**Title:** Ant assemblages have darker and larger members in cold environments

**Running title:** Gradients in ant colour and body size

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

**ABSTRACT**

**Aim** In ectotherms, the colour of an individual’s cuticle may have important thermoregulatory and protective consequences. In cool environments, ectotherms should be darker, to maximise heat gain, and larger, to minimise heat loss. Dark colours should also predominate under high UV-B conditions as melanin offers protection. 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 assemblages (n = 274) along fourteen elevational transects on three continents. Individual assemblages ranged from 250 to 3000 m a.s.l. (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. Annual temporal changes in lightness were examined for a subset of the data.

**Results** Assemblages with large average body sizes were darker in colour than those with small body sizes. Assemblages became lighter in colour with increasing temperature, but darkend 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 are consistent with 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. These findings suggest that there are important constraints on how ectotherm assemblages may be able to respond to rapidly changing environmental conditions.

**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](#_ENREF_48)), attract mates ([Andersson, 1994](#_ENREF_1)) or thermoregulate ([Clusella-Trullas *et al.*, 2007](#_ENREF_19)). For ectotherms, which make up the majority of animal species, 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](#_ENREF_13)). Consequently, the ability to thermoregulate in response to these gradients is critical for ectotherm survival ([Heinrich, 1996](#_ENREF_25)).

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 ([Willmer & Unwin, 1981](#_ENREF_59)). 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](#_ENREF_19)). 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](#_ENREF_10); [Clusella-Trullas *et al.*, 2007](#_ENREF_19)). 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 ([Rapoport, 1969](#_ENREF_44); [Zeuss *et al.*, 2014](#_ENREF_60)). Whilst these effects are a direct result of melanin pigmentation, the melanogenesis pathway itself may also influence cold resistance pleiotropically through its effects on energy homeostasis and metabolic rates ([Ducrest *et al.*, 2008](#_ENREF_20)).

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](#_ENREF_54)). This size effect underpins wide-ranging biogeographical predictions such as Bergmann’s rule which states that organisms should be larger in cold environments ([Chown & Gaston, 2010](#_ENREF_17)).

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. This inverse body mass-heating relationship has been used as an explanation for the apparent lack of support for Bergmann’s rule in ectotherms ([Pincheira-Donoso, 2010](#_ENREF_41)). 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](#_ENREF_19)). This melanism-body size interaction is predicted from both theory and experiments ([Stevenson, 1985](#_ENREF_54); [Shine & Kearney, 2001](#_ENREF_53)) and has been shown to operate across large geographic scales in ectotherms ([Schweiger & Beierkuhnlein, 2015](#_ENREF_50)). 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. 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](#_ENREF_27); [Beckmann *et al.*, 2014](#_ENREF_6); [Williamson *et al.*, 2014](#_ENREF_58)). Both experiments ([Wang *et al.*, 2008](#_ENREF_55)) and correlative studies ([Bastide *et al.*, 2014](#_ENREF_3)) have provided evidence that melanistic individuals or species can be favoured under high UV-B conditions. Gloger’s rule ([Gaston *et al.*, 2008](#_ENREF_23)), 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 ([Caro, 2005](#_ENREF_15)) and, more recently, in plants ([Koski & Ashman, 2015](#_ENREF_31)).

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](#_ENREF_30); [Ellers & Boggs, 2004](#_ENREF_21)). 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 ([Millien *et al.*, 2006](#_ENREF_34)). 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](#_ENREF_18)).

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](#_ENREF_28)) with a wide range of body colours (e.g. www.antweb.org). 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](#_ENREF_5)). 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.

**METHODS**

**Ant assemblage data**

Ant assemblage data were compiled from 14 elevational transects within eight mountain ranges and across three continents (Table 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](#_ENREF_11); [Munyai & Foord, 2012](#_ENREF_36); [Bishop *et al.*, 2014](#_ENREF_7)). 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](#_ENREF_56)). 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](#_ENREF_56)). 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.

**Lightness data**

The colour of each ant species was classified categorically by eye using a predetermined set of colours (Appendix S1). 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 S1), 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.

**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](#_ENREF_12)). 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](#_ENREF_33)) 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](#_ENREF_14)) was performed to estimate the missing Weber’s length for these species (Appendix S2).

**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](#_ENREF_26)). Levels of UV-B irradiance for all assemblages were extracted from the *glUV* dataset ([Beckmann *et al.*, 2014](#_ENREF_6)). 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](#_ENREF_56)). 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](#_ENREF_36); [Bishop *et al.*, 2014](#_ENREF_7)). 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](#_ENREF_29)). Furthermore, the data from southern Africa was used to investigate temporal trends (see below).

**Statistical methods**

All data manipulation and analyses took place in the *R* statistical environment ([R Core Team, 2014](#_ENREF_42)).

*Phylogenetic signal*

A genus level, time calibrated ant phylogeny derived from [Moreau and Bell (2013](#_ENREF_35)) was used to estimate the phylogenetic signal of lightness and body size using Pagel’s λ ([Pagel, 1999](#_ENREF_39)) and Blomberg’s K ([Blomberg *et al.*, 2003](#_ENREF_9)). 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](#_ENREF_46)). 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:

Where *S* is the number of species in an assemblage, *pi* is the proportional abundance of each species and *xi* 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](#_ENREF_32)). 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.

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). 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.

*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](#_ENREF_4)). 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](#_ENREF_49)). 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 (AICc) values were used to compare models. Marginal (due to fixed effects only) and conditional (due to fixed effects and random effects) R2 values were calculated for each model ([Bartoń, 2013](#_ENREF_2); [Nakagawa & Schielzeth, 2013](#_ENREF_38)). Type III tests using Wald X2 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. We chose this rule 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).

**RESULTS**

Across all transects 592 ant species were collected (Table 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.

**Phylogenetic signal**

Lightness was not significantly conserved across the phylogeny – closely related genera do not resemble each other more so than would be expected by chance (Pagel’s λ = 0.32, *p* = 0.06, Blomberg’s K = 0.59, *p* = 0.13). Body size was conserved, however (Pagel’s λ = 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 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.

**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 2). All variables apart from the main effect of residual UV-B radiation were significant according to type III Wald Χ2 tests (Table 3). Assemblage weighted lightness declined with increasing assemblage weighted body size (Fig. 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. 1b). Species richness did not influence these results given the small amount of variation in assemblage lightness that species richness is able to explain (R2m = 0.02, R2c = 0.38.Appendix S5). The same results were found when using *microclim* ([Kearney et al., 2014](#_ENREF_29))temperature data rather than *WorldClim* data (Appendix S6).

**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 (R2m = 0.47, R2C = 0.69, Appendix S7). 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 (R2m = 0.15, R2C = 0.47, Appendix S7).

**Temporal patterns**

The best temporal model included both mean temperature and body size (Table 2). Lightness showed a negative relationship with body size (Fig. 2a) and a positive relationship with data logger derived temperature (Fig. 2b). Both body size and temperature were significant according to type III Wald Χ2 tests (Table 3).

**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](#_ENREF_54); [Shine & Kearney, 2001](#_ENREF_53); [Wang *et al.*, 2008](#_ENREF_55)). 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](#_ENREF_54); [Shine & Kearney, 2001](#_ENREF_53); [Wang *et al.*, 2008](#_ENREF_55)), 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. 1b). In addition, assemblage cuticle lightness was negatively correlated with assemblage body size (Fig. 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. 2a) and positively related to temperature (Fig. 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](#_ENREF_19)), and Gloger’s rule ([Caro, 2005](#_ENREF_15); [Millien *et al.*, 2006](#_ENREF_34)). 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](#_ENREF_15); [Millien *et al.*, 2006](#_ENREF_34); [Koski & Ashman, 2015](#_ENREF_31)). 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. 1b, Table 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](#_ENREF_3); [Koski & Ashman, 2015](#_ENREF_31)).

Comparable results to ours have been found using multiple species across large areas. For example, European insects show positive relationships between cuticle lightness and temperature ([Zeuss *et al.*, 2014](#_ENREF_60)), whilst the cuticle lightness of carabid beetles is negatively related to body size across Europe ([Schweiger & Beierkuhnlein, 2015](#_ENREF_50)). 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](#_ENREF_57)).

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](#_ENREF_40)). 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](#_ENREF_16)) which has tended to focus on the importance of biotic factors such as competition ([but see Gibb, 2011](#_ENREF_24)). 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 ([Reddin *et al.*, 2015](#_ENREF_45)).

Previous studies on this topic ([Zeuss *et al.*, 2014](#_ENREF_60); [Schweiger & Beierkuhnlein, 2015](#_ENREF_50)), and in macroecology in general ([Beck *et al.*, 2012](#_ENREF_5)), 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 km2 scale. Instead, it is the success of individuals at finer grains that determines population viability and ultimately drives ecosystem functioning . It should be noted, however, that despite the large influence that spatial extent and grain size may have in determining geographic patterns ([Rahbek, 2005](#_ENREF_43)), the relationships between lightness, temperature and body size in our dataset (grain size of ~400 m2) are consistent with those studies using a much larger grain size ([Zeuss *et al.*, 2014](#_ENREF_60); [Schweiger & Beierkuhnlein, 2015](#_ENREF_50)). 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](#_ENREF_21)), (2) shape assemblage structure (this study) and (3) determine which species are present in the wider regional pool ([e.g. Zeuss *et al.*, 2014](#_ENREF_60)).

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 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 specialised reflecting hairs ([Shi *et al.*, 2015](#_ENREF_52)) to thermoregulate in hot conditions. In addition, ants have been widely reported to forage at cooler times of the day to avoid peak temperatures ([Fitzpatrick *et al.*, 2014](#_ENREF_22)) 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 ([Hölldobler & Wilson, 1990](#_ENREF_28)), 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 and lighter coloured. The existing literature largely agrees with this decrease in body size ([Sheridan & Bickford, 2011](#_ENREF_51)), but currently suggests that darker melanic individuals will tend to be favoured under climate change scenarios ([Roulin, 2014](#_ENREF_47)). These predicted changes will likely filter certain kinds of species and alter the functional composition and outputs of assemblages.

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**BIOSKETCH**

Tom R. Bishop is interested in using morphology and physiology to understand the distribution of biological diversity, particularly that of the ants.

**TABLES**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 1.** Details on the geographical and elevational characteristics of the transects used in this study. | | | | | | | | |
| Continent | Mountain range | 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 | Sani Pass | 900 | 3000 | 8 | 4 | 92 | [Bishop *et al.* (2014](#_ENREF_7)); [Bishop *et al.* (2015](#_ENREF_8)) |
| Soutpansberg | North Aspect | 800 | 1700 | 5 | 4 | 129 | [Munyai and Foord (2012](#_ENREF_36)); [Munyai and Foord (2015](#_ENREF_37)) |
| South Aspect | 900 | 1600 | 5 | 4 - 8 |
| Cederberg | East Aspect | 500 | 1800 | 6 | 4 | 94 | [Botes *et al.* (2006](#_ENREF_11)) |
| West Aspect | 250 | 1900 | 10 | 4 |
| Mariepskop | Mariepskop | 700 | 1900 | 5 | 4 | 92 | Tshivhandekano & Robertson. unpublished |
| Australia | Snowy Mountains | Back Perisher | 400 | 2000 | 9 | 4 | 109 | Gibb et al. unpublished |
| Ben Lomond plateau, Tasmania | Stack’s Bluff | 400 | 1400 | 6 | 1 - 3 | 12 | Gibb et al. unpublished |
| MacDonnell Ranges | Mt. Zeil | 600 | 1400 | 5 | 4 | 49 | Gibb et al. unpublished |
| South America | Andes, North West Patagonia | Bayo | 900 | 1700 | 9 | 1 | 15 | [Werenkraut *et al.* (2015](#_ENREF_56)) |
| Chall-Huaco | 900 | 2000 | 12 | 1 |
| La Mona | 800 | 1800 | 11 | 1 |
| Lopez | 800 | 1800 | 10 | 1 |
| Pelado | 800 | 1800 | 8 | 1 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 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 + BS2), average summer temperature (T + T2) and average residual UV-B radiation (UV + UV2). 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 R2s. Marginal R2 (R2m) is the amount of variation explained by the fixed effects, conditional R2 (R2c) is that explained by the fixed and random effects. | | | | | | | |
| Model | d.f. | LL | AICc | ΔAICc | wAICc | R2m | R2c |
| Spatial |  |  |  |  |  |  |  |
| ~ (BS + BS2) + (T + T2) X (UV + UV2) | 15 | -301.59 | 635.03 | 0.00 | 1.00 | 0.48 | 0.62 |
| ~ (BS + BS2) + (T + T2) + (UV + UV2) | 11 | -315.07 | 653.14 | 18.11 | 0.00 | 0.38 | 0.56 |
| ~ (T + T2) X (UV + UV2) | 13 | -313.37 | 654.14 | 19.11 | 0.00 | 0.41 | 0.59 |
| ~ (BS + BS2) + (T + T2) | 9 | -321.63 | 661.94 | 26.90 | 0.00 | 0.43 | 0.61 |
| ~ (BS + BS2) + (UV + UV2) | 9 | -322.69 | 664.05 | 29.02 | 0.00 | 0.21 | 0.62 |
| ~ (T + T2) + (UV + UV2) | 9 | -325.24 | 669.17 | 34.14 | 0.00 | 0.28 | 0.52 |
| ~ (T + T2) | 7 | -328.97 | 672.36 | 37.33 | 0.00 | 0.36 | 0.59 |
| ~ (UV + UV2) | 7 | -335.04 | 684.50 | 49.47 | 0.00 | 0.14 | 0.62 |
| ~ (BS + BS2) | 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 + BS2) + (T + T2) | 10 | -112.24 | 245.60 | 0.00 | 0.96 | 0.49 | 0.74 |
| ~ (BS + BS2) | 8 | -117.65 | 252.03 | 6.43 | 0.04 | 0.36 | 0.69 |
| ~ (T + T2) | 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 3.** Test statistics (χ2), 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 2). Explanatory variables were second order orthogonal polynomials and included average body size (BS + BS2), average summer temperature (T + T2) and average residual UV-B radiation (UV + UV2). The temperature variables were derived from *WorldClim* for the spatial models and from data loggers in the temporal models. | | | |
| Spatial | χ2 | d.f. | p |
| T + T2 | 29.77 | 2 | <0.001 |
| UV + UV2 | 3.01 | 2 | 0.22 |
| BS + BS2 | 24.81 | 2 | <0.001 |
| (T + T2) X (UV + UV2) | 29.43 | 4 | <0.001 |
|  |  |  |  |
| Temporal |  |  |  |
| T + T2 | 16.48 | 2 | <0.001 |
| BS + BS2 | 87.85 | 2 | <0.001 |

**FIGURE LEGENDS**

**Figure 1.**

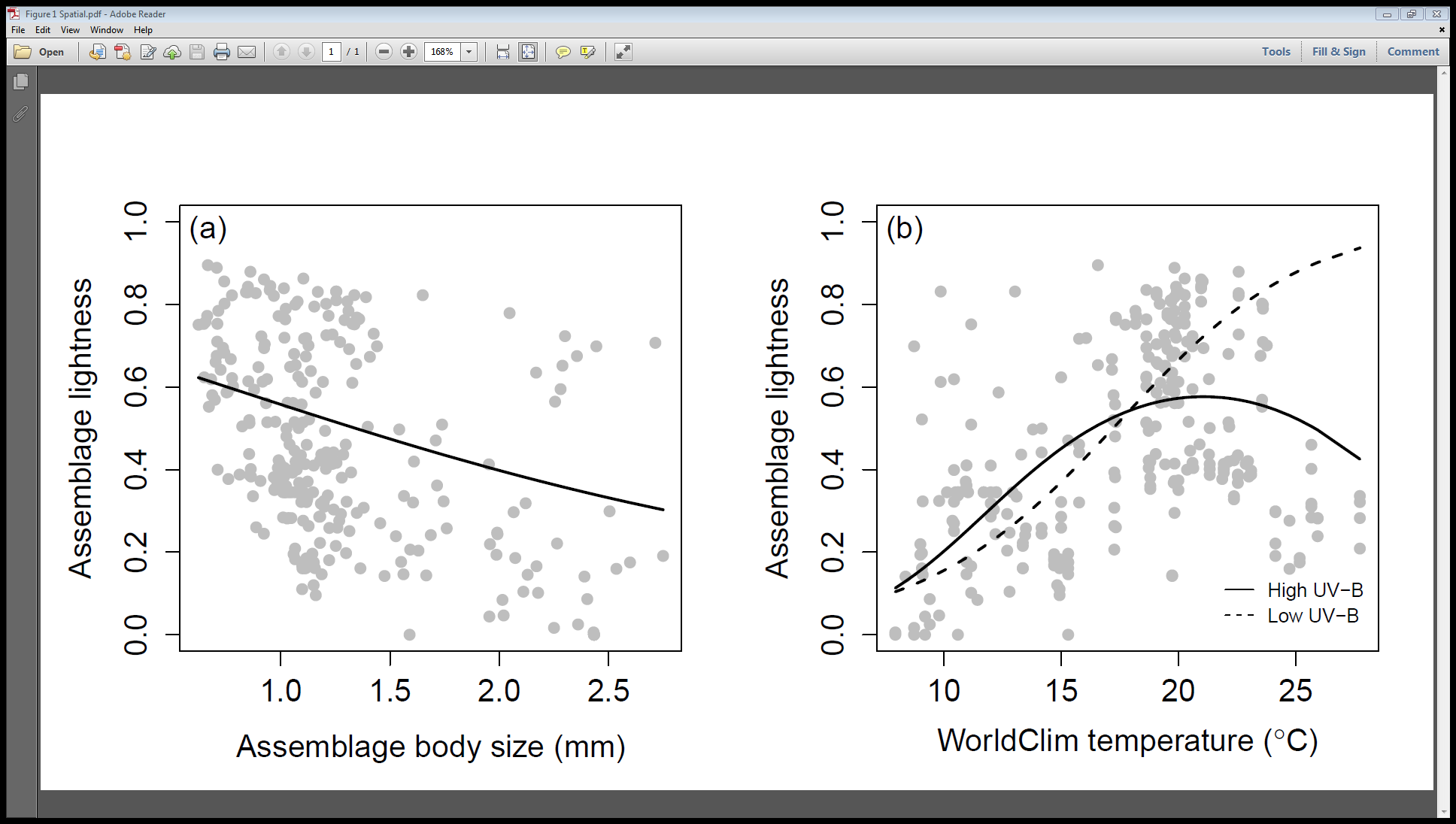
Plots showing the relationship between mean assemblage lightness and body size (a) and mean *WorldClim* derived summer temperature (b). Lines display model predictions. In (b), solid line represents predictions for low levels of UV-B (10th percentile), dashed line represents predictions for high UV-B (90th percentile) (n = 274). R2m (fixed effects) = 0.48, R2c(fixed and random effects) = 0.62.

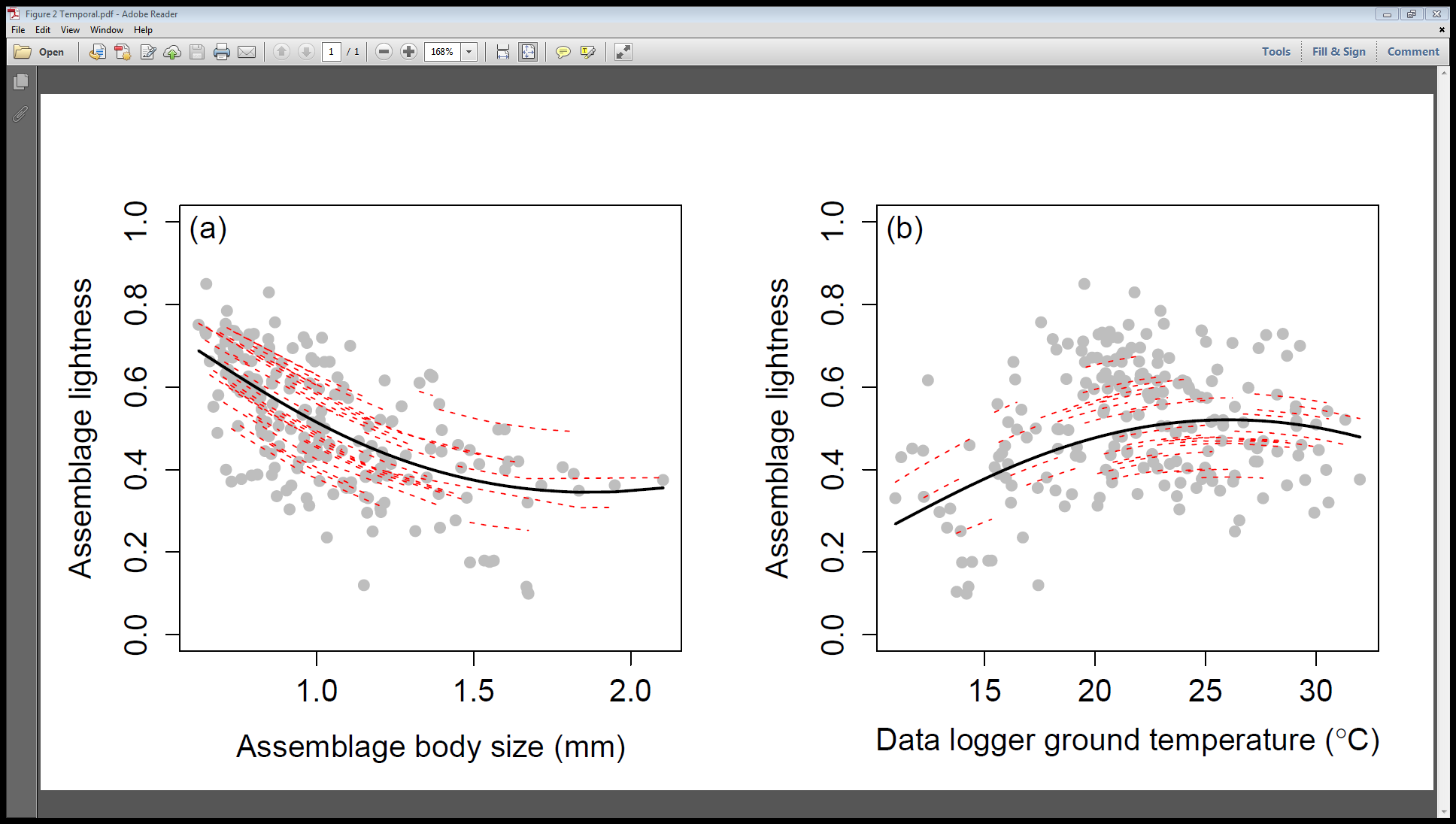
**Figure 2.**

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). R2m (fixed effects) = 0.49, R2c(fixed and random effects) = 0.74.

**FIGURES**

**Figure 1**



**Figure 2**