountain range within continent was used to account for geographic configuration of the study sites. The response variable of assemblage weighted lightness was logit transformed to meet Gaussian assumptions. An information theoretic approach was used to assess models with different combinations of the explanatory variables. Bias corrected Akaike's information criterion (AIC_c) values were used to compare models. Marginal (due to fixed effects only) and conditional (due to fixed effects and random effects) R^2 values were calculated for each model (Bartoń, 2013; Nakagawa & Schielzeth, 2013). Type III tests using Wald X^2 statistics were used to assess the significance of the predictors in the best model. Each of the 274 observations in this analysis was an independent assemblage of ants.

Common and rare species

Two further spatial analyses took place to disentangle which species were driving the spatial patterns. For each assemblage, common species were defined as those making up 90% of the individuals. The remainder were classed as rare species. This is a proportion of sum rule (Gaston, 1994) that we chose to reflect the extremes of the common-rare spectrum. Assemblage weighted lightness and body size were then recalculated using either only the common species, or only the rare species, in each assemblage. Modelling of the modified assemblage weighted lightness (and modified assemblage weighted body size) took place separately for the common and the rare species as described above for the complete spatial analysis.

Temporal patterns

The Maloti-Drakensberg and Soutpansberg ant assemblages and temperature data are available for multiple years (seven and five, respectively). A LMM was used to relate average lightness to average temperature and body size for each assemblage across all years. Modelling took place as described for the spatial analysis but the random effects structure was modified to take into account temporal pseudoreplication: sampling grid was nested within transect within mountain range. This model allows us to detect whether the lightness values of each assemblage covary according to temporal changes in temperature and body size. There were 206 observations in this analysis representing 41 different replicate assemblages sampled over a number of years (Maloti-Drakensberg = 19 assemblages over 7 years, Soutpansberg = 22 assemblages over 5 years. There were 243 space/time samples available but 37 caught no ants, leading to 206 usable observations).

4.4 RESULTS

Across all transects 592 ant species were collected (Table 4.1). These species spanned the full range of possible lightness values (0 - 1). Weber's length varied from 0.25 to 6.48 mm. Assemblage weighted lightness ranged from 0 to 0.9 whilst assemblage weighted body size ranged from 0.62 to 2.88 mm.

4.4.1 Phylogenetic signal

Lightness was not significantly conserved across the phylogeny (Pagel's $\lambda = 0.32$, $p = 0.06$, Blomberg's K = 0.59, $p = 0.13$). Body size was conserved, however (Pagel's $\lambda = 0.81$, $p = 0.001$, Blomberg's K = 0.86, $p = 0.002$). This signal was due to genera in the Ponerinae subfamily tending to be larger than those in other subfamilies (Appendix S3). We do not consider this to confound the analyses because proportional representation of Ponerinae in the sampled assemblages does not correlate strongly with their average assemblage body sizes ($r = -0.003$, $p = 0.96$). A strong correlation between the proportions of an assemblage that are Ponerines and average body size would have indicated that this phylogenetic signal was influencing the results.

4.4.2 Data loggers vs *WorldClim*

There was a strong correlation between the temperature values obtained from the data loggers and those extracted from *WorldClim* ($r = 0.94$, $p < 0.001$, Appendix S4.4). The intercept did not differ from 0 (95% CIs intercept = -2.69, 0.03) while the slope differed from 1, if only slightly (95% CIs slope = 1.11, 1.13). Thus *WorldClim* temperatures slightly underestimated the data logger temperatures.

4.4.3 Spatial patterns

The best spatial model was also the most complicated. It contained the main effects of temperature, residual UV-B, body size and also included an interaction between temperature and UV-B (Table 4.2). All variables apart from the main effect of residual UV-B radiation were significant according to type III Wald X^2 tests (Table 4.3). Assemblage weighted lightness declined with increasing assemblage weighted body size (Fig. 4.1a). At low levels of residual UV-B, assemblage weighted lightness increased with increasing temperature. At high levels of residual UV-B the relationship between lightness and temperature was unimodal - at higher temperatures lightness declined (Fig. 4.1b). Species richness did not influence these results given the small amount of variation in assemblage lightness that species richness is able to explain (R^2_m = 0.02, R^2_c = 0.38. Appendix S4.5). The same results were found when using *microclim* (Kearney et al., 2014) temperature data rather than *WorldClim* data (Appendix S4.6).

4.4.4 Common and rare species

The best model for common species was exactly the same as the overall spatial model (which used all species) and also explained a similar amount of variance (R^2_m = 0.47, R^2_C = 0.69, Appendix S4.7). For the rare species, the best model contained assemblage body size and residual UV-B. Lightness declined with increasing average body size and formed a U-shaped relationship with residual UV-B. This model did not explain much variation (R^2_m = 0.15, R^2_C = 0.47, Appendix S4.7).

4.4.5 Temporal patterns

The best temporal model included both mean temperature and body size (Table 4.2). Lightness showed a negative relationship with body size (Fig. 4.2a) and a positive relationship with data logger derived temperature (Fig. 4.2b). Both body size and temperature were significant according to type III Wald X^2 tests (Table 4.3).

Table 4.2 Comparative and summary statistics for linear mixed models explaining variation in ant assemblage colour across space or through time. Predictors were all second order orthogonal polynomials and included average body size ($BS + BS^2$), average summer temperature ($T + T^2$) and average residual UV-B radiation ($UV + UV^2$). The temperature variables were derived from *WorldClim* for the spatial models and from data loggers in the temporal models. Listed are the degrees of freedom (d.f.), maximum log-likelihood (LL), Akaike's bias corrected information criterion (AICc) and it's change relative to the top ranked model (ΔAICc), the model probabilities (wAICc) and the marginal and conditional R^2s. Marginal R^2 (R^2_m) is the amount of variation explained by the fixed effects, conditional R^2 (R^2_c) is that explained by the fixed and random effects.

Model	d.f.	LL	AICc	ΔAICc	wAICc	R^2_m	R^2_c
Spatial							
~ $(BS + BS^2) + (T + T^2) \times (UV + UV^2)$	15	-301.59	635.03	0.00	1.00	0.48	0.62
~ $(BS + BS^2) + (T + T^2) + (UV + UV^2)$	11	-315.07	653.14	18.11	0.00	0.38	0.56
~ $(T + T^2) \times (UV + UV^2)$	13	-313.37	654.14	19.11	0.00	0.41	0.59
~ $(BS + BS^2) + (T + T^2)$	9	-321.63	661.94	26.90	0.00	0.43	0.61
~ $(BS + BS^2) + (UV + UV^2)$	9	-322.69	664.05	29.02	0.00	0.21	0.62
~ $(T + T^2) + (UV + UV^2)$	9	-325.24	669.17	34.14	0.00	0.28	0.52
~ $(T + T^2)$	7	-328.97	672.36	37.33	0.00	0.36	0.59
~ $(UV + UV^2)$	7	-335.04	684.50	49.47	0.00	0.14	0.62
~ $(BS + BS^2)$	7	-347.08	708.58	73.55	0.00	0.07	0.41
~ 1	5	-356.71	723.64	88.61	0.00	0.00	0.44
Temporal							
~ $(BS + BS^2) + (T + T^2)$	10	-112.24	245.60	0.00	0.96	0.49	0.74
~ $(BS + BS^2)$	8	-117.65	252.03	6.43	0.04	0.36	0.69
~ $(T + T^2)$	8	-145.36	307.46	61.85	0.00	0.11	0.59
~ 1	6	-149.12	310.66	65.06	0.00	0.00	0.61

Table 4.3 Test statistics (χ^2), degrees of freedom (d.f.) and p values from type III Wald tests on the best spatial and temporal models (top ranked spatial and temporal models from Table 4.2). Explanatory variables were second order orthogonal polynomials and included average body size ($BS + BS^2$), average summer temperature ($T + T^2$) and average residual UV-B radiation ($UV + UV^2$). The temperature variables were derived from *WorldClim* for the spatial models and from data loggers in the temporal models.

Spatial	χ^2	d.f.	p
$T + T^2$	29.77	2	<0.001
$UV + UV^2$	3.01	2	0.22
$BS + BS^2$	24.81	2	<0.001
$(T + T^2) X (UV + UV^2)$	29.43	4	<0.001
Temporal			
$T + T^2$	16.48	2	<0.001
$BS + BS^2$	87.85	2	<0.001

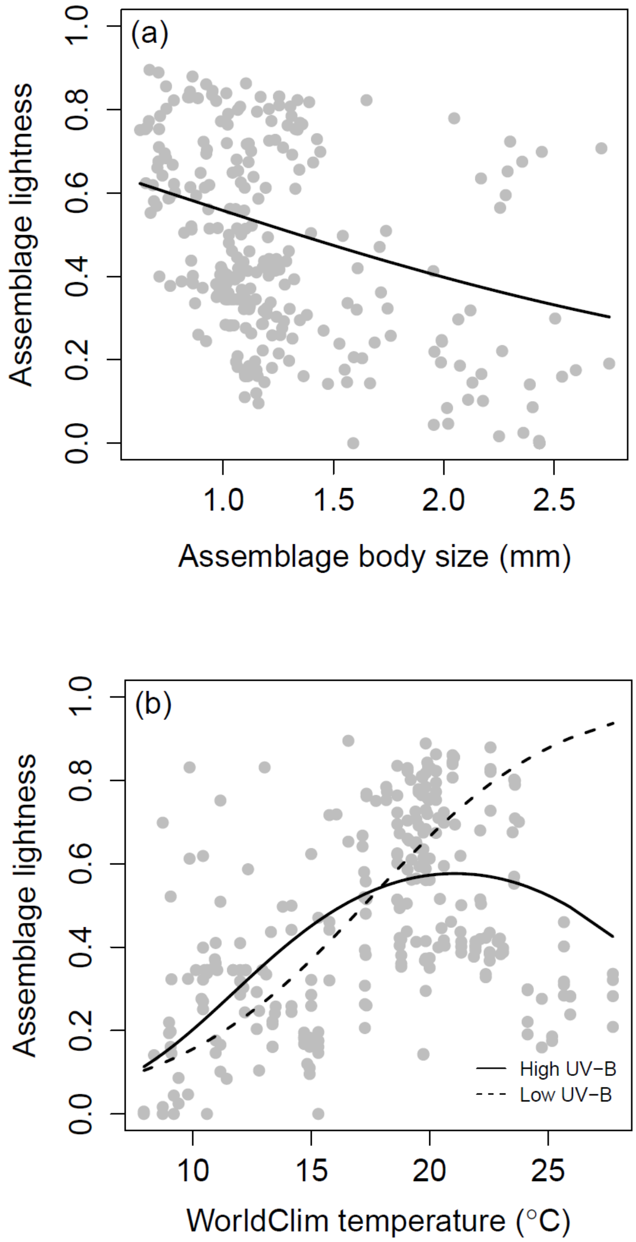


Figure 4.1 Plots showing the relationship between mean assemblage lightness and body size (a) and mean *WorldClim* derived summer temperature (b). Lines display model predictions. In (b), solid line represents predictions for low levels of UV-B (10th percentile), dashed line represents predictions for high UV-B (90th percentile) (n = 274). R^2_m (fixed effects) = 0.48, R^2_c (fixed and random effects) = 0.62.

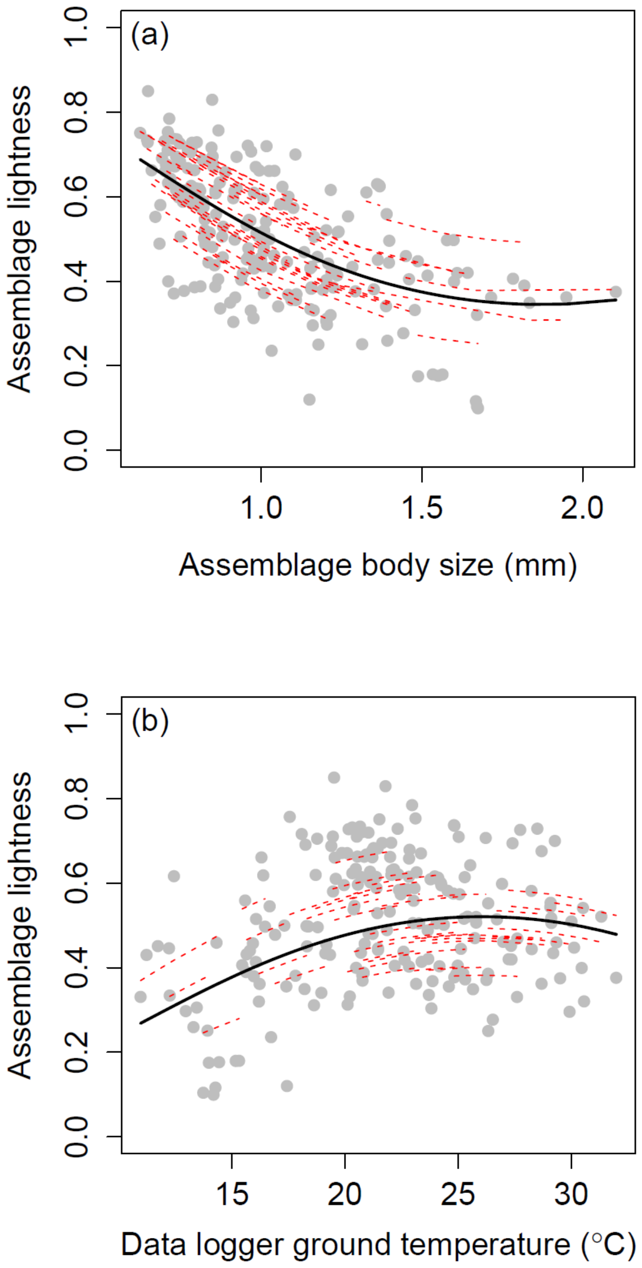


Figure 4.2 Plots showing the relationship between mean assemblage lightness and body size (a) and mean data logger derived summer temperature (b) through time for the Maloti-Drakensberg and Soutpansberg mountain ranges of southern Africa (n = 206). Solid black lines display the average model predictions. Red dashed lines display predictions for each individual assemblage (41 unique assemblages). R^2_m (fixed effects) = 0.49, R^2_c (fixed and random effects) = 0.74.

4.5 DISCUSSION

Our study shows that broad geographic patterns of cuticle colour in ants are consistent with a thermoregulatory and a UV-B protection role, as predicted by experiment and theory (Stevenson, 1985; Shine & Kearney, 2001; Wang *et al.*, 2008). Furthermore, the effects that we detected were at the assemblage level and therefore reflect changes in the relative abundances of species. Generally, the most abundant species are those whose cuticle colour is best suited, in a thermoregulatory or protective sense (Stevenson, 1985; Shine & Kearney, 2001; Wang *et al.*, 2008), for the prevailing environmental conditions. This suggests that assemblage structure will change as the optimum cuticle lightness changes depending on the climate. Our temporal data show that this can happen over a relatively short timescale through shifts in species abundance. Such shifts in assemblage structure under predicted levels of climate change may have cascading effects on ecosystem functioning and integrity.

Across space, we find that, on average, assemblages have lighter cuticles in warm environments and darker cuticles where it is cooler. High UV-B irradiance makes a difference where it is hot, and is associated with darker cuticles (Fig. 4.1b). In addition, assemblage cuticle lightness was negatively correlated with assemblage body size (Fig. 4.1a). We find similar results through time. Our data show that temporal changes in the assemblage cuticle lightness were negatively related to body size (Fig. 4.2a) and positively related to temperature (Fig. 4.2b).

Our data can be interpreted in light of both of the two major contrasting ecogeographic rules that describe and explain colour variation. These are the thermal melanism hypothesis, or Bogert's rule (Clusella-Trullas *et al.*, 2007; Gaston *et al.*, 2009), and Gloger's rule (Caro, 2005; Millien *et al.*, 2006). The two rules differ in their target animal groups and in their principal underlying mechanisms. The thermal melanism hypothesis is usually applied to ectotherms and proposes that darker colours should dominate in cold environments (usually high latitudes or elevations) because of the thermoregulatory benefits of being dark. Gloger's rule is typically applied to endotherms and states that darker colours are found closer to the equator in warmer environments. This pattern may be caused by UV-B protection, camouflage or thermoregulatory needs - white fur can scatter radiation toward the skin for heat gain whilst dark fur can enhance cooling via evaporation (Caro, 2005; Millien *et al.*, 2006; Koski & Ashman, 2015). Whilst the majority of our dataset supports the thermal melanism hypothesis (ants are darker in colder environments) the significant interaction of temperature and UV-B in our modelling procedure (Fig. 4.1b, Table 4.3) suggests that the UV-B protection mechanism of Gloger's rule may also be applicable to ant assemblages (e.g. Bastide *et al.*, 2014; Koski & Ashman, 2015).

Comparable results to ours have been found using multiple species across large areas. For example, South American reptiles and European insects show positive relationships between cuticle or skin lightness and temperature (Clusella-Trullas *et al.*, 2008; Zeuss *et al.*, 2014; Moreno Azócar *et al.*, 2015), whilst the cuticle lightness of carabid beetles is negatively related to body size across Europe (Schweiger & Beierkuhnlein, 2015). Our results are in agreement with these previous findings, but take them a step further by using assemblage level data. This provides information on the identities and relative abundances of the species (and their cuticle lightness) that were active at the time of our sampling. As a consequence, the performance of different lightness values in different environments is captured by our assemblage average. This point is illustrated well in our temporal analysis. The same point in space shows different lightness values under different temperatures - species with the right cuticle lightness are able to rapidly take advantage of altered thermal conditions. The agreement that we find between the spatial and temporal patterns greatly strengthens the power that we have to infer a process of assemblage change mediated by ant physiology than either pattern would in isolation (White *et al.*, 2010).

By restricting our assemblage data to the most common species, we find the same patterns in cuticle lightness. This implies that it is the dominant ant species that are driving the relationships between cuticle lightness, temperature and UV-B. This is important as the dominant species are consuming most of the energy in the system and can structure the rest of the assemblage (Parr, 2008). This finding emphasises the importance of the abiotic environment in structuring local assemblages and contrasts with the majority of the existing literature on ants (e.g. Cerdá *et al.*, 2013) which has tended to focus on the importance of biotic factors such as competition (but see Gibb, 2011). The importance of the common species in driving these macrophysiological patterns echoes similar findings in macroecology where it is also the common species which drive assemblage diversity patterns (Lennon *et al.*, 2004; Vázquez & Gaston, 2004; Reddin *et al.*, 2015).

Previous studies on this topic (Cushman *et al.*, 1993; Zeuss *et al.*, 2014; Schweiger & Beierkuhnlein, 2015), and in macroecology in general (Beck *et al.*, 2012), rarely have the kind of data to draw conclusions at the assemblage level. We argue that understanding this fine spatial and temporal scale of variation is crucial for appreciating how, and why, organisms respond to the environment. Most ectotherms do not interact with each other, or their environment, at the 50 km^2 scale. Instead, it is the success of individuals at finer grains that determines population viability and ultimately drives ecosystem functioning (Ricklefs, 2008). It should be noted, however, that despite the large influence that spatial extent and grain size may have in determining geographic patterns (Rahbek, 2005), the relationships between lightness, temperature and body size in our dataset (grain size of ~400 m^2) are consistent with those studies using a much larger grain size (Zeuss *et al.*, 2014; Schweiger & Beierkuhnlein, 2015). This combination of evidence suggests that the thermoregulatory role of colour in ectotherms may scale consistently to (1) influence the success of individuals (e.g. Ellers & Boggs, 2004), (2) shape assemblage structure (this study) and (3) determine which species are present in the wider regional pool (e.g. Zeuss *et al.*, 2014).

Although our spatial and temporal models explain a large amount of the assemblage level variation in cuticle lightness in our dataset (~50% for fixed effects, Table 4.2), a considerable portion of the variation remains unexplained. There are likely to be two main sources for this variation. The first is methodological. Our use of global surfaces (*WorldClim* and *glUV*) in the spatial analysis is likely to have underestimated the true range of temperatures and UV-B levels that the sampled ant assemblages encounter. This could lead to assemblages appearing lighter or darker than expected for their estimated temperatures. This is less of an issue for our temporal analysis as we used data loggers to track temperature. Secondly, we may be underappreciating the ability of ants to thermoregulate without the use of cuticle colour. A range of other morphological and behavioural mechanisms can play a role in ant thermoregulation. This has been reported mainly for extremely hot conditions. For example, *Cataglyphis* species have been recorded to use body posture, low cuticular transpiration and metabolic rates (Cerdá & Retana, 2000) and specialised reflecting hairs (Shi *et al.*, 2015) to thermoregulate in hot conditions. In addition, ants have been widely reported to forage at cooler times of the day to avoid peak temperatures (Cerdá *et al.*, 1997; Fitzpatrick *et al.*, 2014) which may completely decouple the biophysical link between their morphological thermoregulatory traits and the environment. In cold environments, nest architecture and building materials can keep colonies warm (Kadochová & Frouz, 2013), but there is little reporting of individual worker traits that allow activity to be maintained in the cold. We assume that these mechanisms are the exception rather than the rule but this may not be the case.

In summary, we have shown that the structure of assemblages can be driven by the differential performance of species based on their thermoregulatory traits. This finding suggests that ant assemblages will have to shift in ways consistent with thermoregulatory and protective needs as the climate changes. Under warmer conditions, ants should become smaller (Gibb *et al.*, in review) and lighter coloured. This will likely filter certain kinds of species and alter the functional composition and outputs of assemblages.

4.6 ACKNOWLEDGEMENTS

We thank the DST-NRF Centre of Excellence for Invasion Biology, the University of Pretoria, Chantal Ferreira, the Mazda Wildlife Fund, Ezemvelo KZN Wildlife and the Lesotho Ministry of Tourism, Environment and Culture for their various roles in supporting the Maloti-Drakensberg, Soutpansberg and Cederberg transects. The Mariepskop transect was financially supported by the NRF and 19 Helicopter Squadron at Hoedspruit provided logistical support. T.R.B. was supported by a NERC studentship. Theo Evans and Chris Jeffs provided stimulating discussion that much improved the direction of the manuscript.

4.7 REFERENCES

Alho, J.S., Herczeg, G., Söderman, F., Laurila, A., Jönsson, K.I. & Merilä, J. (2010) Increasing melanism along a latitudinal gradient in a widespread amphibian: local adaptation, ontogenic or environmental plasticity? *BMC Evolutionary Biology*, **10**, 317.

Andersson, M.B. (1994) *Sexual selection*. Princeton University Press, Princeton.

Bartoń, K. (2013) *MuMIn: Multi-Model Inference*. Available at: http://www.cran.r-project.org/.

Bastide, H., Yassin, A., Johanning, E.J. & Pool, J.E. (2014) Pigmentation in Drosophila melanogaster reaches its maximum in Ethiopia and correlates most strongly with ultra-violet radiation in sub-Saharan Africa. *BMC Evolutionary Biology*, **14**, 179.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H. & Dai, B. (2014) *lme4: Linear mixed-effects models using Eigen and S4*. Available at: http://www.cran.r-project.org/.

Beck, J., Ballesteros-Mejia, L., Buchmann, C.M., Dengler, J., Fritz, S.A., Gruber, B., Hof, C., Jansen, F., Knapp, S. & Kreft, H. (2012) What's on the horizon for macroecology? *Ecography*, **35**, 673-683.

Beckmann, M., Václavík, T., Manceur, A.M., Šprtová, L., Wehrden, H., Welk, E. & Cord, A.F. (2014) glUV: a global UV-B radiation data set for macroecological studies. *Methods in Ecology and Evolution*, **5**, 372-383.

Bishop, T.R., Robertson, M.P., van Rensburg, B.J. & Parr, C.L. (2014) Elevation-diversity patterns through space and time: ant communities of the Maloti-Drakensberg Mountains of southern Africa. *Journal of Biogeography*, **41**, 2256-2268.

Bishop, T.R., Robertson, M.P., van Rensburg, B.J. & Parr, C.L. (2015) Contrasting species and functional beta diversity in montane ant assemblages. *Journal of Biogeography*, **42**, 1776-1786.

Blackburn, T.M., Gaston, K.J. & Loder, N. (1999) Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity and Distributions*, **5**, 165-174.

Blomberg, S.P., Garland Jr, T., Ives, A.R. & Crespi, B. (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, **57**, 717-745.

Bogert, C.M. (1949) Thermoregulation in reptiles, a factor in evolution. *Evolution*, **3**, 195-211.

Botes, A., McGeoch, M.A., Robertson, H.G., van Niekerk, A., Davids, H.P. & Chown, S.L. (2006) Ants, altitude and change in the northern Cape Floristic Region. *Journal of Biogeography*, **33**, 71-90.

Brown, W.L. (1953) Revisionary studies in the ant tribe Dacetini. *American Midland Naturalist*, **50**, 1-137.

Buckley, L.B., Hurlbert, A.H. & Jetz, W. (2012) Broad-scale ecological implications of ectothermy and endothermy in changing environments. *Global Ecology and Biogeography*, **21**, 873-885.

Burtt, E.H. (1981) The adaptiveness of animal colors. *Bioscience*, **31**, 723-729.

Buuren, S. & Groothuis-Oudshoorn, K. (2011) mice: Multivariate imputation by chained equations in R. *Journal of Statistical Software*, **45**, 1-67.

Caro, T. (2005) The adaptive significance of coloration in mammals. *Bioscience*, **55**, 125-136.

Cerdá, X. & Retana, J. (2000) Alternative strategies by thermophilic ants to cope with extreme heat: individual versus colony level traits. *Oikos*, **89**, 155-163.

Cerdá, X., Retana, J. & Cros, S. (1997) Thermal disruption of transitive hierarchies in Mediterranean ant communities. *Journal of Animal Ecology*, **66**, 363-374.

Cerdá, X., Arnan, X. & Retana, J. (2013) Is competition a significant hallmark of ant (Hymenoptera: Formicidae) ecology. *Myrmecological News*, **18**, 131-147.

Chown, S.L. & Nicolson, S.W. (2004) *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, Oxford.

Chown, S.L. & Gaston, K.J. (2010) Body size variation in insects: a macroecological perspective. *Biological Reviews*, **85**, 139-169.

Chown, S.L. & Gaston, K.J. (2015) Macrophysiology - progress and prospects. *Functional Ecology*, **30**, 330-344.

Clusella-Trullas, S., van Wyk, J.H. & Spotila, J.R. (2007) Thermal melanism in ectotherms. *Journal of Thermal Biology*, **32**, 235-245.

Clusella-Trullas, S., Terblanche, J., Blackburn, T. & Chown, S. (2008) Testing the thermal melanism hypothesis: a macrophysiological approach. *Functional Ecology*, **22**, 232-238.

Collingwood, C.A. (1979) *The Formicidae (Hymenoptera) of Fennoscandia and Denmark*. Scandinavian Science Press, Klampenborg.

Cushman, J.H., Lawton, J.H. & Manly, B.F. (1993) Latitudinal patterns in European ant assemblages: variation in species richness and body size. *Oecologia*, **95**, 30-37.

Dzialowski, E.M. (2005) Use of operative temperature and standard operative temperature models in thermal biology. *Journal of Thermal Biology*, **30**, 317-334.

Ellers, J. & Boggs, C.L. (2004) Functional ecological implications of intraspecific differences in wing melanization in Colias butterflies. *Biological Journal of the Linnean Society*, **82**, 79-87.

Fitzpatrick, G., Lanan, M.C. & Bronstein, J.L. (2014) Thermal tolerance affects mutualist attendance in an ant-plant protection mutualism. *Oecologia*, **176**, 129-138.

Gaston, K.J. (1994) *Rarity*. Chapman & Hall, London.

Gaston, K.J., Chown, S.L. & Evans, K.L. (2008) Ecogeographical rules: elements of a synthesis. *Journal of Biogeography*, **35**, 483-500.

Gaston, K.J., Chown, S.L., Calosi, P., Bernardo, J., Bilton, D.T., Clarke, A., Clusella-Trullas, S., Ghalambor, C.K., Konarzewski, M. & Peck, L.S. (2009) Macrophysiology: a conceptual reunification. *The American Naturalist*, **174**, 595-612.

Gates, D.M. (1980) *Biophysical Ecology*. Springer, New York.

Gibb, H. (2011) Experimental evidence for mediation of competition by habitat succession. *Ecology*, **92**, 1871-1878.

Gibb, H., Sanders, N.J., Dunn, R.R., Arnan, X., Vasconcelos, H.L., Donoso, D.A., Andersen, A.N., Silva, R.R., Bishop, T.R., Gomez, C., Grossman, B.F., Yusah, K.M., Luke, S.H., Pacheco, R., Pearce-Duvet, J., Retana, J., Tista, M. & Parr, C.L. (in review) Habitat disturbance selects against both small and large body size across climates.

Heinrich, B. (1996) *The Thermal Warriors: Strategies of Insect Survival*. Harvard University Press, Cambridge, Massachusetts.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965-1978.

Hodkinson, I.D. (2005) Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews*, **80**, 489-513.

Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Springer-Verlag, Berlin.

Kadochová, Š. & Frouz, J. (2013) Thermoregulation strategies in ants in comparison to other social insects, with a focus on red wood ants (Formica rufa group) [version 1; referees: 2 approved, 1 approved with reservations]. *F1000Research*, **2**, 280.

Kearney, M.R., Isaac, A.P. & Porter, W.P. (2014) microclim: Global estimates of hourly microclimate based on long-term monthly climate averages. *Scientific Data*, **1**, 140006.

Kingsolver, J.G. (1995) Fitness consequences of seasonal polyphenism in western white butterflies. *Evolution*, **49**, 942-954.

Koski, M.H. & Ashman, T.-L. (2015) Floral pigmentation patterns provide an example of Gloger's rule in plants. *Nature Plants*, **1**, 1-5.

Legendre, P. (2008) *lmodel2: Model II Regression*. Available at: http://www.cran.r-project.org/.

Lennon, J.J., Koleff, P., Greenwood, J.J. & Gaston, K.J. (2004) Contribution of rarity and commonness to patterns of species richness. *Ecology Letters*, **7**, 81-87.

Mani, M.S. (1968) *Ecology and Biogeography of High Altitude Insects*, 1st edn. Springer Netherlands, The Hague.

Mbanyana, N. & Robertson, H.G. (2008) Review of the ant genus Nesomyrmex (Hymenoptera: Formicidae: Myrmicinae) in southern Africa. *African Natural History*, **4**, 35-55.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006) Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, **21**, 178-185.

Millien, V., Kathleen Lyons, S., Olson, L., Smith, F.A., Wilson, A.B. & Yom-Tov, Y. (2006) Ecotypic variation in the context of global climate change: revisiting the rules. *Ecology Letters*, **9**, 853-869.

Moreau, C.S. & Bell, C.D. (2013) Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. *Evolution*, **67**, 2240-2257.

Moreno Azócar, D., Perotti, M., Bonino, M., Schulte, J., Abdala, C. & Cruz, F. (2015) Variation in body size and degree of melanism within a lizards clade: is it driven by latitudinal and climatic gradients? *Journal of Zoology*, **295**, 243-253.

Munyai, T.C. & Foord, S.H. (2012) Ants on a mountain: spatial, environmental and habitat associations along an altitudinal transect in a centre of endemism. *Journal of Insect Conservation*, **16**, 677-695.

Munyai, T.C. & Foord, S.H. (2015) Temporal Patterns of Ant Diversity across a Mountain with Climatically Contrasting Aspects in the Tropics of Africa. *PloS One*, **10**, e0122035.

Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, **4**, 133-142.

Olalla-Tárraga, M.Á. & Rodríguez, M.Á. (2007) Energy and interspecific body size patterns of amphibian faunas in Europe and North America: anurans follow Bergmann's rule, urodeles its converse. *Global Ecology and Biogeography*, **16**, 606-617.

Overgaard, J., Kearney, M.R. & Hoffmann, A.A. (2014) Sensitivity to thermal extremes in Australian Drosophila implies similar impacts of climate change on the distribution of widespread and tropical species. *Global Change Biology*, **20**, 1738-1750.

Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature*, **401**, 877-884.

Parr, C.L. (2008) Dominant ants can control assemblage species richness in a South African savanna. *Journal of Animal Ecology*, **77**, 1191-1198.

R Core Team (2014) *R: A language and environment for Statistical Computing*. R Foundation for Statistical Computing. Available at: http://www.r-project.org/.

Rahbek, C. (2005) The role of spatial scale and the perception of large-scale species-richness patterns. *Ecology Letters*, **8**, 224-239.

Rapoport, E. (1969) Gloger's rule and pigmentation of Collembola. *Evolution*, **23**, 622-626.

Reddin, C., Bothwell, J. & Lennon, J. (2015) Between-taxon matching of common and rare species richness patterns. *Global Ecology and Biogeography*, **24**, 1476-1486.

Revell, L.J. (2012) phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**, 217-223.

Ricklefs, R.E. (2008) Disintegration of the ecological community. *The American Naturalist*, **172**, 741-750.

Ruxton, G.D., Sherratt, T.N. & Speed, M.P. (2004) *Avoiding attack*. Oxford University Press, Oxford.

Schielzeth, H. (2010) Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, **1**, 103-113.

Schweiger, A.H. & Beierkuhnlein, C. (2015) Size dependency in colour patterns of Western Palearctic carabids. *Ecography*, (early view as of 29/03/2016).

Shi, N.N., Tsai, C.-C., Camino, F., Bernard, G.D., Yu, N. & Wehner, R. (2015) Keeping cool: Enhanced optical reflection and heat dissipation in silver ants. *Science*, **349**, 298-301.

Shine, R. & Kearney, M. (2001) Field studies of reptile thermoregulation: how well do physical models predict operative temperatures? *Functional Ecology*, **15**, 282-288.

Stevenson, R. (1985) The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. *American Naturalist*, **126**, 362-386.

Vázquez, L.B. & Gaston, K.J. (2004) Rarity, commonness, and patterns of species richness: the mammals of Mexico. *Global Ecology and Biogeography*, **13**, 535-542.

Wang, Z., Liu, R., Wang, A., Du, L. & Deng, X. (2008) Phototoxic effect of UVR on wild type, ebony and yellow mutants of Drosophila melanogaster: Life Span, fertility, courtship and biochemical aspects. *Science in China Series C: Life Sciences*, **51**, 885-893.

Warren, R.J. & Chick, L. (2013) Upward ant distribution shift corresponds with minimum, not maximum, temperature tolerance. *Global Change Biology*, **19**, 2082-2088.

Werenkraut, V., Fergnani, P.N. & Ruggiero, A. (2015) Ants at the edge: a sharp forest-steppe boundary influences the taxonomic and functional organization of ant species assemblages along elevational gradients in northwestern Patagonia (Argentina). *Biodiversity and Conservation*, **24**, 287308.

White, E.P., Ernest, S.K.M., Adler, P.B., Hurlbert, A.H. & Lyons, S.K. (2010) Integrating spatial and temporal approaches to understanding species richness. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **365**, 3633-3643.

Williamson, C.E., Zepp, R.G., Lucas, R.M., Madronich, S., Austin, A.T., Ballaré, C.L., Norval, M., Sulzberger, B., Bais, A.F. & McKenzie, R.L. (2014) Solar ultraviolet radiation in a changing climate. *Nature Climate Change*, **4**, 434-441.

Willmer, P. & Unwin, D. (1981) Field analyses of insect heat budgets: reflectance, size and heating rates. *Oecologia*, **50**, 250-255.

Wilson, E.O. (1999) *The diversity of life*. WW Norton & Company, New York.

Zeuss, D., Brandl, R., Brändle, M., Rahbek, C. & Brunzel, S. (2014) Global warming favours light-coloured insects in Europe. *Nature Communications*, **5**, 3874.

4.8 APPENDICES

Appendix S4.1 Colour assignment.

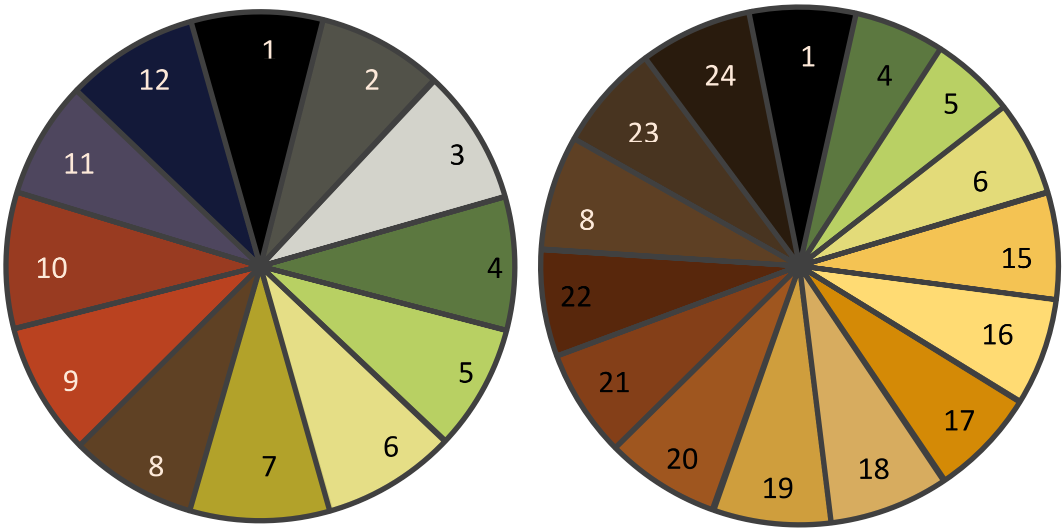


Figure S4.1.1 Colour wheels used to categorise the colour of ant species across the three continents.

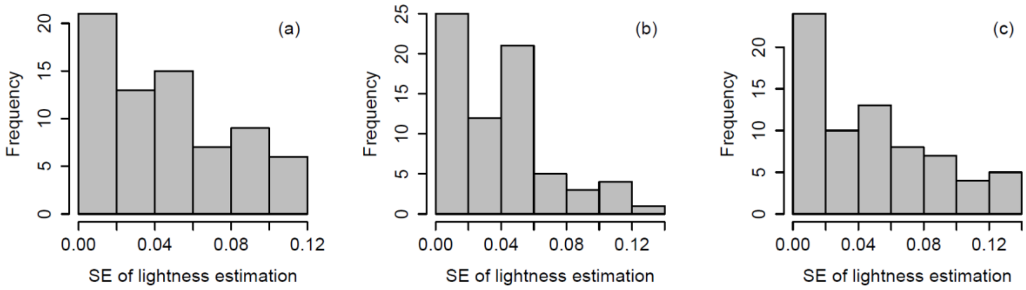


Figure S4.1.2 Histograms showing the standard errors of lightness values estimated for the (a) head, (b) mesosoma and (c) gaster by the five observers used in this study on a set of 71 photographs of ants from antweb.org. Mean standard error for the head is 0.04, mesosoma is 0.036, and gaster is 0.045.

Appendix S4.2 Multivariate imputation using chained equations (MICE) of MacDonnell Ranges body size.

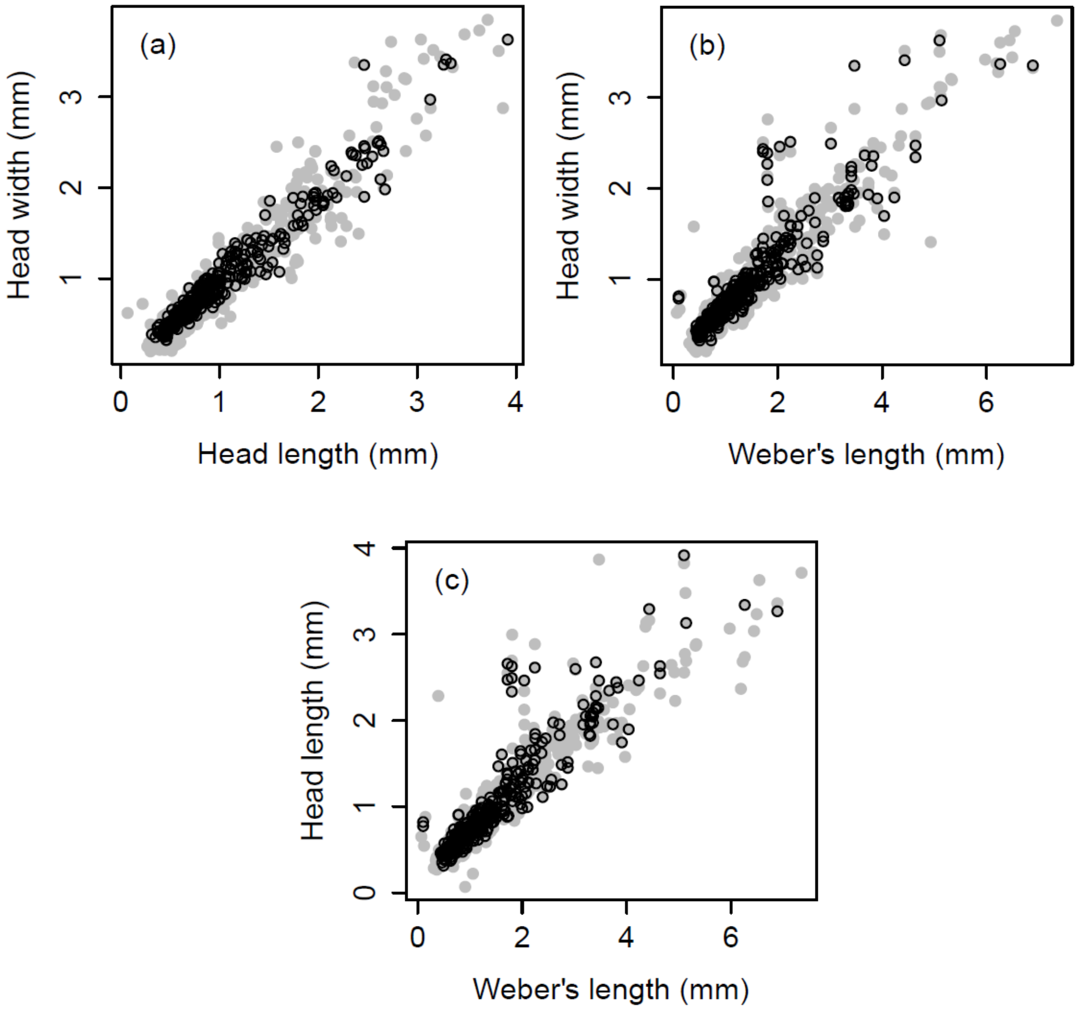


Figure S4.2.1 Plots showing relationship between morphological traits for Australian ants. Values for the MacDonnell Ranges ants are circled in black. For plots (b) and (c) the Weber's length values (x-axis) for the MacDonnell species (black circles) were estimated using a MICE procedure. Plot (a) illustrates that the head width-length relationship for the MacDonnell ants is similar to that for the other Australian sites. Plots (b) and (c) show that the estimated values of Weber's length for the MacDonnell ants conform to the existing relationships between Weber's length, head width and head length. Consequently, the Weber's length values estimated using the MICE procedure are reasonable.

Appendix S4.3 Phylogenetic signal.

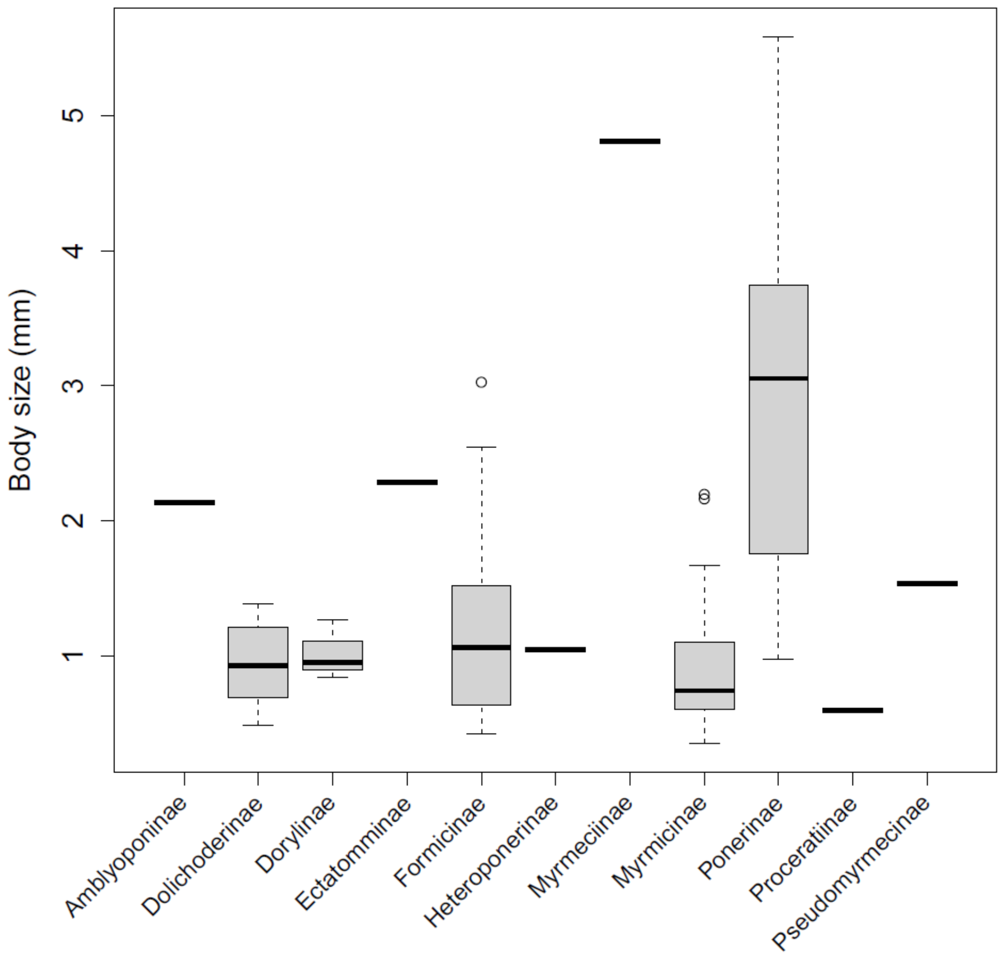


Figure S4.3.1 Plot showing the distribution of average genus body sizes across the ant subfamilies present in this study.

Appendix S4.4 Relationship between temperature values obtained from data loggers in the Maloti-Drakensberg, Soutpansberg and Patagonian Andes and those extracted from *WorldClim*.

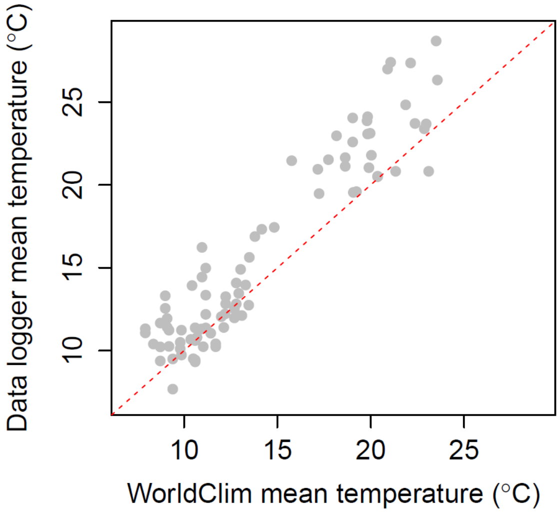


Figure S4.4.1 Relationship between temperature values obtained from data loggers and those extracted from *WorldClim*, ($r = 0.94$, $p < 0.001$). Red line indicates a 1:1 relationship.

Appendix S4.5 Species richness effects.

The distribution of lightness is not evenly spread across the species in the dataset (Fig. S4.5.1). This may produce biased results if sampling effects occur and influence the lightness value of assemblages. For example, an assemblage may be dark in colour simply because a large proportion of the species able to colonise it are themselves dark.

A linear mixed model (LMM) of assemblage lightness as a function of assemblage species richness was run. The random effects structure was the same as that for the spatial model in the main text: transect was nested within mountain range within continent. The effect of species richness was significant according to a type III Wald χ^2 test ($\chi^2 = 5.62$, $p = 0.02$) and had a positive influence (Fig. S4.5.2) on assemblage lightness but actually explained very little variation in assemblage lightness ($R^2_m = 0.02$, $R^2_c = 0.38$). This small R^2_m suggests that richness does not have a large influence on assemblage lightness patterns.

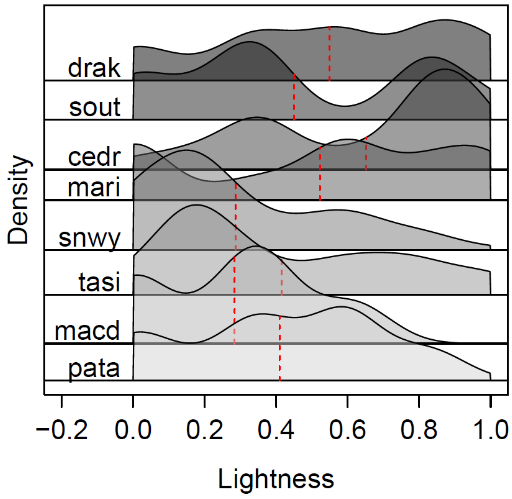


Figure S4.5.1 Stacked density plot showing the distribution of lightness values for each mountain range. Underlying data is at the morphospecies level. Bandwidth used was 0.1. Codes and number of species as follows: drak = Maloti-Drakensberg (n = 92); sout = Soutpansberg (n = 129); cedr = Cederberg (n = 94); mari = Mariepskop (n = 92), snwy = Snowy Mountains (n = 109); tasi = Ben Lomond plateau, Tasmania (n = 12); macd = MacDonnell Ranges (n = 49); pata = Andes, North West Patagonia (n = 15).

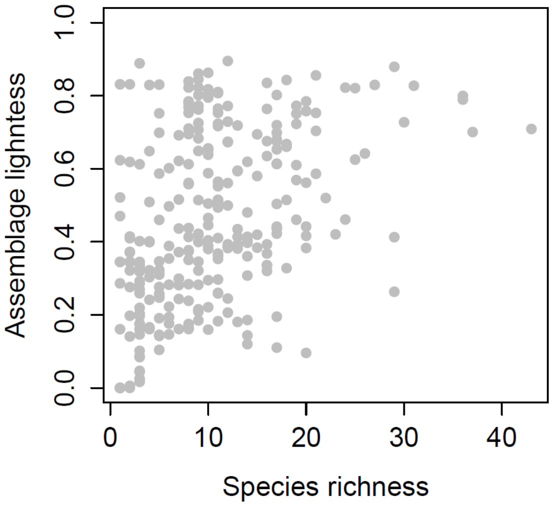


Figure S4.5.2 Plot showing the relationship between assemblage lightness and species richness.

Appendix S4.6 Modelling of lightness across space using *microclim* temperature data.

Data

Soil temperatures at 1 cm above the soil under 0% shade were extracted from the *microclim* dataset (Kearney *et al.*, 2014). A single average was calculated per sampling grid using data from January to March.

Data loggers vs microclim

There was a strong and significant positive correlation between data logger temperature values and *microclim* estimates (r = 0.92, p < 0.001, Fig. S4.6.1). Major axis regression showed that the intercept was greater than zero (95% CIs intercept = 1.12, 3.62) and the slope was slightly less than one (95% CIs slope = 0.76, 0.91).

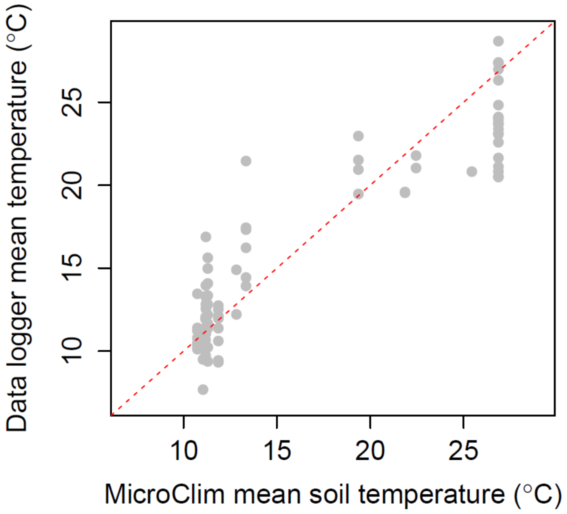


Figure S4.6.1 Relationship between temperature values obtained from data loggers and those extracted from microc*lim*, (r = 0.92, p < 0.001). Red line indicates a 1:1 relationship.

Modelling

Spatial modelling took place as described in the main text. Linear mixed models (LMMs) were used to assess how much variation in assemblage weighted lightness could be explained by *microclim* estimates of temperature, amount of UV-B radiation and assemblage weighted body size. This was done using the lme4 package in R (Bates *et al.*, 2014). An interaction term between temperature and UV-B was also fitted. All explanatory variables were scaled and standardised in order to allow greater interpretability of the regression coefficients (Schielzeth, 2010). Explanatory variables were coded as second order orthogonal polynomials in order to detect curvature in the relationships between them and assemblage weighted lightness. A nested random effects structure of transect within mountain range within continent was used to account for geographic configuration of the study sites. The response variable of assemblage weighted lightness was logit transformed to meet Gaussian assumptions. An information theoretic approach was used to assess models with different combinations of the explanatory variables. Bias corrected Akaike's information criterion (AICc) values were used to compare models. Marginal (due to fixed effects only) and conditional (due to fixed effects and random effects) R^2 values were calculated for each model (Bartoń, 2013; Nakagawa & Schielzeth, 2013). Type III tests using Wald χ^2 statistics were used to assess the significance of the predictors in the "best" model. Each of the 274 observations in this analysis was an independent assemblage of ants.

The best model was the same as when using *WorldClim* data. It contained the main effects of temperature, UV-B, body size and also included an interaction between temperature and UV (Table S4.6.1). All variables were significant according to type III Wald X2 tests (Table S4.6.2). Assemblage weighted lightness declined with increasing assemblage weighted body size (Fig. S4.6.2a). At low levels of UV-B, assemblage weighted lightness increased with increasing temperature. At high levels of UV-B there was a hump-shaped relationship between lightness and temperature - at higher temperatures lightness declined (Fig. S4.6.2b).

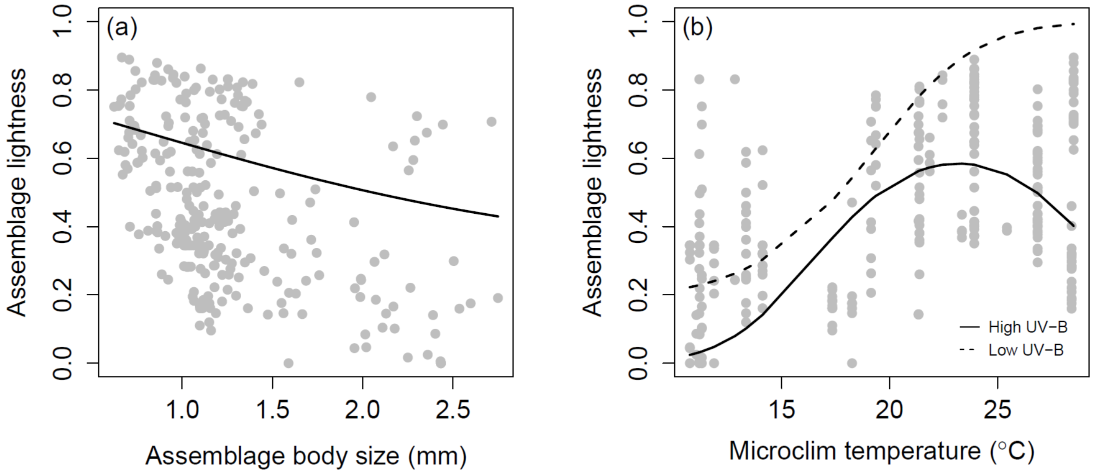


Figure S4.6.2 Plots showing the relationship between assemblage lightness and body size (a) and average microclim derived summer temperature (b). Lines display model predictions. In (b), solid line represents predictions for low levels of UV-B, dashed line represents predictions for high UV-B (n = 274).

Table S4.6.1 Comparative and summary statistics for linear mixed models explaining variation in ant assemblage colour across space. Predictors were all second order orthogonal polynomials and included average body size (BS + BS^2), average summer temperature (T + T^2) and average UV-B radiation (UV + UV^2). The temperature variables were derived from *microclim*. Listed are the degrees of freedom (d.f.), maximum log-likelihood (LL), Akaike's bias corrected information criterion (AICc) and it's change relative to the top ranked model (ΔAICc), the model probabilities (wAICc) and the marginal and conditional R^{2}s. Marginal R^2 (R^{2}m) is the amount of variation explained by the fixed effects, conditional R^2 (R^2_c) is that explained by the fixed and random effects.

Model	d.f.	LL	AICc	ΔAICc	wAICc	R^2_m	R^2_c
Spatial							
~ (BS + BS^2) + (T + T^2) X (UV + UV^2)	15	-321.19	674.25	0.00	0.90	0.50	0.53
~ (BS + BS^2) + (T + T^2) + (UV + UV^2)	11	-328.40	679.80	5.55	0.06	0.46	0.62
~ (BS + BS^2) + (T + T^2)	9	-330.82	680.32	6.08	0.04	0.48	0.70
~ (T + T^2) X (UV + UV^2)	13	-330.46	688.32	14.07	0.00	0.38	0.51
~ (T + T^2) + (UV + UV^2)	9	-336.42	691.52	17.27	0.00	0.33	0.58
~ (T + T^2)	7	-338.83	692.07	17.82	0.00	0.42	0.68
~ (BS + BS^2)	7	-347.08	708.58	34.33	0.00	0.07	0.41
~ (BS + BS^2) + (UV + UV^2)	9	-346.38	711.44	37.19	0.00	0.21	0.42
~ 1	5	-356.71	723.64	49.39	0.00	0.00	0.44
~ (UV + UV^2)	7	-356.50	727.41	53.17	0.00	0.01	0.41

Table S4.6.2 Test statistics (χ^2), degrees of freedom (d.f.) and p values from type III Wald tests on the best spatial model (top ranked from Table S4.6.1). Explanatory variables were second order orthogonal polynomials and included average body size (BS + BS^2), average summer temperature (T + T^2) and average UV-B radiation (UV + UV^2). The temperature variables were derived from microclim.

Spatial	χ^2	d.f.	p
T + T^2	16.04	2	<0.001
UV + UV^2	15.80	2	<0.001
BS + BS^2	20.17	2	<0.001
(T + T^2) X (UV + UV^2)	35.86	4	<0.001

Appendix S4.7 Modelling of lightness across space for common and rare species.

Table S4.7.1 Comparative and summary statistics for linear mixed models explaining variation in ant assemblage colour across space for either common or rare species. Predictors were all second order orthogonal polynomials and included average body size ($BS + BS^2$), average summer temperature ($T + T^2$) and average residual UV-B radiation ($UV + UV^2$). Assemblage lightness and body size were recalculated for common and rare models separately. Common species in assemblages were those that made up to 90% of the individuals. Rare species were the remainder. Listed are the degrees of freedom (d.f.), maximum log-likelihood (LL), Akaike's bias corrected information criterion (AICc) and it's change relative to the top ranked model (ΔAICc), the model probabilities (wAICc) and the marginal and conditional R^2s. Marginal R^2 (R^2_m) is the amount of variation explained by the fixed effects, conditional R^2 (R^2_c) is that explained by the fixed and random effects.

Common species	d.f.	LL	AICc	ΔAICc	wAICc	R^2_m	R^2_c
~ $(BS + BS^2) + (T + T^2) X (UV + UV^2)$	15	-326.12	684.27	0.00	0.98	0.47	0.69
~ $(BS + BS^2) + (UV + UV^2)$	9	-337.26	693.27	8.99	0.01	0.22	0.70
~ $(BS + BS^2) + (T + T^2) + (UV + UV^2)$	11	-335.82	694.74	10.46	0.01	0.29	0.65
~ $(T + T^2) X (UV + UV^2)$	13	-342.03	711.60	27.33	0.00	0.13	0.71
~ $(BS + BS^2) + (T + T^2)$	9	-346.45	711.64	27.37	0.00	0.35	0.69
~ $(UV + UV^2)$	7	-350.73	715.93	31.66	0.00	0.10	0.72
~ $(T + T^2) + (UV + UV^2)$	9	-350.31	719.37	35.10	0.00	0.09	0.68
~ $(T + T^2)$	7	-355.53	725.52	41.25	0.00	0.20	0.68
~ $(BS + BS^2)$	7	-364.22	742.90	58.63	0.00	0.05	0.60
~ 1	5	-370.62	751.49	67.22	0.00	0.00	0.66
Rare species							
~ $(BS + BS^2) + (UV + UV^2)$	9	-322.72	664.16	0.00	0.47	0.15	0.47
~ $(BS + BS^2) + (T + T^2) X (UV + UV^2)$	15	-316.59	665.12	0.96	0.29	0.17	0.54
~ $(BS + BS^2) + (T + T^2) + (UV + UV^2)$	11	-322.09	667.24	3.08	0.10	0.16	0.46
~ $(BS + BS^2)$	7	-326.56	667.56	3.40	0.09	0.13	0.45
~ $(BS + BS^2) + (T + T^2)$	9	-325.09	668.89	4.73	0.04	0.14	0.45
~ $(UV + UV^2)$	7	-342.69	699.82	35.66	0.00	0.04	0.42
~ $(T + T^2) + (UV + UV^2)$	9	-342.18	703.06	38.91	0.00	0.06	0.41
~ 1	5	-348.90	708.04	43.88	0.00	0.00	0.40
~ $(T + T^2)$	7	-346.83	708.11	43.95	0.00	0.03	0.41
~ $(T + T^2) X (UV + UV^2)$	13	-340.79	709.03	44.88	0.00	0.06	0.46

Table S4.7.2 Test statistics (χ^2), degrees of freedom (d.f.) and p values from type III Wald tests on the best spatial models for common and rare species subsets (top ranked from Table S4.7.1). Explanatory variables were second order orthogonal polynomials and included average body size (BS + BS^2), average summer temperature (T + T^2) and average residual UV-B radiation (UV + UV^2).

Common	χ^2	d.f.	p
T + T^2	18.49	2	<0.001
UV + UV^2	1.19	2	0.55
BS + BS^2	39.81	2	<0.001
(T + T^2) X (UV + UV^2)	21.67	4	<0.001
Rare			
BS + BS^2	74.44	2	<0.001
UV + UV^2	7.86	2	0.02

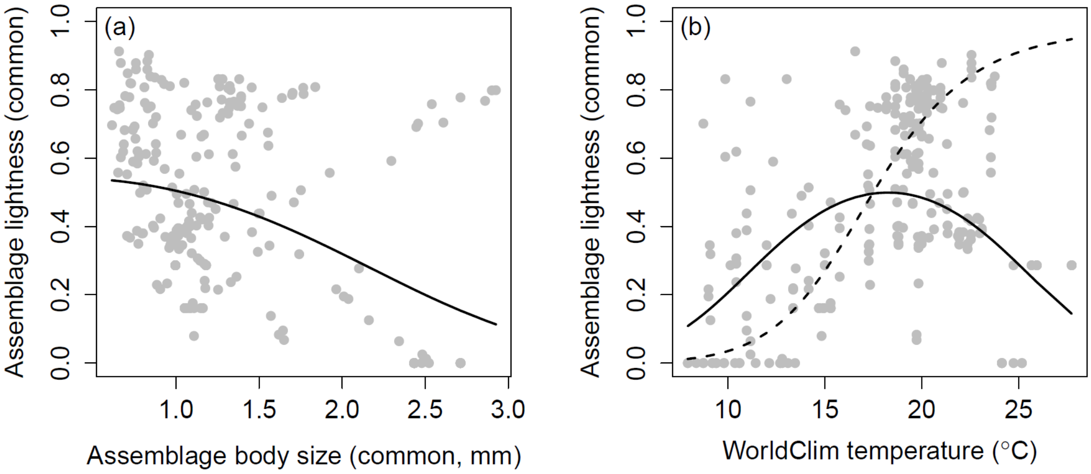


Figure S4.7.1 Plots showing the relationship between assemblage lightness and body size (a) and average *WorldClim* derived summer temperature (b). Only common species were used in the calculation of lightness and body size in plots (a) and (b). Lines display model predictions. In (b), solid line represents predictions for low levels of UV-B, dashed line represents predictions for high UV-B.

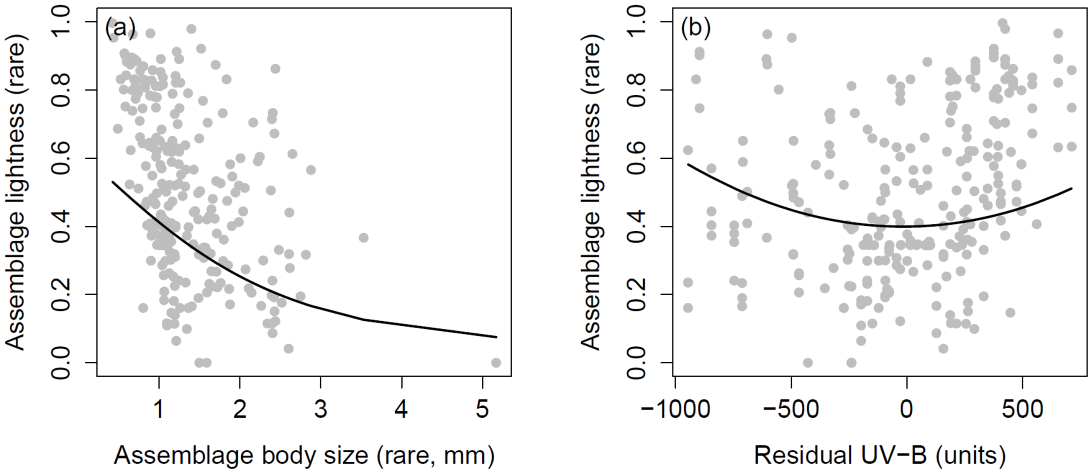


Figure S4.7.2 Plots showing the relationship between assemblage lightness and body size (a) and residual summer UV-B (b). Only rare species were used in the calculation of lightness and body size in plots (a) and (b). Lines display model predictions.

References

Bartoń, K. (2013) *MuMIn: Multi-model inference*.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H. & Dai, B. (2014) *lme4: Linear mixed-effects models using Eigen and S4*.

Kearney, M.R., Isaac, A.P. & Porter, W.P. (2014) microclim: Global estimates of hourly microclimate based on long-term monthly climate averages. *Scientific Data*, **1**, 140006.

Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, **4**, 133-142.

Schielzeth, H. (2010) Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, **1**, 103-113.

[Intentionally left blank]

Chapter 5

Coping with the cold: minimum temperatures and thermal tolerances dominate the ecology of mountain ants

Images: Glen McLean and Kate Parr

Coping with the cold: minimum temperatures and thermal tolerances dominate the ecology of mountain ants

Tom R. Bishop[1, 2*], Mark P. Robertson[2], Berndt J. van Rensburg[3, 4], and Catherine L. Parr[1, 5]

[1] *Department of Earth, Ocean and Ecological Sciences, University of Liverpool, Liverpool, L69 3GP, UK*

[2] *Centre for Invasion Biology, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa*

[3] *School of Biological Sciences, University of Queensland, St. Lucia, Queensland 4072, Australia*

[4] *Centre for Invasion Biology, Department of Zoology, University of Johannesburg, Auckland Park, Johannesburg, 2006, South Africa*

[5] *School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag X3, Wits 2050, South Africa*

**Correspondence: tbish@liv.ac.uk / thomasrhys.bishop@gmail.com*

Submitted to *Ecological Entomology* 25th March 2016

5.1 ABSTRACT

1. Ants (Hymenoptera: Formicidae) are often cited as highly thermophilic and this has led to a range of studies investigating their thermal tolerances. It is unknown, however, if the geographic distribution of ant thermal tolerance conforms to the two major macropyhsiological rules that have been found in other taxa: Janzen's and Brett's rules. In addition, there is a paucity of data on how the lower thermal tolerances of ants are able to influence behaviour.

2. We addressed these two knowledge gaps by sampling ants across a 1500 m elevational gradient in southern Africa and estimating the upper (CTmax) and lower (CTmin) thermal tolerances of 31 and 28 species, respectively. We also recorded ant abundances and soil temperatures across the gradient over six years.

3. We found that the average CTmin of the ants declined with elevation along with environmental temperatures. We also found that the correlation between abundance and local temperature depended on the ant species' CTmin. The activity of species with a low CTmin was not constrained by temperature, whereas those with a high CTmin were limited by low temperatures.

4. For the first time, we provide evidence that the thermal tolerances of ants are consistent with two major macrophysiological rules: Brett's rule and Janzen's rule. We also show a mechanistic link between physiology, behaviour and the environment which highlights that the ability of ants to deal with the cold may be a key, but often overlooked, factor allowing multiple ant species to succeed within an environment.

Author contributions: T.R.B. and C.L.P. conceived the research questions. M.P.R and B.J.v.R. oversaw historical data collection. T.R.B. performed experiments, analysed the data and wrote the manuscript. All authors contributed to the final draft.

5.2 INTRODUCTION

Variation in temperature is important for all of life. It dictates the metabolic rate of organisms (Huey & Kingsolver, 1989), influences activity patterns and can impose controls on geographic distributions (Gaston & Chown, 1999; Jenkins & Hoffmann, 1999). If an environment is too hot or too cold for a species then it will not perform well and, potentially, will not be able to exist there at all. As a result, managing for extremes of temperature is critical for organisms and has led to a diversity of thermoregulatory adaptations (Angilletta, 2009). Globally, insects are one of the most diverse and functionally important groups of animals (Wilson, 1987) and have been characterised as "thermal warriors" given that their lifestyle and success is so reliant on maintaining optimal temperatures (Heinrich, 1996). Understanding how the diversity of insect thermal tolerance is distributed across space, and the consequences this has for species and communities is central to many basic and applied goals in ecology and entomology. Current and potential future insect geographic distributions, species interactions and ecosystem functions that they mediate are all linked to thermal tolerances (Heinrich, 1996; Chown & Nicolson, 2004).

The ants (Hymenoptera: Formicidae) are a group of insects for which the broad patterns of diversity in thermal tolerance have only recently being revealed (e.g. Diamond *et al.*, 2012; Kaspari *et al.*, 2015). Ants are abundant and ubiquitous on nearly all continents (Fisher, 2010), display a fascinating diversity of life history strategies (Hölldobler & Wilson, 1990) and are believed to be functionally critical organisms in a range of environments (Evans *et al.*, 2011; Zelikova *et al.*, 2011; McGlynn & Poirson, 2012). Moreover, ants are commonly cited to be a thermophilic group (heat loving; Hölldobler & Wilson, 1990; Kaspari *et al.*, 2000). Ant diversity increases with temperature at a range of spatial scales (Sanders *et al.*, 2007; Jenkins *et al.*, 2011; Bishop *et al.*, 2014) and temperature has been shown to positively influence ant running speed (Hurlbert *et al.*, 2008; Kaspari *et al.*, 2016). There are also a variety of both individual (Cerdá & Retana, 2000; Shi *et al.*, 2015) and colony level (Kadochová & Frouz, 2013) thermoregulatory mechanisms that ants use to maintain optimum temperatures in both hot and cold conditions.

Despite the well-established link between many aspects of ant ecology and temperature, there are two key areas of ant-thermal tolerance research that remain poorly understood. The first of these is whether broad spatial patterns in the diversity of ant thermal tolerance are consistent with those reported for other taxa. The second is our relative lack of knowledge on how lower thermal limits influence ant ecology.

There are two broad scale geographic patterns in thermal tolerance that have emerged. Janzen's rule (Janzen, 1967), commonly called the climate variability hypothesis, states that greater variation in environmental temperatures is matched by a greater range in organismal thermal tolerances (Stevens, 1989; Gaston *et al.*, 2009). Brett's rule states that there is less geographic variation in upper than lower thermal tolerances (Brett, 1956; Gaston *et al.*, 2009). A range of terrestrial vertebrate, invertebrate and plant species show patterns which match the predictions of Janzen's and Brett's rules (Addo-Bediako *et al.*, 2000; Sunday *et al.*, 2011; Araújo *et al.*, 2013; Hoffmann *et al.*, 2013). Organisms tend to have larger thermal tolerance ranges in environments known to be more variable and this is due to greater variation in lower thermal tolerance limits. For ants, it has been shown that there is little geographic variation in upper thermal limits (Diamond *et al.*, 2012), as measured by their critical thermal maximum (CTmax). How the lower limits or the range of tolerance changes over environmental gradients in ants is unknown.

The thermophilic characterisation of the ants has led to a number of studies that largely focus on their upper thermal limits and what they mean in the context of climate change. Perhaps unsurprisingly, hotter conditions tend to favour ants with a higher CTmax. This effect can be seen when comparing different microhabitats within the same ecosystem (Baudier *et al.*, 2015; Kaspari *et al.*, 2015), under experimental shade or heating regimes (Wittman *et al.*, 2010; Stuble *et al.*, 2013) and in the different daily activity rhythms of ant species (Fitzpatrick *et al.*, 2014). Similarly to other taxa (Sunday *et al.*, 2014), it appears that tropical lowland ant species may be the most physiologically susceptible to future climate warming (Diamond *et al.*, 2012).

What often goes unappreciated is that the thermophilic nature of ants must also mean that they are cryophobic (cold fearing). Cool temperatures should also constrain ant activity and performance. The ant thermal tolerance literature, however, tends to focus on species and environments where extreme heat is more likely to be a limiting factor (Cerdá & Retana, 2000; Arnan & Blüthgen, 2015; Kaspari *et al.*, 2015). This is despite the fact that lower thermal tolerance may play a key role in maintaining global ant diversity and influencing species ranges. For example, in the Appalachian mountains of the USA, Warren and Chick (2013) showed that the critical thermal minimum temperature (CTmin) of the montane *Aphaenogaster picea* was consistently ~2°C lower than that of the coastal *A. rudis.* As minimum temperatures rose in this region over a period of 40 years, the cold intolerant *A. rudis* gained access to higher elevational sites and has now begun to displace the cold tolerant *A. picea*. The CTmin of ants clearly has the ability to influence their demography and distribution but is a largely unexplored topic.

Here, we start to address these shortcomings in the ant thermal tolerance literature whilst also testing for phylogenetic signal in tolerance measures. We investigate how both the CTmax and CTmin of ants changes along a 1500 m elevational gradient to tackle three specific aims:

1. Do patterns of ant thermal tolerance conform to the predictions of Janzen's rule across elevation?

2. Do patterns of ant thermal tolerance conform to the predictions of Brett's rule across elevation?

3. Do the CTmin and CTmax of ants influence their foraging behaviour under different temperatures?

We predict that (1) the thermal tolerance range will correlate with greater environmental temperature variability; (2) in line with other taxa, lower thermal tolerance limits will vary much more than the upper limits over the elevational gradient; and (3) that in our mountain ecosystem, the CTmin of species, but not their CTmax, will impose constraints on the ability of species to forage under different temperature regimes.

5.3 MATERIALS AND METHODS

5.3.1 Study site

Field and experimental work took place in the Sani Pass of the Maloti-Drakensberg mountains. The Sani Pass is the only road running through the Maloti-Drakensberg Transfrontier Conservation Area of South Africa and Lesotho. The Pass ranges in elevation from 1500 m a.s.l. (29°64' S 29°45' E) to 2874 m a.s.l. (29°60' S 29°29' E). This area is part of the grassland biome of southern Africa (Cowling *et al.*, 1997) and is recognised as a centre of endemism (Carbutt & Edwards, 2006; Kuhlmann, 2009).

5.3.2 Live ant sampling

Live ants were sampled from four different elevations (1500, 1800, 2400 and 3000 m a.s.l.) in January and February 2014 using a combination of baits (sugar water and cat food) and active searching. Ants were transported back to the laboratory and kept in perforated containers with soil and a damp piece of cotton wool. The thermal tolerances of the live ants were tested within 24 hours of their collection from the field.

5.3.3 Thermal tolerance experiments

Thermal tolerances were measured as the ants' critical thermal maximum (CTmax) and critical thermal minimum (CTmin). Individual ants experienced only a single experimental run (either CTmin or CTmax) and were then were not subjected to further testing. A dry heat bath (Tropicooler 260014-2, Boekel Scientific, Feasterville, PA, USA) was used to estimate CTmax and CTmin. The heat bath has a temperature range of -19 to 69°C with an accuracy of ±1°C. The heat bath contains two wells of 8.7 X 5.7 X 3.7 cm into which fit an aluminium heating block. Each heating block has 14 wells, each of which hold a single 1.5 ml microcentrifuge tube. At full capacity, a single experiment could test 28 individual ants (1 ant per tube X 2 blocks X 14 tubes = 28).

An experimental run testing either CTmin or CTmax took place as follows. A single worker was placed into a microcentrifuge tube. The microcentrifuge tube was plugged with a small piece of cotton wool to prevent the worker ant from climbing to the top of the tube and finding a thermal refuge and placed into one of the aluminium heating blocks within the heat bath unit. Ants were acclimatised for 15 minutes at 15 or 25°C for CTmin and CTmax, respectively. After this, the temperature was lowered (CTmin) or raised (CTmax) by 1°C. The heat bath was kept at the new temperature for 3 minutes. The ants were then checked for the loss of muscle coordination (CTmax, Lutterschmidt & Hutchison, 1997) or the absence of any movement at all (CTmin, Hazell & Bale, 2011) by quickly removing and flicking the individual microcentrifuge tubes. If an individual ant met these criteria this was deemed to be their critical temperature and was recorded. The experimental run stopped once all individuals had reached their critical temperatures.

Individuals of different species were randomly assigned to wells and positions within the aluminium heating blocks and heat bath unit for each experimental run. This was to ensure there was no systematic bias was introduced in case different parts of the heat bath unit heated or cooled at different rates. Where possible we repeated each experiment (CTmin and CTmax) three times for each species from each elevation. During each run of an experiment there were five individuals of each species that were being tested at that time. Consequently, 30 individuals of a species were tested from a single elevation when they could be found in sufficient numbers (5 individuals X 3 replicates X 2 experiments = 30).

We recognise the range of pitfalls associated with determining insect physiological tolerances experimentally - including the effects that the rate of temperature ramping may have on the results (Terblanche *et al.*, 2011). We emphasise the logistical constraints imposed on performing these experiments with field caught animals and argue that our thermal tolerance estimates are comparable within this study but caution against direct numerical comparison with other insect (including ant) thermal tolerance results which have used different experimental details and approaches.

5.3.4 Time series data

Data on forager abundance/activity were obtained by sampling epigaeic (ground-dwelling) ants biannually for six years from January 2006 to September 2012. The two sampling periods in each year represent the hotter and wetter season (January) and the colder and drier season (September/October). Two replicate blocks, spaced at least 300 m apart, were established at four different elevations. The four elevations were at 1500, 1800, 2400 and 3000 m a.s.l., the same as those which were sampled for live ants. At each block, 10 pitfall traps were set in two parallel lines with 10 m separating each adjacent trap. Traps were 150 ml in volume with a diameter of 55 mm and a depth of 70 mm. Rain guards supported on wire legs were placed over each trap to prevent flooding. All traps contained a 50% ethylene glycol preservative and were left out for five trapping nights in total. Traps were checked and replaced every two to three days to prevent overfilling. Ants were transferred to 70% ethanol in the laboratory and identified to morphospecies or species level where possible. This ant abundance data is a subset of that described and analysed in Bishop *et al.* (2014) and Bishop *et al.* (2015). In those studies, data from an additional two replicate blocks at each elevation and four more elevational sites were analysed. This study only analyses time series data from replicates for which we had iButton data and elevational sites where we sampled live ants for the thermal tolerances.

Thermocron iButtons (DS1921G, Semiconductor Corporation, Dallas/Maxim, TX, USA) were used to record soil temperatures through time at each replicate block. The iButtons were buried 10 mm below the soil surface and recorded the temperature every 1.5 hours. From January 2010 hourly readings were taken as a higher capacity iButton (DS1922L) was phased into use. The iButton data was inspected for cases where the unit had clearly malfunctioned or been directly exposed to the sun. These cases were removed from the temperature time series before analysis.

5.3.5 Phylogenetic signal

A genus level, time-calibrated phylogeny from Moreau and Bell (2013) was used to estimate phylogenetic signal in CTmin and CTmax. This was done in two ways. Firstly, we added species from this study as polytomies onto the original genus phylogeny and the calculated phylogenetic signal. Secondly, we calculated genus level means in the thermal tolerance measures and used the original genus level phylogeny to calculate phylogenetic signal. Two genera, *Lepisiota* and *Streblognathus* were not present on the original phylogeny. These genera were inserted as tips next to their closest relative. *Lepisiota* was inserted as a sister to *Plagiolepis* (Ward *et al.*, 2016) and *Streblognathus* was inserted as a sister to *Odontomachus (Schmidt, 2013)*. Phylogenetic signal was calculated using Pagel's λ (Pagel, 1999) and Blomberg's K (Blomberg *et al.*, 2003). A likelihood ratio test was used to test for a significant departure of both of these statistics from 0 (no phylogenetic signal). The *phytools* package in *R* was used to manipulate the phylogeny and perform the phylogenetic signal tests (Revell, 2012).

5.3.6 Temperature and elevation

The minimum, maximum and variance in temperatures were calculated for January (the month during which our thermal tolerance sampling and experiments took place) for each elevational site from the iButton data logger time series over the years 2006 - 2012 inclusive. Differences in these temperature variables across elevation were not statistically analysed due to temporal pseudoreplication. There were only two true (spatial) replicates per elevation.

5.3.7 Thermal tolerance and elevation

Differences in the average thermal tolerances of the ants between elevations were tested using ANOVA. Average CTmin and CTmax for each species within each elevation were calculated from the individual level data. These averages were used as independent data points. CTrange was calculated as the difference between CTmax and CTmin for each species within each elevation. Three separate ANOVA analyses were used to test for differences in (1) CTmin, (2) CTmax and (3) CTrange across the four elevational classes. Tukey's honest significant difference tests were performed on significant ANOVAs to reveal which elevations were different from each other. We did not perform phylogenetically controlled analyses as there was no phylogenetic signal in the thermal tolerance traits (see results). We also performed this analysis using mixed effects models to control for the fact that some species are present at multiple elevations. The results did not differ (Appendix S1) and so we present this simpler analysis in the main text.

5.3.8 Thermal tolerance and foraging behaviour

To assess how thermal tolerance is related to foraging behaviour we first calculated a species-specific temperature response. The temperature response was defined as the Pearson's correlation coefficient between log transformed abundance and temperature for each species over the time series. Abundance was log transformed to achieve normality. A positive correlation coefficient indicates that a species is more abundant at higher temperatures. Temperature was calculated as the mean, minimum and maximum temperature at each replicate block (2 replicates X 4 elevations = 8 replicates) during all of the pitfall sampling periods (6 years X 2 seasons = 12 time periods). We excluded species from the analysis if they were not detected in five or more sampling periods. We also excluded time periods for which a given species had an abundance of zero as we were interested in how abundances changed with temperature rather than if occurrences were affected.

We related variation in species' temperature response to their CTmin and CTmax using linear regression. This led to six regression models as the temperature response was calculated three times using different temperature data (mean, minimum and maximum). Species that were collected at multiple elevations were treated as separate species to allow incorporation of the different thermal tolerance estimates that we gathered at different elevations. Similar to the ANOVA analysis above, we also performed a mixed model regression to control for the non-independence of some species that were present at multiple elevations. The result was the same as the linear regression analysis (Appendix S2) and so we present the simpler analysis in the main text.

All data manipulation and analyses were performed in the *R* statistical environment (R Core Team, 2014).

5.4 RESULTS

We collected CTmin estimates for 28 species and CTmax estimates for 31 species (Appendix S3). We could not collect CTmin for three species. Thermal tolerance was estimated at more than one elevation for eight and nine species for CTmin and CTmax, respectively.

5.4.1 Phylogenetic signal

Using the species level polytomy tree neither CTmin (Pagel's $\lambda = 0.21$, $p = 0.39$, Blomberg's $K < 0.01$, $p = 0.26$) or CTmax (Pagel's $\lambda < 0.01$, $p = 1$, Blomberg's $K < 0.01$, $p = 0.42$) displayed significant phylogenetic signal. Similar results were found for CTmin (Pagel's $\lambda = 0.21$, $p = 0.49$, Blomberg's $K = 0.59$, $p = 0.57$) and CTmax (Pagel's $\lambda < 0.01$, $p = 1$, Blomberg's $K = 0.64$, $p = 0.43$) on the genus level phylogeny using genus averages.

5.4.2 Temperature and elevation

Minimum temperature clearly differed across elevation. The minimum temperature declined from 14.7°C at 1500 m a.s.l. to 5.9°C at 3000 m a.s.l. (Fig. 1a). The maximum and variance in temperature showed much greater overlap between elevations compared to the minimum temperature (Fig. 1b, c). Maximum temperature tended to decline with increasing elevation (from 34.9 to 26.7°C) but to a lesser extent than minimum temperature. Variance in temperature showed no clear trend with elevation (Fig. 1c).

5.4.3 Thermal tolerance and elevation

CTmin differed significantly across elevation (ANOVA, $F_{3,\,33} = 11.98$, $p < 0.01$). Tukey HSD tests revealed that these differences were between 1500 and 2400 m a.s.l. ($p < 0.01$), 1500 and 3000 m a.s.l. ($p = 0.02$), 1800 and 2400 m a.s.l. ($p < 0.01$), and 1800 and 3000 m a.s.l. ($p = 0.02$). This splits the elevations into two groups. CTmin was significantly lower at 2400 and 3000 m a.s.l. than at 1500 and 1800 m a.s.l. (Fig. 2a). CTmax (ANOVA, $F_{3,\,37} = 0.9$, $p = 0.45$, Fig. 2b) and CTrange (ANOVA, $F_{3,\,33} = 2.58$, $p = 0.07$, Fig. 2c) did not differ across elevation but both tended to increase with increasing elevation.

5.4.4 Thermal tolerance and foraging behaviour

After filtering species with insufficient time series data (see Methods), 25 species/elevation combinations were used for CTmin and 25 were used for CTmax. There was a significant relationship between CTmin and the temperature response when using mean temperatures (linear regression, df = 23, t = 2.12, $p = 0.046$, adjusted $R^2 = 0.13$ Fig. 3a) and minimum temperatures (linear regression, df = 23, t = 2.4, $p = 0.025$, adjusted $R^2 =$

0.17, Fig. 3b). There was no significant relationship between CTmin and the temperature response when using maximum temperatures ($p > 0.05$, Fig. 3c) or between any of the three temperature response variables and CTmax ($p > 0.05$, Fig. 4).

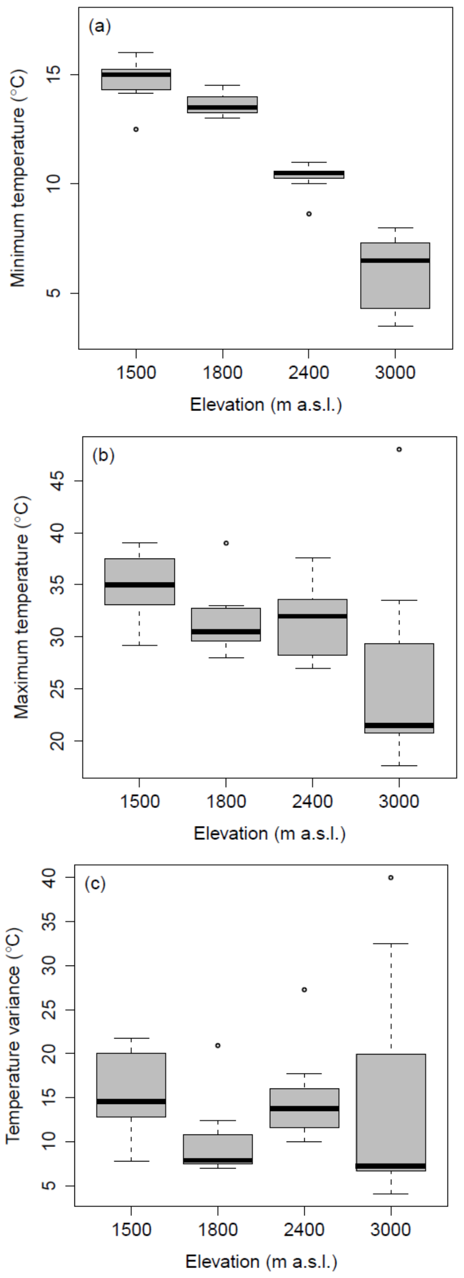


Figure 5.1 Box plots showing (a) minimum, (b) maximum and (c) variance in temperature in January across different elevations in the Sani Pass of the Maloti-Drakensberg mountains, southern Africa. Box plots show the median (central band), 25th and 75th percentiles (bottom and top of boxes) and 1.5 times the interquartile range above or below the 25th and 75th percentiles (whiskers). A single data point is a temperature estimate from one replicate during one year.

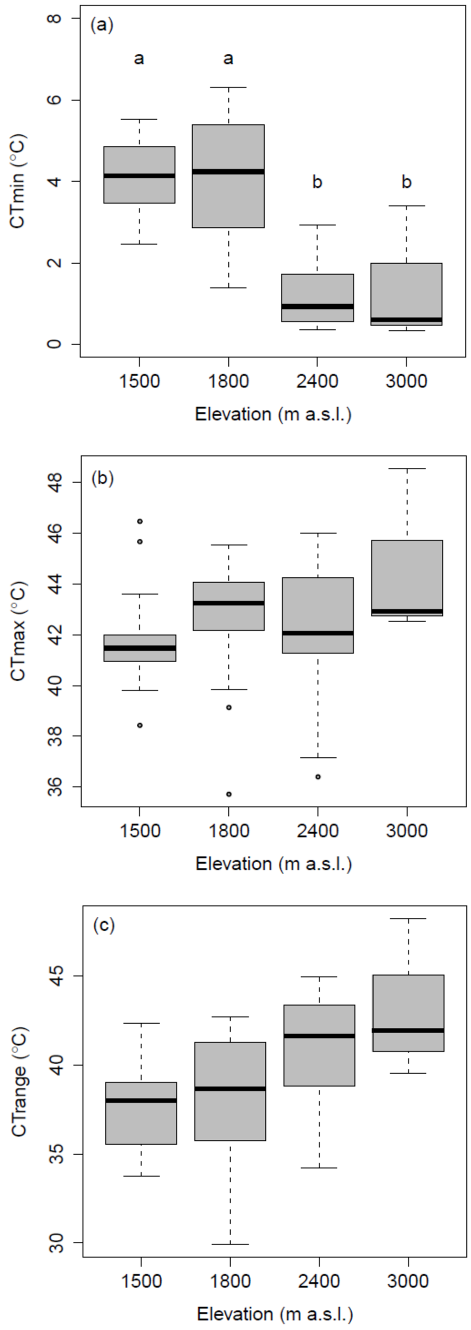


Figure 5.2 Box plots showing (a) CTmin, (b) CTmax and (c) CTrange (calculated as the difference between CTmax and CTmin) at different elevations in the Sani Pass of the Maloti-Drakensberg mountains, southern Africa. In (a), letters above boxes indicate significantly different groupings. Box plots show the median (central band), 25th and 75th percentiles (bottom and top of boxes) and 1.5 times the interquartile range above or below the 25th and 75th percentiles (whiskers).

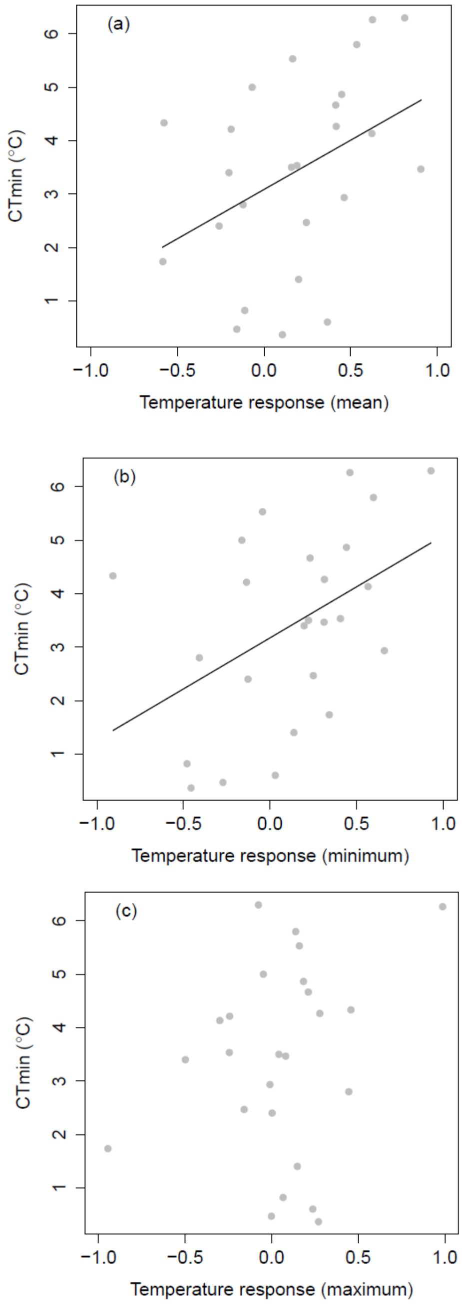


Figure 5.3 Plots showing the relationship between CTmin and species temperature responses. Mean (a), minimum (b) and maximum (c) temperatures were used to calculate the temperature responses in each panel. The temperature response is defined as the Pearson's correlation coefficient between log transformed abundance and temperature (mean, min. or max.) for each species over the time series. Regression lines indicate significant linear relationships.

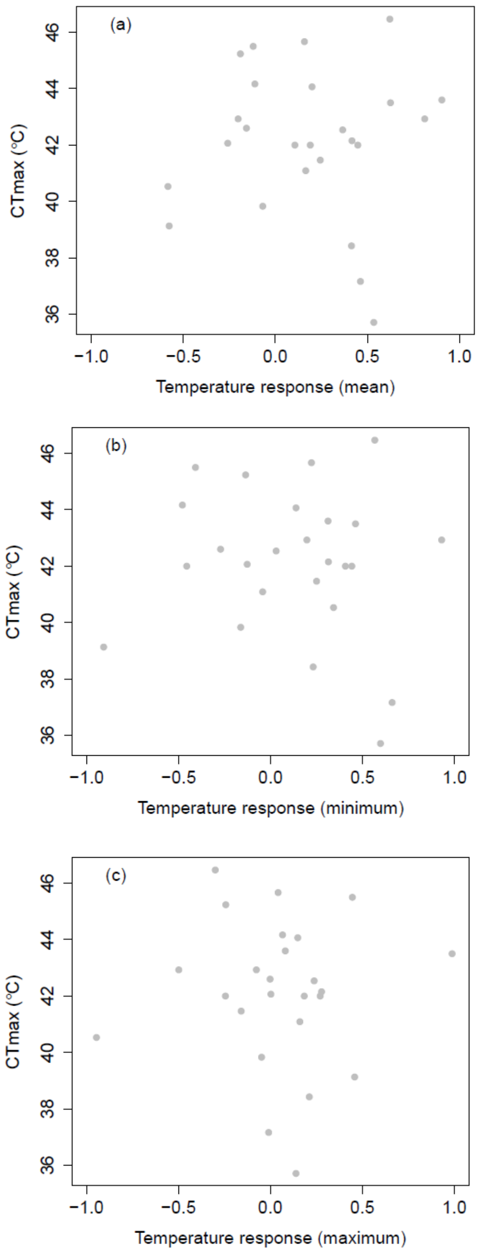


Figure 5.4 Plots showing the relationship between CTmax and species temperature responses. Mean (a), minimum (b) and maximum (c) temperatures were used to calculate the temperature responses in each panel. The temperature response is defined as the Pearson's correlation coefficient between log transformed abundance and temperature (mean, min. or max.) for each species over the time series.

5.5 DISCUSSION

We provide evidence that the thermal tolerances of ants are consistent with two major macrophysiological rules: Brett’s rule and Janzen’s rule. Whilst previous studies have begun to reveal the diversity present in ant thermal tolerances, it has not been clear how this diversity may be structured geographically. We also found that the foraging behaviour of ants under different temperatures can be mediated by their CTmin, but not their CTmax. Combined, these results show that there is not only more spatially structured variation in lower thermal limits in ants, but that this variation is able to control a key aspect of their ecology.

It is important to note that there is no phylogenetic signal in our estimates of CTmin or CTmax. Consequently, our main analyses did not control for any phylogenetic effects. This is in contrast to Diamond et al. (2012) who found small, but significant, phylogenetic signal in CTmax for ants in their global dataset. Their data show that closely related species had CTmax estimates that were more dissimilar to each other than expected under Brownian motion. The lack of any statistically signficant signal in our study is likely due to the realtively small sample size of 28 species used compared to the 156 species analysed by Diamond *et al.* (2012).

Whilst we find evidence that is consistent with both Brett’s rule and Janzen’s rule, the level of support differs for each. This is likely due to the nature of the temperature gradient that we have captured. Our results fully support Brett’s rule that there is greater geographic variation in lower than in upper thermal tolerance limits (Brett, 1956; Gaston *et al.*, 2009). CTmin, but not CTmax, significantly changes with elevation (Fig. 2a, b). At higher elevations ants tend to have a lower CTmin. This makes sense in terms of the environmental temperatures recorded at the different elevations. High elevations have a much lower minimum temperature compared to low elevation sites, but the change in maximum temperature is not as pronounced (Fig. 1a, b). This asymmetric change in both tolerance limits and environmental temperature extremes, especially minimum temperature, mirrors that which is often reported across latitudinal gradients (Addo-Bediako *et al.*, 2000; Hoffmann *et al.*, 2013).

Our data are consistent with Janzen's rule, which predicts a greater thermal tolerance range where there is a more variable temperature environment (Janzen, 1967; Gaston *et al.*, 2009), but does not provide unequivocal support. We find that neither thermal tolerance range (Fig. 2c), nor variation in environmental temperature changes significantly across elevation (Fig. 1c). This conclusion is at odds with a previous study, which used part of the same elevational gradient used here. Gaston and Chown (1999) found evidence for both Brett's and Janzen's rule in dung beetles. Their data shows that the range in temperature does not change between 1500 - 3000 m a.s.l. (from an interpolated climatic surface, see Fig. 3 in Gaston & Chown, 1999); we find the same in our study using data loggers. For Gaston and Chown (1999), it is their inclusion of sites close to sea level which reveal increases in both temperature variation and thermal tolerance range with elevation. This implies that the collection of ant thermal tolerance data from a more extensive elevational gradient may reveal stronger evidence in support of Janzen's rule.

We find that CTmin mediates the relationship that species have with temperature (Fig. 3a, b), but that CTmax does not (Fig. 4). A lower CTmin means that species' abundances are less affected by changes in temperature. This is most evident when comparing the relationship between abundance and minimum temperature with CTmin (Fig. 3b). The temperature response variable indicates whether abundances correlate positively (>0) or negatively (<0) with increasing temperature. Figure 3b shows that species which respond negatively or are invariant to minimum temperatures have a low CTmin. Species with a high CTmin, on the other hand, respond positively to increasing minimum temperatures. A similar pattern is seen when using mean temperature (Fig. 3a).

This pattern, that foraging activity is constrained by the link between temperature and species' physiological tolerances, is perhaps not surprising given the widespread understanding that ants are generally thermophilic (Hölldobler & Wilson, 1990). This is one of the first times, however, that a mechanistic link between physiology, behaviour and the environment has been made for ants. In the fauna we have sampled here, it is the CTmin of species and the coldest environmental temperatures that appear to determine how many foragers are active. Previously, it has been shown that ants change their foraging abundances over a range of timescales and that this is often linked to concurrent changes in temperature (Andersen, 1983; Fellers, 1989; Cerdá *et al.*, 1997; Dunn *et al.*, 2007). Extreme temperatures are viewed as marginal environments, which only subdominant or subordinate species will forage in (e.g. Cerdá *et al.*, 1997). The data we present here suggests that these differences in preferred foraging times may be underpinned by differences in species' physiology. This conclusion can help us to better understand the current and future distributions of ants and their interactions with each other (Warren & Chick, 2013).

These results linking physiology to foraging behaviour are based on a relatively small sample size (25 species) yet we still detect significant effects (mean temperature $p = 0.046$, minimum temperature $p = 0.025$). We argue that this is an impressive signal given the inherent noise present in abundance data. It is likely that factors such as disturbance (Andersen *et al.*, 2014), rainfall (Holway, 1998; Kaspari & Valone, 2002) and competitive interactions (Parr & Gibb, 2010) have left their mark on the abundance data of each species. A next step in this research would be to evaluate the physiology-foraging link under controlled laboratory conditions and to also investigate a larger number of species over a larger area (elevation and temperature range) whilst also recording detailed environmental information.

We found no link between CTmax and forager abundances. In contrast, Stuble *et al.* (2013) reported that species with a higher CTmax were found foraging most at higher temperatures. We suspect that the CTmax plays a smaller role in our analyses because it is never actually consistently hot enough at our field site over our five day sampling periods. The study by Stuble *et al.* (2013), in contrast, concerns an experimental warming treatment. Comparing the influence of CTmin and CTmax on foraging behaviour in a number of different locations, and recording the influence this has on colony performance, would be a fruitful step forward for the ant thermal tolerance field.

Finally, we emphasise that further work understanding fine scale variation in thermal tolerance, behaviour and colony performance over extensive gradients would be of use. We have begun to reveal patterns of thermal tolerance variation with this study but extending the range of thermal environments sampled and increasing the sample size would be beneficial. For example, we find what looks like a step change in CTmin (Fig. 2a) but this is likely an artefact of our sampling regime. Sampling more species and individuals would allow much more scope to assess the relative importance of inter- vs intraspecific variation and also strengthen any conclusions that can be made in relation to behaviour and performance.

In summary, we find that patterns of ant thermal tolerance largely conform to existing macrophysiological rules. We find greater geographic variation in CTmin than in CTmax, which supports Brett's rule. We also find that thermal tolerance range does not change across environments that have equally variable temperatures, which is consistent with the predictions of Janzen's rule. Finally, we show that the physiology of ants, in particular their CTmin, imposes constraints on their ability to forage at different temperatures. This effect may have widespread consequences for our understanding of contemporary ant species diversity and coexistence and for the way in which it may change in the future.

5.6 ACKNOWLEDGEMENTS

We thank the DST-NRF Centre of Excellence for Invasion Biology, the University of Pretoria, Chantal Ferreira, the Mazda Wildlife Fund, Ezemvelo KZN Wildlife and the Lesotho Ministry of Tourism, Environment and Culture for their various roles in supporting the Maloti-Drakensberg ant sampling transect. Glen McLean and Bradley Reynolds provided invaluable assistance and great company in the field and lab during the collection of the thermal tolerance data. Chantal Ferreira deserves a double acknowledgement for keeping everything ticking over and everyone in line.

5.7 REFERENCES

Addo-Bediako, A., Chown, S.L. & Gaston, K.J. (2000) Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B: Biological Sciences*, **267**, 739-745.

Andersen, A.N. (1983) Species diversity and temporal distribution of ants in the semi-arid mallee region of northwestern Victoria. *Australian Journal of Ecology*, **8**, 127-137.

Andersen, A.N., Ribbons, R.R., Pettit, M. & Parr, C.L. (2014) Burning for biodiversity: highly resilient ant communities respond only to strongly contrasting fire regimes in Australia's seasonal tropics. *Journal of Applied Ecology*, **51**, 1406-1413.

Angilletta, M.J. (2009) *Thermal adaptation: a theoretical and empirical synthesis*. Oxford University Press, Oxford.

Araújo, M.B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P.A., Valladares, F. & Chown, S.L. (2013) Heat freezes niche evolution. *Ecology Letters*, **16**, 1206-1219.

Arnan, X. & Blüthgen, N. (2015) Using ecophysiological traits to predict climatic and activity niches: lethal temperature and water loss in Mediterranean ants. *Global Ecology and Biogeography*, **24**, 1454-1464.

Baudier, K.M., Mudd, A.E., Erickson, S.C. & O'Donnell, S. (2015) Microhabitat and body size effects on heat tolerance: implications for responses to climate change (army ants: Formicidae, Ecitoninae). *Journal of Animal Ecology*, **84**, 1322-1330.

Bishop, T.R., Robertson, M.P., van Rensburg, B.J. & Parr, C.L. (2014) Elevation-diversity patterns through space and time: ant communities of the Maloti-Drakensberg Mountains of southern Africa. *Journal of Biogeography*, **41**, 2256-2268.

Bishop, T.R., Robertson, M.P., van Rensburg, B.J. & Parr, C.L. (2015) Contrasting species and functional beta diversity in montane ant assemblages. *Journal of Biogeography*, **42**, 1776-1786.

Blomberg, S.P., Garland Jr, T., Ives, A.R. & Crespi, B. (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, **57**, 717-745.

Brett, J. (1956) Some principles in the thermal requirements of fishes. *The Quarterly Review of Biology*, **31**, 75-87.

Carbutt, C. & Edwards, T.J. (2006) The endemic and near-endemic angiosperms of the Drakensberg Alpine Centre. *South African Journal of Botany*, **72**, 105-132.

Cerdá, X. & Retana, J. (2000) Alternative strategies by thermophilic ants to cope with extreme heat: individual versus colony level traits. *Oikos*, **89**, 155-163.

Cerdá, X., Retana, J. & Cros, S. (1997) Thermal disruption of transitive hierarchies in Mediterranean ant communities. *Journal of Animal Ecology*, **66**, 363-374.

Chown, S.L. & Nicolson, S.W. (2004) *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, Oxford.

Cowling, R.M., Richardson, D.M. & Pierce, S.M. (1997) *Vegetation of Southern Africa*. Cambridge University Press, Cambridge.

Diamond, S.E., Sorger, D.M., Hulcr, J., Pelini, S.L., Toro, I.D., Hirsch, C., Oberg, E. & Dunn, R.R. (2012) Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Global Change Biology*, **18**, 448-456.

Dunn, R.R., Parker, C.R. & Sanders, N.J. (2007) Temporal patterns of diversity: assessing the biotic and abiotic controls on ant assemblages. *Biological Journal of the Linnean Society*, **91**, 191-201.

Evans, T.A., Dawes, T.Z., Ward, P.R. & Lo, N. (2011) Ants and termites increase crop yield in a dry climate. *Nature Communications*, **2**, 262.

Fellers, J.H. (1989) Daily and seasonal activity in woodland ants. *Oecologia*, **78**, 69-76.

Fisher, B. (2010) Biogeography. *Ant Ecology* (ed. by L. Lach, C.L. Parr and K.L. Abbott). Oxford University Press, Oxford.

Fitzpatrick, G., Lanan, M.C. & Bronstein, J.L. (2014) Thermal tolerance affects mutualist attendance in an ant-plant protection mutualism. *Oecologia*, **176**, 129-138.

Gaston, K.J. & Chown, S.L. (1999) Elevation and climatic tolerance: a test using dung beetles. *Oikos*, **86**, 584-590.

Gaston, K.J., Chown, S.L., Calosi, P., Bernardo, J., Bilton, D.T., Clarke, A., Clusella-Trullas, S., Ghalambor, C.K., Konarzewski, M. & Peck, L.S. (2009) Macrophysiology: a conceptual reunification. *The American Naturalist*, **174**, 595-612.

Hazell, S.P. & Bale, J.S. (2011) Low temperature thresholds: Are chill coma and CT min synonymous? *Journal of Insect Physiology*, **57**, 1085-1089.

Heinrich, B. (1996) *The Thermal Warriors: Strategies of Insect Survival*. Harvard University Press, Cambridge, Massachusetts.

Hoffmann, A.A., Chown, S.L. & Clusella-Trullas, S. (2013) Upper thermal limits in terrestrial ectotherms: how constrained are they? *Functional Ecology*, **27**, 934-949.

Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Springer-Verlag, Berlin.

Holway, D.A. (1998) Factors governing rate of invasion: a natural experiment using Argentine ants. *Oecologia*, **115**, 206-212.

Huey, R.B. & Kingsolver, J.G. (1989) Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology & Evolution*, **4**, 131-135.

Hurlbert, A.H., Ballantyne, F. & Powell, S. (2008) Shaking a leg and hot to trot: the effects of body size and temperature on running speed in ants. *Ecological Entomology*, **33**, 144-154.

Janzen, D.H. (1967) Why mountain passes are higher in the tropics. *The American Naturalist*, **101**, 233-249.

Jenkins, C.N., Sanders, N.J., Andersen, A.N., et al. (2011) Global diversity in light of climate change: the case of ants. *Diversity and Distributions*, **17**, 652-662.

Jenkins, N.L. & Hoffmann, A.A. (1999) Limits to the Southern Border of Drosophila serrata: Cold Resistance, Heritable Variation, and Trade-Offs. *Evolution*, **53**, 1823-1834.

Kadochová, Š. & Frouz, J. (2013) Thermoregulation strategies in ants in comparison to other social insects, with a focus on red wood ants (Formica rufa group) [version 1; referees: 2 approved, 1 approved with reservations]. *F1000Research*, **2**, 280.

Kaspari, M. & Valone, T.J. (2002) On ectotherm abundance in a seasonal environment-studies of a desert ant assemblage. *Ecology*, **83**, 2991-2996.

Kaspari, M., Alonso, L. & O'Donnell, S. (2000) Three energy variables predict ant abundance at a geographical scale. *Proceedings of the Royal Society B-Biological Sciences*, **267**, 485-489.

Kaspari, M., Clay, N.A., Lucas, J., Yanoviak, S.P. & Kay, A. (2015) Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. *Global Change Biology*, **21**, 1092-1102.

Kaspari, M., Clay, N.A., Lucas, J., Revzen, S., Kay, A. & Yanoviak, S.P. (2016) Thermal adaptation and phosphorus shape thermal performance in an assemblage of rainforest ants. *Ecology*, (early view as of 29/03/2016).

Kuhlmann, M. (2009) Patterns of diversity, endemism and distribution of bees (Insecta: Hymenoptera: Anthophila) in southern Africa. *South African Journal of Botany*, **75**, 726-738.

Lutterschmidt, W.I. & Hutchison, V.H. (1997) The critical thermal maximum: data to support the onset of spasms as the definitive end point. *Canadian Journal of Zoology*, **75**, 1553-1560.

McGlynn, T.P. & Poirson, E.K. (2012) Ants accelerate litter decomposition in a Costa Rican lowland tropical rain forest. *Journal of Tropical Ecology*, **28**, 437-443.

Moreau, C.S. & Bell, C.D. (2013) Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. *Evolution*, **67**, 2240-2257.

Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature*, **401**, 877-884.

Parr, C.L. & Gibb, H. (2010) Competition and the role of dominant ants. *Ant ecology. Oxford University Press, Oxford*, 77-96.

R Core Team (2014) *R: A language and environment for Statistical Computing.* R Foundation for Statistical Computing. Available at: http://www.R-project.org/.

Revell, L.J. (2012) phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**, 217-223.

Sanders, N.J., Lessard, J.-P., Fitzpatrick, M.C. & Dunn, R.R. (2007) Temperature, but not productivity or geometry, predicts elevational diversity gradients in ants across spatial grains. *Global Ecology and Biogeography*, **16**, 640-649.

Schmidt, C. (2013) Molecular phylogenetics of ponerine ants (Hymenoptera: Formicidae: Ponerinae). *Zootaxa*, **3647**, 201-250.

Shi, N.N., Tsai, C.-C., Camino, F., Bernard, G.D., Yu, N. & Wehner, R. (2015) Keeping cool: Enhanced optical reflection and heat dissipation in silver ants. *Science*, **349**, 298-301.

Stevens, G.C. (1989) The latitudinal gradient in geographical range: how so many species coexist in the tropics. *American Naturalist*, **133**, 240-256.

Stuble, K.L., Pelini, S.L., Diamond, S.E., Fowler, D.A., Dunn, R.R. & Sanders, N.J. (2013) Foraging by forest ants under experimental climatic warming: a test at two sites. *Ecology and evolution*, **3**, 482-491.

Sunday, J.M., Bates, A.E. & Dulvy, N.K. (2011) Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society of London B: Biological Sciences*, **278**, 1823-1830.

Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T. & Huey, R.B. (2014) Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences*, **111**, 5610-5615.

Terblanche, J.S., Hoffmann, A.A., Mitchell, K.A., Rako, L., le Roux, P.C. & Chown, S.L. (2011) Ecologically relevant measures of tolerance to potentially lethal temperatures. *The Journal of experimental biology*, **214**, 3713-3725.

Ward, P.S., Blaimer, B.B. & Fisher, B.L. (2016) A revised phylogenetic classification of the ant subfamily Formicinae (Hymenoptera: Formicidae), with resurrection of the genera Colobopsis and Dinomyrmex. *Zootaxa*, **4072**, 343-357.

Warren, R.J. & Chick, L. (2013) Upward ant distribution shift corresponds with minimum, not maximum, temperature tolerance. *Global Change Biology*, **19**, 2082-2088.

Wilson, E.O. (1987) The little things that run the world (the importance and conservation of invertebrates). *Conservation Biology*, **1**, 344-346.

Wittman, S.E., Sanders, N.J., Ellison, A.M., Jules, E.S., Ratchford, J.S. & Gotelli, N.J. (2010) Species interactions and thermal constraints on ant community structure. *Oikos*, **119**, 551-559.

Zelikova, T.J., Sanders, N.J. & Dunn, R.R. (2011) The mixed effects of experimental ant removal on seedling distribution, belowground invertebrates, and soil nutrients. *Ecosphere*, **2**, art63.

5.8 APPENDICES

Appendix S5.1 Thermal tolerance and elevation

Table S5.1.1 shows a mixed effects model analysis of the thermal tolerance data. Species was used as a random effect. The results are the same as those presented in the main text using general linear models. Elevation explains variation in CTmin, but not in CTmax or CTrange.

Table S5.1.1 Comparative and summary statistics for linear mixed models explaining variation in ant thermal tolerance across elevation. Models for each response variable (CTmin, CTmax and CTrange) are listed in rank order - with the best model according to AICc listed first. Listed are the degrees of freedom (d.f.), maximum log-likelihood (LL), Akaike's bias corrected information criterion (AICc) and it's change relative to the top ranked model (ΔAICc), the model probabilities (wAICc) and the marginal and conditional R^2s. Marginal R^2 (R^2_m) is the amount of variation explained by the fixed effects, conditional R^2 (R^2_c) is that explained by the fixed and random effects.

CTmin	d.f.	LL	AICc	ΔAICc	wAICc	R^2_m	R^2_c
~ elevation	6	-58.92	132.64	0	1	0.52	0.71
~ 1	3	-72.79	152.31	19.67	0	0	0.45
CTmax							
~ 1	3	-93.36	193.37	0	0.93	0	0.71
~ elevation	6	-92.06	198.59	5.22	0.07	0.06	0.72
CTrange							
~ 1	3	-96.55	199.84	0	0.68	0	0.66
~ elevation	6	-93.25	201.30	1.47	0.32	0.17	0.66

Appendix S5.2 Thermal tolerance and foraging behaviour

Table S5.2.1 shows a mixed effects model analysis of the thermal tolerance and temperature response data. Species was used as a random effect. The results are the same as those presented in the main text using linear regression. Mean and minimum temperature response explain variation in CTmin, but maximum temperature response does not. None of the temperature responses explain variation in CTmax.

Table S5.2.1 Comparative and summary statistics for linear mixed models relating variation in ant thermal tolerance to variation in species' temperature response. The temperature response in the Pearsons correlation coefficient between abundance and temperature (mean, minimum or maximum) for each species. Six modelling procedures took places - three models (mean, minimum and maximum temperature responses) for each thermal tolerance measure (CTmin and CTmax). Models are listed in rank order - with the best model according to AICc listed first. Listed are the degrees of freedom (d.f.), maximum log-likelihood (LL), Akaike's bias corrected information criterion (AICc) and it's change relative to the top ranked model (ΔAICc), the model probabilities (wAICc) and the marginal and conditional R^2s. Marginal R^2 (R^2_m) is the amount of variation explained by the fixed effects, conditional R^2 (R^2_c) is that explained by the fixed and random effects.

		d.f.	LL	AICc	ΔAICc	wAICc	R^2_m	R^2_c
CTmin	Mean							
	~ Temperature response (mean)	4	-47.40	104.80	0	0.61	0.17	0.17
	~ 1	3	-49.28	105.71	0.90	0.39	0	0.36
	Minimum							
	~ Temperature response (minimum)	4	-46.84	103.68	0	0.73	0.21	0.21
	~ 1	3	-49.28	105.70	2.03	0.24	0	0.36
	Maximum							
	~ 1	3	-49.28	105.71	0	0.66	0	0.36
	~ Temperature response (maximum)	4	-48.51	107.02	1.32	0.34	0.06	0.49
CTmax	Mean							
	~ 1	3	-55.17	117.47	0	0.76	0	0.66
	~ Temperature response (mean)	4	-54.90	119.79	2.32	0.24	0.02	0.68
	Minimum							
	~ 1	3	-55.17	117.47	0	0.80	0	0.66
	~ Temperature response (minimum)	4	-55.15	120.31	2.83	0.19	0	0.66
	Maximum							
	~ 1	3	-55.17	117.47	0	0.80	0	0.66
	~ Temperature response (maximum)	4	-55.13	120.27	2.79	0.20	0	0.67

Appendix S5.3 Thermal tolerances of ant species in the Sani Pass

Table S5.3.1 Table showing the critical thermal minimum (CTmin) and critical thermal maximum (CTmax) for ants of the Sani Pass. Elevation from which individuals were tested and the number of individuals tested also given.

Species	Elevation (m a.s.l.)	CTmin (°C)	CTmin *n*	CTmax (°C)	CTmax *n*
Anoplolepis custodiens	1500	4.1	15	46.5	15
Camponotus 1	3000	0.6	15	42.5	15
Camponotus 3	1800	1.5	15	43.0	17
Camponotus 3	1500	2.8	15	40.8	15
Camponotus havilandi	2400	0.6	14	45.5	15
Camponotus irredux	2400	0.4	11	42.0	10
Camponotus irredux	1800	2.8	20	45.5	19
Camponotus un10	2400	0.8	12	44.2	20
Cerapachys un01	1500	3.0	1	41.7	15
Crematogaster 6	3000	0.3	15	48.5	15
Crematogaster natalensis	1800	6.3	15	43.5	15
Crematogaster natalensis	1500	4.9	15	42.0	15
Crematogaster un02	1800	4.3	15	42.2	15
Crematogaster un02	1500	3.5	15	43.6	15
Lepisiota 1	2400	NA	15	46.0	2
Lepisiota 1	1800	4.2	15	45.2	17
Lepisiota un03	1500	5.5	15	41.1	15
Lepisiota un05	2400	0.9	15	44.3	10
Leptogenys intermedia	2400	1.7	15	40.5	15
Meranoplus peringueyi	1500	3.5	15	45.7	15
Messor 1	1800	3.2	15	42.7	15
Messor 1	1500	5.5	15	40.9	15
Monomorium 1	3000	3.4	15	42.9	15
Monomorium un01	1800	NA	15	45.5	15
Pachycondyla caffraria	1800	4.3	15	39.1	15
Pachycondyla caffraria	1500	4.3	15	41.4	14
Pachycondyla pumicosa	2400	NA	15	42.0	4
Pachycondyla wroughtonii	2400	1.0	5	36.4	10
Pheidole 4	2400	2.4	15	42.1	15
Pheidole un01	1800	5.0	5	39.8	16
Pheidole un01	1500	2.5	15	41.5	15
Plagiolepis un01	1800	6.3	15	42.9	15
Plectroctena mandibularis	1800	2.9	11	43.7	15
Pristomyrmex fossulatus	1500	4.9	9	39.8	15
Solenopsis 2	2400	2.9	15	37.2	15
Solenopsis 2	1800	5.8	15	35.7	15

Solenopsis 2	1500	4.7	15	38.4	15
Streblognathus peetersi	2400	0.5	15	42.6	15
Tetramorium new sp	1800	NA	15	43.7	15
Tetramorium frenchi	1500	3.5	15	42.0	5
Tetramorium sericeiventre	1800	1.4	5	44.1	15

[Intentionally left blank]

Chapter 6

General discussion

Images: Mark Robertson and Tom Rhys Bishop

6.1 KEY FINDINGS

The central aim of this thesis was to understand the mechanisms that underpin the distribution of biodiversity. Specifically, I used mountain ecosystems to describe and test various aspects of ant ecology from both a taxonomic and a functional traits perspective. Combined, the four data chapters show that it is principally temperature, and the ability of species to respond to it, that drives patterns of diversity. Chapters 2 and 3 describe how diversity at the community (alpha diversity) and inter-community (beta diversity) levels change in response to temperature gradients. Chapters 4 and 5 highlight the importance of thermoregulatory functional traits in determining the abundance and performance of species in different thermal environments. In mountain ecosystems, such as that studied here, it would appear that minimum temperatures were most limiting - a fact not often explicitly recognised in the literature. Below, I highlight the key findings from each data chapter that contribute to these conclusions.

6.1.1 Chapter 2: Alpha diversity

This chapter is only one of a few in the diversity-elevation literature to explore variation through time as well as through space. In addition, this chapter analyses patterns of evenness and abundance. Species density showed hump-shaped patterns with respect to elevation, and was also strongly affected by season. These patterns were explained largely by variation in mean temperature - available area, which may operate via a species-area mechanism, only had a small effect. Evenness also showed a hump-shaped elevational pattern but tended not to vary though time. Finally, patterns of abundance were highly variable through space and time.

The main finding of this chapter is that temperature appeared to control both spatial and temporal variation. For example, there were fewer ant species at higher elevations, where temperatures were low, but also in the dry season, when temperatures were lower than in the wet season. This is one of the first times such an effect has been shown across an elevational gradient, but similar patterns of temporal changes have been detected in other contexts (e.g. in Australian savannas Andersen, 1983). What is interesting about this combination of temporal and elevational data was its ability to discount geometric constraint models of diversity (Colwell & Lees, 2000). Geometric constraints suggest that diversity patterns arise simply as a product of absolute range limits and variation in species range sizes. They cannot explain temporal changes. Consequently, this chapter clearly emphasises the link between temperature and ant diversity patterns.

6.1.2 Chapter 3: Beta diversity

This chapter focussed on different types of beta diversity and how they respond to an elevational gradient. Beta diversity was split into species (taxonomic) and functional descriptions, and also into turnover and nestedness components (Baselga, 2010; Villéger *et al.*, 2013). Species beta diversity looks at how species identities change, whilst functional beta diversity characterises how the diversity of functional traits differs between samples. Turnover defines the changes that occur when new species or traits are found in one sample but, not another. Nestedness describes the situation where one sample is simply a depauperate subset of another.

Species beta diversity was largely caused by turnover across the elevational gradient, whilst functional beta diversity was characterised by nestedness patterns. In other words, different species were found at different elevations but they represent an increasingly nested subset of the available functional strategies. This classic distance decay of similarity has been reported for a range of other organisms and functional traits (Swenson *et al.*, 2011; Wang *et al.*, 2012). Furthermore, the species changes appeared deterministic, but the functional changes were easily replicated by a null model. This implies that the functional traits analysed here cannot explain the species changes across the elevational gradient adequately.

The most interesting parts of the results from this chapter are the new questions generated. For example, **why** do ant species turnover across elevation? The results presented here provide a glimpse at the answer. They narrow down the possible list of factors that could be controlling ant species turnover across elevation. The traits used here correspond to diet and habitat preferences, but it is found that they bear no relation to species turnover. This is interesting as both diet and habitat preferences have been suggested as the likely factors in determining and structuring the diversity and ecology of montane insects (Mani, 1968), but they are ruled out in this case. Instead, I suggest that unrecorded traits such as physiological thermal tolerances may be controlling which species exist at which elevations. Chapters 4 and 5 go some way to following up this hypothesis.

6.1.3 Chapter 4: Colour

This chapter was inspired by a pure natural history question. **Why are ants so many different colours?** I constructed a global-scale dataset of ant assemblage, colour and body size data to answer this question. The results showed that the colour of ants varies in accordance with known biophysical principles that relate to thermoregulation and UV-B protection. Ants were darker and larger where it was cold. They were also dark in colour where both UV-B irradiances and temperatures were high. Theory tells us that dark cuticle colouration speeds up the rate at which an individual can gain heat from incoming solar radiation whilst large body sizes slow down the rate of heat loss (Clusella-Trullas *et al.*, 2007). My findings support these predictions.

The result presented in this Chapter is powerful for two key reasons. First, the geographic scale of the dataset was large. The data were taken from across three different continents, which have different ant faunas, ecological contexts and evolutionary histories. Despite this, consistent patterns were found. This result implies that the functionality of colour in ants is global. Thus, the data used here go beyond the majority of previous studies investigating geographic colour variation which have either been Euro-centric or of limited scale of (e.g. Zeuss *et al.*, 2014; Moreno Azócar *et al.*, 2015).

Second, the data analysed were at the assemblage level. This is an important, yet subtle, distinction. The measures of assemblage lightness and body size do not only reflect what the “average ant” may look like at a given location. They are also capturing the very structure of the assemblage. Changes in the proportions of light and dark, or small and large species feed directly into the assemblage level averages of lightness and body size. Consequently, changes in assemblage lightness reflect the changing dominance of species with different phenotypes. This point is reinforced by the use of temporal data from the Maloti-Drakensberg and Soutpansberg Mountain ranges. In these cases, the same physical point in space has a different assemblage lightness value at different times and temperatures. What this means is that when it is hotter, a greater proportion of small, light-coloured ants are seen. Conversely, when it is colder, a greater proportion of large, dark-coloured ants can be found.

This assemblage level finding places constraints on the ways in which ant communities, and the ecological functions they provide, may change in the future. Notably, the results presented here suggest that if the climate warms, then smaller and lighter-coloured ants should be favoured over larger and darker species. This may seriously disrupt the existing structure of many ant communities. In addition, some localities may not even have ant species with the right phenotypes to achieve predicted "optimum" assemblage lightness or body size values under future climates.

6.1.4 Chapter 5: Thermal tolerance

This final data chapter takes the theme of temperature to its logical conclusion. The previous chapters investigated how temperature relates to diversity patterns (chapters 2 and 3) and how it may interact with functional traits related to thermoregulation (chapter 4). Chapter 5 looks directly at how ants are able to cope physiologically with extreme temperatures. I find evidence that supports two major macrophysiological rules: Janzen's and Brett's rules. In addition, I find that the activity patterns of ants can be influenced by low temperatures depending on the lower thermal limits of the ants.

The findings from this chapter contribute in two ways toward existing theory. First, the consistency with Janzen's and Brett's rules brings our knowledge of ant thermal tolerances for ants in line with that of other groups (Addo-Bediako *et al.*, 2000; Araújo *et al.*, 2013). This is important because it suggests that the general principles underlying the evolution and distribution of thermal physiology in other groups are likely to hold in the ants.

Second, I stress the importance of the cold on ant ecology. Most studies of ant thermal physiology are concerned with high temperatures, ants' thermophilic nature and what this may mean for niche partitioning and dominance (Cerdá *et al.*, 1997; Stuble *et al.*, 2013; Arnan & Blüthgen, 2015). This view is not necessarily wrong, but that it misses part of what it means to be thermophilic. Being thermophilic means that the ants respond positively to the heat, but also that they respond negatively to cold! It is this second part of the idea which has not received much attention. In the Sani Pass low temperatures coupled with the ants' lower thermal limits dictate their response to changing temperatures and are more important in understanding the ants' activity patterns. It is hoped that the work presented in chapter 5 will stimulate further research into the lower thermal limits of ants and how they may, or may not, limit their performance in natural and laboratory settings.

6.2 POINTS OF SYNTHESIS

All of the data chapters presented here were undertaken and written as independent research projects dealing with related, but separate, research questions. When viewing the thesis as a whole there are a number of links between the chapters that are interesting.

Most notably, chapter 3 (beta diversity) questions what factors may be driving the turnover of ant species with elevation. There is no evidence that morphological traits related to feeding or habitat use are involved. It is suggested that physiological traits may be causing the turnover. Indirectly, the data from chapter 5 (thermal tolerance) provide support for this idea. Chapter 5 shows that species moderate their activity based on their ability to tolerate low temperatures. It is conceivable that as minimum temperatures get colder with increasing elevation (Fig. 5.1) only ants with adequate thermal physiology will be able to exist at these sites -

driving the observed species turnover. There is currently not enough available data on the thermal tolerances of the ants in the Sani Pass to explore this idea quantitatively.

A further point of synthesis is between chapters 4 (colour) and 5 (thermal tolerance). Chapter 4 shows that the relative abundances or identities of species in a local assemblage can change depending on their colour and the ambient temperature. Chapter 5 discusses how species' thermal physiology can constrain when (and, by extension, where) they are actively foraging. Together, these results suggest that there may be a coupled evolution of thermoregulatory traits that ultimately have an effect on the success of a species in a given context. This idea is not new, but the data presented in these two chapters provides insights into the mechanisms behind thermal partitioning in ants for the first time - colour and thermal tolerance.

6.3 FUTURE RESEARCH

The work presented here highlights the importance of thermoregulatory traits in structuring ant diversity patterns. It also generated a variety of ideas for future research programs which would expand our knowledge in the fields of ant ecology, functional traits, macroecology and macrophysiology significantly. Based on this work, three major avenues on ant ecology and the link to functional traits are suggested for future research, these are:.

6.2.1 Abiotic vs biotic constraints

Both chapters 4 and 5 highlight how ant behaviour and community structure can be influenced by abiotic conditions. In those cases, temperature had a strong influence on which species could forage at which times, and at what abundances. In the ant ecology literature, however, biotic interactions often take centre stage (Cerdá *et al.*, 2013) but research presented in this thesis highlight that thermoregulatory traits and the abiotic environment can be central to ant ecology. Consequently, I suggest that further work investigating the balance between abiotic and biotic constraints in ant communities would be useful. Are there contexts when biotic constraints are paramount? Or, conversely, negligible? Some of this work has already started to take place (Gibb, 2011) and hints that the strength of biotic constraints can be dependent on context. The research questions is:

Under what conditions are abiotic or biotic factors more important for structuring ant diversity?

6.2.2 Intra-specific variation and plasticity

All of the functional trait work in this thesis is at the inter-specific level and effectively ignores intra-specific variation. Intra-specific variation is commonly assumed not to be as large as inter-specific variation. Additionally, intra-specific variation is much harder to quantify and collect data on than inter-specific variation. Ant ecology is in desperate need of a greater understanding of intra-specific variation. Linked to this theme is the concept of plasticity. Can any of the functional traits considered in this thesis change in response to the pressures of the environment? In many aspects of their ecology, ants are considered to behave like plants (Andersen, 1991). Plants have been demonstrated to be highly plastic in a range of the functional traits and life history strategies, can the same be said of ants? One may hypothesise that their eusocial nature is in itself a mechanism of plasticity. Colonies can respond to their environment by foraging in greater or smaller numbers, or raising more workers of a particular caste (Hölldobler & Wilson, 1990). Because of the flexibility afforded to them by eusociality their morphological or physiological functional traits may be relatively fixed over ecological time scales. This is unknown, however the general research question is:

How do ant functional traits vary intraspecifically and how plastic are they?

6.2.3 Colony-level performance

Most work on functional traits in ants, including that reported in this thesis, focuses on individual level traits and how these relate to community level metrics (species richness, abundance etc). By doing this, we have learned a great deal about how the adaptations of ants may prepare them for particular environments (e.g. chapters 4 and 5; Gibb & Parr, 2013). What does this mean for the demography and reproductive success of colonies? For ants the colony is the effectively the reproductive unit. Just how important is it, in terms of a colony's reproductive output, for its workers to have optimal body sizes, cuticle colouration or thermal physiology for a given environment? The answers to this question are almost entirely unknown but are likely to be extremely important for a comprehensive understanding of ant biology and ecology. The research question is:

How do functional traits map onto the performance and success of colonies?

6.3 CONCLUDING REMARKS

This thesis builds on the strong foundations of functional ecology, biogeography, macroecology and macrophysiology to describe patterns of biodiversity and to explore mechanisms that may be underpinning them. The use of an extensive spatio-temporal dataset across an elevational gradient and linking this to the functional traits of species has contributed to our general understanding of how biodiversity is organised. Perhaps what is most exciting are the questions that remain to be answered in ant functional and montane ecology.

6.4 REFERENCES

Addo-Bediako, A., Chown, S.L. & Gaston, K.J. (2000) Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B: Biological Sciences*, **267**, 739-745.

Andersen, A.N. (1983) Species diversity and temporal distribution of ants in the semi-arid mallee region of northwestern Victoria. *Australian Journal of Ecology*, **8**, 127-137.

Andersen, A.N. (1991) Parallels between ants and plants: implications for community ecology. *Ant-Plant Interactions* (ed. by C.R. Huxley and D.F. Cutler), pp. 539-558. Oxford University Press, Oxford.

Araújo, M.B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P.A., Valladares, F. & Chown, S.L. (2013) Heat freezes niche evolution. *Ecology Letters*, **16**, 1206-1219.

Arnan, X. & Blüthgen, N. (2015) Using ecophysiological traits to predict climatic and activity niches: lethal temperature and water loss in Mediterranean ants. *Global Ecology and Biogeography*, **24**, 1454-1464.

Baselga, A. (2010) Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, **19**, 134-143.

Cerdá, X., Retana, J. & Cros, S. (1997) Thermal disruption of transitive hierarchies in Mediterranean ant communities. *Journal of Animal Ecology*, **66**, 363-374.

Cerdá, X., Arnan, X. & Retana, J. (2013) Is competition a significant hallmark of ant (Hymenoptera: Formicidae) ecology. *Myrmecological News*, **18**, 131-147.

Clusella-Trullas, S., van Wyk, J.H. & Spotila, J.R. (2007) Thermal melanism in ectotherms. *Journal of Thermal Biology*, **32**, 235-245.

Colwell, R.K. & Lees, D.C. (2000) The mid-domain effect: geometric constraints on the geography of species richness. *Trends in Ecology & Evolution*, **15**, 70-76.

Gibb, H. (2011) Experimental evidence for mediation of competition by habitat succession. *Ecology*, **92**, 1871-1878.

Gibb, H. & Parr, C.L. (2013) Does structural complexity determine the morphology of assemblages? An experimental test on three continents. *Plos One*, **8**, e64005.

Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Springer-Verlag, Berlin.

Mani, M.S. (1968) *Ecology and Biogeography of High Altitude Insects*, 1st edn. Springer Netherlands, The Hague.

Moreno Azócar, D., Perotti, M., Bonino, M., Schulte, J., Abdala, C. & Cruz, F. (2015) Variation in body size and degree of melanism within a lizards clade: is it driven by latitudinal and climatic gradients? *Journal of Zoology*, **295**, 243-253.

Stuble, K.L., Rodriguez-Cabal, M.A., McCormick, G.L., Jurić, I., Dunn, R.R. & Sanders, N.J. (2013) Tradeoffs, competition, and coexistence in eastern deciduous forest ant communities. *Oecologia*, **171**, 981-992.

Swenson, N.G., Anglada-Cordero, P. & Barone, J.A. (2011) Deterministic tropical tree community turnover: evidence from patterns of functional beta diversity along an elevational gradient. *Proceedings of the Royal Society B-Biological Sciences*, **278**, 877-884.

Villéger, S., Grenouillet, G. & Brosse, S. (2013) Decomposing functional β-diversity reveals that low functional β-diversity is driven by low functional turnover in European fish assemblages. *Global Ecology and Biogeography*, **22**, 671-681.

Wang, J., Soininen, J., Zhang, Y., Wang, B., Yang, X. & Shen, J. (2012) Patterns of elevational beta diversity in micro- and macroorganisms. *Global Ecology and Biogeography*, **21**, 743-750.

Zeuss, D., Brandl, R., Brändle, M., Rahbek, C. & Brunzel, S. (2014) Global warming favours light-coloured insects in Europe. *Nature Communications*, **5,** 3874.

[Intentionally left blank]

Tom Rhys Bishop

University of Liverpool

March 2016

[Intentionally left blank]