Functional implications of temperature-correlated colour polymorphism in an iridescent, scarabaeine dung beetle

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Abstract. 1. In many Coleoptera, iridescent colouration is generated by exoskeleton ultra-structure, within which multilayer interference reflects only certain wavelengths. Published work indicates that the colour polymorphism shown by some iridescent beetles is under genetic control. However, the present study suggests environmental involvement in the polymorphic southern African dung beetle, *Gymnopleurus humanus* Macleay.

2. At 24 study sites across a 1000-km latitudinal temperature gradient, population samples of *G. humanus* were dominated by blue individuals in the cooler south, by cupreous individuals in the warmer north, and by locally co-occurring blue, green and cupreous individuals in intermediate situations.

3. Using digital reflectance spectrophotometry, we measured wavelength intensity values across the visible spectrum (400–800 nm) and used the 70 measured specimens to estimate maximum reflectance from a further 3912 beetles. Differences in mean reflectance values between 24 populations were strongly correlated with average annual temperatures at study sites.

4. Much stronger correlations between mean reflectance values and average temperatures of the cool dry season months suggest that the cross-climatic patterns may be related to interaction between breeding seasonality and development under different cooler temperatures.

5. Published evidence suggests that inherent physical properties of cholesteric liquid crystals and their responses to different thermal conditions could, potentially, generate the different exocuticle ultra-structure responsible for different reflected colour wavelengths. Furthermore, colour polymorphism could be advantageous across a gradient from cooler to warmer climate as a result of the different thermal properties of different colours.

6. Given the correlation with temperature, it is predicted that the prevailing reflected colour balance in southern populations would shift in response to global climatic change.

Key words. Colour polymorphism, dung, exoskeleton, *Gymnopleurus*, iridescent, Scarabaeinae, southern Africa, temperature.

Introduction

Iridescent colouration is present in a number of insect orders and has received a certain amount of attention, particularly in Lepidoptera (e.g. Ghiradella, 1985; Brink & Lee, 1998; Sweeney *et al.*, 2003; Kemp, 2007a) and Coleoptera (e.g. Parker *et al.*, 1998; Kurachi *et al.*, 2002; Vigneron *et al.*, 2006; Brink *et al.*, 2007). These perceived colours are produced not by the chemical composition of the exoskeleton, but by specific cuticular ultra-structure that generates colour through the selective reflection of particular visible wavelengths. The ultra-structure and optical processes vary between insect taxa and include light-diffraction

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grating arrangements on the exoskeleton surface; spectral effects from light scattering structures or photonic crystals within the cuticle; and cuticular ultra-structure that induces thin-film or multilayer interference (Kinoshita & Yoshioka, 2005). Colour generated by multilayer interference is characteristic of many beetle families (Kinoshita & Yoshioka, 2005) including the Scarabaeidae (Neville & Caveney, 1969; Hegedüs *et al.*, 2006) and dung beetles of the scarabaeid subfamily, Scarabaeinae (Neville & Caveney, 1969; Brink *et al.*, 2007). Such multiple cuticular layers, with their different refractive indices, cause greater transmission or absorption of certain wavelengths across the visible light spectrum as well as the greater reflection of others, thus generating both the perceived exoskeleton colour and its intensity (Kinoshita & Yoshioka, 2005).

In iridescent scarabaeid beetles, the ultra-structure responsible for reflectance shares properties with cholesteric liquid crystals (Neville & Caveney, 1969). These optically active structures occur in the exocuticle and comprise of identical microfibrillae arranged in parallel. They give the impression of having been laid down in sheets, as the microfibrillae of each successive level are offset to the left at a slight angle until they describe a complete 360 degree rotation, and form multiple helicoidal structures (Neville & Caveney, 1969). This left-aligned spiral structure of the exocuticle causes left circular polarisation of light, first noted by Michelson (1911), which is rare in nature (Hegedüs *et al.*, 2006) but is the rule in all iridescent dung beetles studied to date, including *Phanaeus*, *Onitis* (Neville & Caveney, 1969), and *Gymnopleurus* species (Brink *et al.*, 2007).

The pitch or perpendicular thickness of each complete 360 degree helix in the optically active layers, dictates which wavelengths of light are absorbed and which are reflected (Neville & Caveney, 1969; Brink *et al.*, 2007). Thus, differences in pitch lead to differences in wavelengths of reflected light and the perceived colour of the beetle. In scarabaeid beetles, pitch has been manipulated experimentally by boiling a portion of green-reflecting cuticle (Neville & Caveney, 1969). This caused it to expand and reflect longer red wavelengths, whereas applying finger pressure caused it to contract and reflect shorter blue wavelengths. However, natural differences in pitch and the perceived reflected colour would presumably arise primarily from variation in the development of ultra-structure within the nascent adult exoskeleton during the pupal stage.

Dung beetles show a good deal of variation in exoskeleton colour, although the manner of its generation is mostly speculative. All 12 tribes of Scarabaeinae include non-iridescent taxa in which colour is presumably generated through pigmentation, probably mainly melanin (black to brown) but with a partial contribution in some species by other probable colour pigments (e.g. red, yellow, white). A very small number of dark dung beetles with a metallic sheen, may represent cases of colouration induced by mixed melanic pigmentation and iridescence from ultra-structure, such as has been described for some carabid beetles (Lindroth, 1974; Paarmann *et al.*, 2007). However, the present work and discussion is restricted to those representatives of six tribes in which both the ground colour and sheen are apparently generated predominantly or entirely by ultra-structural iridescence, as exemplified by the photograph in Brink *et al.* (2007). In some iridescent species, different colours are re-

flected by different sclerites at the same angle of incidence, or they show mixed iridescent and pigmented sclerites, particularly prothoracic discs and elytra. However, individuals of many iridescent species are essentially monochromatic. Although some monochromatic species show the same dominant colour in all individuals, many are polymorphic, comprising two or three colour varieties (Ferreira, 1972). Thus, the same species may be represented by individuals that are entirely cupreous, entirely green, or entirely blue. However, it should be emphasised that although these colour varieties are essentially monochromatic, if the dominant reflected wavelengths are close to the spectral transition between different perceived colours, then there is a bi-chromatic reflectance effect, particularly if viewing the exoskeleton at different angles.

In iridescent dung beetles, limited attention has been paid to the biological attributes or environmental responses that might drive developmental differences in ultra-structure and the resulting variation in exoskeleton colour. As regards biological attributes, breeding ratios in *Phanaeus difformis* Leconte (Blume & Aga, 1976) and experimental cross-breeding ratios in *Canthon cyanellus cyanellus* LeConte (Favila *et al.*, 2000) indicate that colour polymorphism may be under simple Mendelian genetic control. Furthermore, according to Paarmann *et al.* (2007) cross-breeding results for the polymorphic carabid beetle, *Poecilus lepidus* Leske, suggest alleles for certain colours that are either dominant or recessive, with further variation in colour of the phenotype attributable to whether individuals are homozygous or heterozygous. However, observations in Africa suggest that environmental influences may also play a role in colour polymorphism, particularly in those polymorphic dung beetle species in which a single colour variety dominates populations, e.g. *Gymnopleurus humeralis* Klug (Davis & Génier, 2007). In such species, geographical change from one colour to another appears to parallel altitude, rainfall, and temperature regimes. However, patterns are variable. In species such as *Gymnopleurus humanus* Macleay, some populations are dominated by a single colour variety, whereas others comprise more than one colour morph (Davis & Génier, 2007), which may, or may not, represent points of transitional environmental conditions. In still other species, two or more colour varieties frequently co-occur and the patterns are less obviously attributable to environmental variation at a macro-scale. Therefore, a quantitative study was conducted in order to support or disprove some of the observations.

The monochromatic species, *Gymnopleurus humanus*, was selected for this preliminary study, as it is a dominant member of dung beetle assemblages in the arid southwest of Africa, where it appears to show a latitudinal gradient in colour polymorphism from the warm temperate southern Nama Karoo in South Africa (32–33°S) to the subtropical southwest of Angola (14–15°S) (Davis & Génier, 2007). Furthermore, the low physiognomy of the scattered vegetation of this arid late summer rainfall region would not unduly influence results, as it offers little shade and would therefore, be a limited modifier of local microclimate and, exclude any possible, linked exoskeleton colour response. Across the distributional range of *G. humanus*, altitude varies from approximately 600 to 1700 m and rainfall from 75 to 425 mm per annum. However, greatest abundance is centred in

annual rainfall regimes below 300 mm on a mosaic of deep and stony soils and this is where study sites were selected.

Sampling from populations across the latitudinal gradient was designed in such a way to ask two questions. First, as extreme dominance of cupreous individuals had been observed in northern populations (central Namibia), and other colour morphs had only been recorded in the south (southern South Africa), would blue individuals dominate southern populations? Second, although geographical variation in exoskeleton colour of *G. humanus* is supported by observations, would this variation be correlated with or independent of physical variables? The discussion considers the functional implications of the results.

Methods

Study region and selection of study sites using GIS

Study material of *G. humanus* was collected in the southern half of its range (Fig. 1) from southern South Africa (32°S) as far north as central Namibia (23°S), where previous observations showed that populations were dominated by individuals with cupreous exoskeletons. This latitudinal range comprised the Nama Karoo uplands in the south and the central Namibian uplands in the north, with intervening latitudes occupied by the lower-lying river valleys of the Fish, Orange, Hartbees, and their tributaries. Study sites were selected using GIS maps to ensure that material was sampled from across the entire range of climatic conditions

present in the study region. These vary with topography, latitude, and rainfall, all of which may have an influence on temperature. Owing to the irregular distribution of topography, study sites were selected by treating the altitude/rainfall regimes in uplands separately from temperature regimes.

Maps of altitude and average annual rainfall regimes were created by reclassifying measured or interpolated data (5×5 km polygons) on GIS maps of the study region. A reclassified map of altitude regimes was graduated at 100-m intervals across the range from 600 to 1700 m. A similar reclassified map of average annual rainfall regimes was graduated at 50-mm intervals across the range from 75 to 325 mm. These two maps were crosstabulated to create 51 altitude/average annual rainfall regimes that occurred in diffuse patches. A subset of 18 upland regimes was selected according to lower to higher altitude ranges (1000–1100 m, 1200–1300 m, 1400–1500 m), and lower to higher average annual rainfall ranges (125–175 mm, 175–225 mm, 225–275 mm). These were intended to comprise duplicate sets of nine identical altitude/rainfall regimes in the Upper Nama Karoo (30–32°S) and the central Namibian uplands (23–25°S). However, this ideal design was only partially realised, as certain regimes were not represented in both regions or were found to coincide with unsuitable situations. For instance, the deep Kalahari sands, which necessitated adjustments to selection of study sites in the field. In each existing selected regime (eight in the south and six in the north = 14 study sites), a single study site was selected along roads as close as possible to the geographical centre of a representative patch.
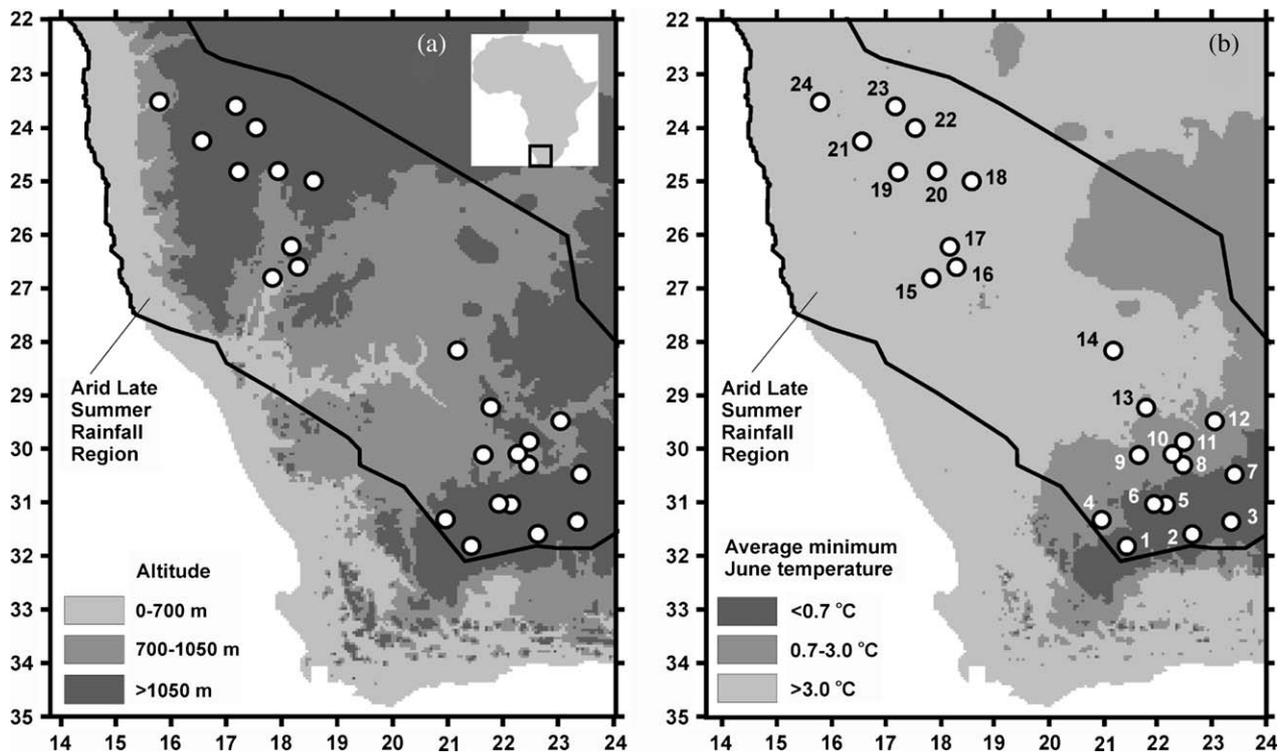
Fig. 1. Maps of south-western Africa showing the location of study sites in relation to (a) altitude and (b) mid-winter minimum temperature regimes (See Fig. 5 for key to sites).

A GIS map of interpolated, mid-winter, average minimum temperatures for June was reclassified to create eight temperature regimes for the study region. These were graduated at 1.5-°C intervals across a range from −2.0 to 8.5 °C. Study sites were selected from each temperature regime. The coolest southern and warmest northern regimes were already well represented by the sites selected from the altitude/rainfall regimes in the uplands between 30–32°S and 23–25°S. Therefore, an extra 11 sites were added primarily to sample from intermediate temperature regimes that were missed or under-represented. The majority occurred at lower altitudes (700–1100 m) in valleys at intermediate latitudes (26–30°S) across a range of average annual rainfall (90–250 mm) that was similar to that of the altitude/rainfall regimes. Again, study sites were selected along roads as close as possible to the geographical centre of patches representing selected temperature regimes.

Collection of study material

Grid references for the 25 selected study sites were generated from the GIS maps. These were entered into a GPS, which was used to pinpoint the selected locations in the field. Sampling of beetles was conducted from 24 February until 17 March 2006. It coincided with the February-to-April seasonal peak in average annual precipitation that is experienced in the arid late summer rainfall region (Davis, 2002, derived from the climatic classification of Walter & Lieth, 1964). As *G. humanus* is an abundant and adaptable species, five baited pitfall traps were placed 50 m apart along the roadside verge at each study site and exposed for the duration of flight activity by the species, which shows a peak from mid to late morning if the weather is sunny. Each trap comprised a small plastic container (120 mm top diameter, 125 mm deep) with a plastic funnel (50 mm deep at apex) suspended from its inner rim, which prevented the escape of trapped beetles. The bait comprised 50 ml of cloth-wrapped pig dung that was suspended immediately above the centre of each trap from an inverted u-shaped wire. A shade cover was supported over each trap. At sites where a large enough sample was obtained from fewer than five traps ($>$140), all beetles in other traps were released. The retained trap material represented a standardised sample from each local population, assuming that proportional colour morph composition was independent of the number of traps and the number of trapped individuals. This material was preserved in 96% alcohol and then dried before measurements of reflectance were made. No observable colour change resulted from this treatment.

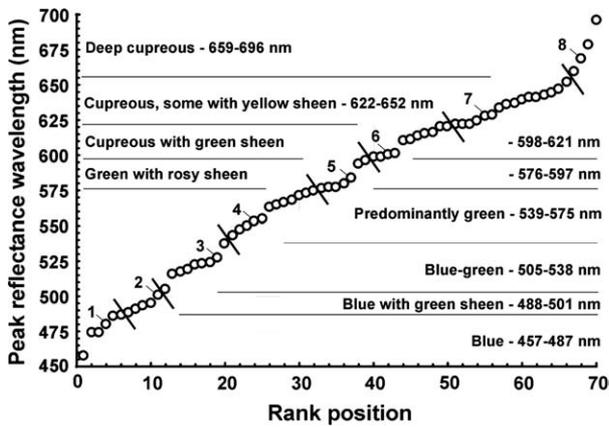### Measurements of exoskeleton reflectance

The beetle exoskeletons were measured for intensity of reflected photo-wavelengths across the entire visible spectrum, using digital reflectance spectrophotometry as described by Brink *et al.* (2007). Measurements were made using a 2-mm diameter collimated beam from a white Tungsten-halogen light source, that was focused to illuminate a 0.3-mm diameter spot on the beetle exoskeleton. The incident and reflected beams were right circularly polarised by passing them through a plane polariser and quarter-wave plate combination, to compensate for the left circular polarised light induced and emitted by the left-skewed helicoidal ultra-structure of the exocuticle in *Gymnopleurus* species (Brink *et al.*, 2007). The reflected beam was directed through a 3 mm slit, 60 cm from the sample, and focused on the entrance cone of a 0.64-m HR 640 monochromator (HORIBA Jobin Yvon Inc., Edison, NJ) measuring device coupled to a TE-410RF S20 photomultiplier (Products for Research Inc., Danvers, MA). This arrangement limited the angular spread of the recorded beam to no more than 3°. Samples and illumination source were mounted on interlinked turn-tables which allowed us to record spectra at a standard angle of incidence of 10°. All recorded spectra were normalised by dividing point by point with the spectrum of the light source polarised in the same way as during recordings. This compensates for wavelength-dependent detector response and monochromator transmission. The averaging option on Dilor software (HORIBA Jobin Yvon Inc.) was used to smooth the data.

Measurements were standardised for all specimens by taking readings from the same body sclerite, i.e. a relatively horizontal point just anterior to the centre of the posterior margin of the prothoracic disc, which overlies the flight and walking muscles. As these measurements took several hours for each beetle, logistics demanded that they were made of only a selected subsample of the trapped material [70 specimens from 18 sites, southern (1–12), and northern (16, 18, 21–24 – see Fig. 1)] with each individual measured on only a single occasion. This is considered sufficient to characterise the reflectance from each individual as each measurement comprised 801 separate readings of relative intensity at 0.5-nm intervals across the visible spectrum from 400 to 800 nm. The readings are also considered to be representative for each individual as the underlying colour at the same angle of incidence (10°) appeared uniform across the entire dorsal surface of each beetle.

Analytical methods

The measured beetles were ranked according to peak values in magnitude of reflected wavelengths (Fig. 2). They were used as a colour template against which the peak reflected colour wavelengths of all other material, were estimated by comparison by eye in natural sunlight. The wavelengths at the peak magnitude of reflectance intensity in these 70 beetles were tightly distributed across the measured range (Fig. 2) and were, therefore, a valid sample on which to base these estimates. The measured or estimated maximum reflectance values for each individual were used to calculate mean colour bias and its standard deviation within each of 24 study sites (mean 165.9 individuals per study site, range 43–329, one site with only 19 individuals was deleted). Despite the imprecision of estimation, it is considered that mean values derived from large samples generate a reliable approximation of the overall colour bias in each of the 24 populations. These $\log_{10}$ transformed mean colour reflectance values were tested for correlation with $\log_{10}$ transformed physical factors for each of the 24 study sites (average annual minimum temperature, average annual rainfall, altitude, and latitude) using both multiple and linear regression. Coefficients of determination (r^2) from separate

Fig. 2. Rank measured values (nm) for peak intensity of reflectance from 70 individuals of *Gymnopleurus humanus*. These beetles are divided into eight waveband categories on the basis of perceived colour of the exoskeleton. Patterns of intensity of wavelength reflectance for beetles one to eight are depicted in Fig. 3.

linear regressions of $\log_{10}$ transformed mean colour reflectance values on $\log_{10}$ transformed average temperatures for each month (maximum, minimum, and maximum + minimum/2) were used to graph the seasonal variation in patterns of correlation.

As there was extensive spatial variation in population density, it was necessary to standardise results to the same scale for detailed between-site comparisons. This was done by dividing the ranked beetles (Fig. 2) into eight wavelength ranges on the basis of perceived colour of their exoskeletons. The abundances of individuals in each category were then standardised by converting them to a percentage scale for each of the 24 study sites. The patterns at each site were compared using bar diagrams of percentage data. MDS ordination of a correlation matrix derived from untransformed abundance data was also used for each of the eight categories at each of the 24 study sites.

Results

It was confirmed that, although muted, exoskeleton colour in *G. humanus* is iridescent. Measured specimens mostly reflected across a narrow range of wavelengths with peaks in magnitude of intensity that varied from the blue to the red ends of the visible light spectrum in different individuals (Figs 2 and 3).

Populations of *G. humanus* showed different patterns of colour distribution between individuals at different study sites across a south–north geographical gradient (Figs 1, 4 and 5). These different patterns were divisible into four clusters on an ordination plot (Fig. 4). Blue, or a combination of blue and green individuals (Fig. 2 – categories 1–4), dominated populations at Sites 1–6 (Fig. 5), which were centred on southern uplands with low minimum June temperatures (Fig. 1). Beetles with cupreous exoskeletons were absent or extremely rare at these sites. Sites 7–12 were centred just to the north of Sites 1–6 at lower altitude with warmer minimum June temperatures (Fig. 1). For the most part, all eight colour categories co-occurred at Sites 7–10, al-
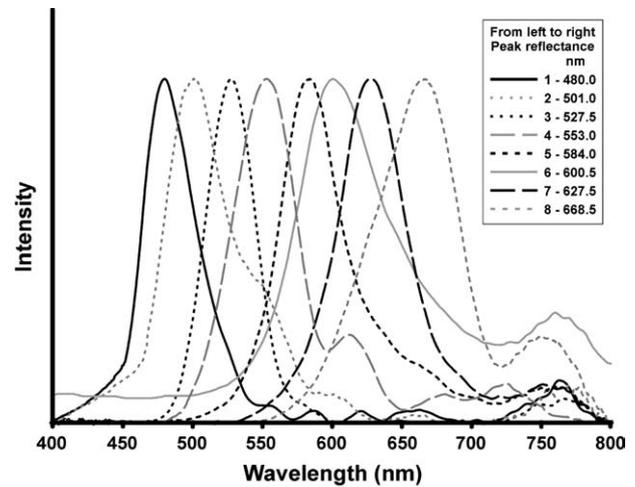

Fig. 3. Patterns of intensity of reflectance from eight beetles across the visible wavelengths, each representing one of eight bandwidth categories defined according to perceived exoskeleton colour (see Fig. 2) [n.b. This diagram merely illustrates different patterns on a standardised scale (0–1 – all nm values divided by maximum value). Between-beetle differences in intensity of reflectance are possible, but these are unknown as measurements were made to an arbitrary scale unique to each trace. Optical surfaces of biological samples are variable and unsuited to measurements on an absolute scale (Brink *et al.* 2007)].

though most populations were dominated by individuals with rosy-green or cupreous-green exoskeletons (categories 5 and 6) (Figs 2 and 5). At the slightly more northerly Sites 11 and 12, populations were dominated by cupreous-green and cupreous individuals (Fig. 2 – categories 6 and 7) with blue individuals extremely rare or absent. Sites 13–24 are centred on a still more northerly region with variable altitude, but warmer minimum June temperatures than the southern area occupied by Sites 1–12 (Fig. 1). Populations at these sites are dominated by individuals with cupreous exoskeletons (Fig. 2 – category 7) although several more southerly sites showed sizeable proportions of cupreous-green individuals (Figs 2 and 5). Blue and green individuals were rare or absent from these sites.
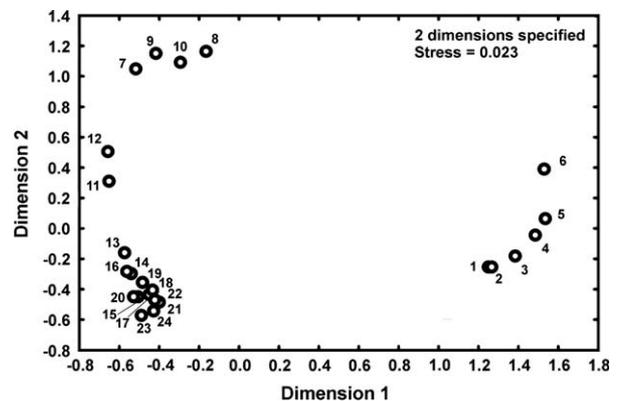
Fig. 4. MDS ordination plot depicting the dissimilarity between patterns of colour polymorphism in populations of *Gymnopleurus humanus* Macleay from 24 study sites in south-western Africa.
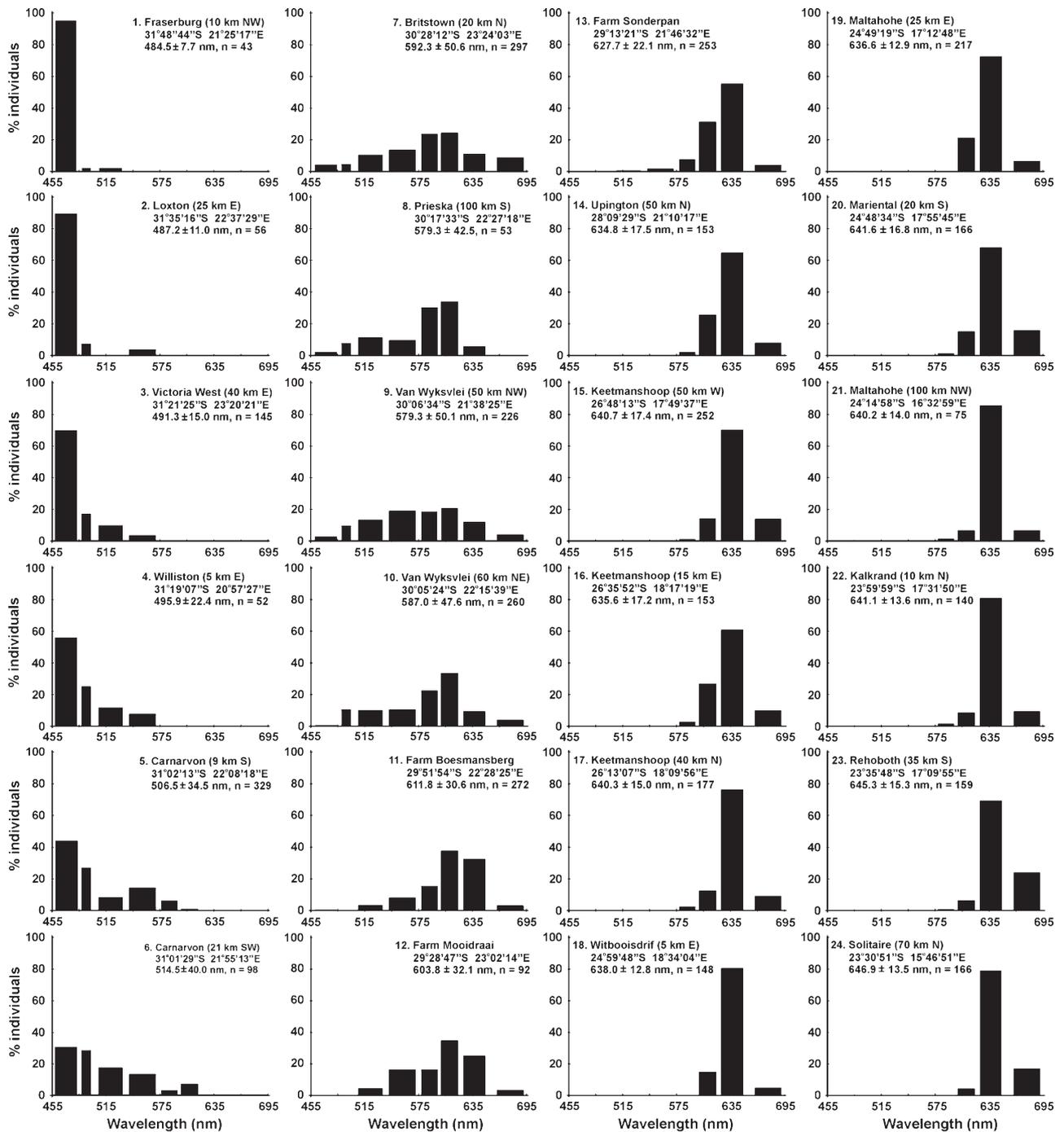
Fig. 5. Patterns of iridescent colour polymorphism in populations of *Gymnopleurus humanus* MacLeay from 24 study sites in south-western Africa ranked from south (1) to north (24). Each bar represents one colour bandwidth defined in Fig. 2 (n.b. to clarify the patterns, bars are reduced to 0.8 of each bandwidth). Mean values for estimated peak intensity of reflectance ± SD (nm) are also given for each site together with the number of individual beetles from which this mean was derived (*n*).

Multiple regression indicated that mean exoskeleton colour bias at the 24 sites was strongly and significantly correlated with average annual minimum temperatures, but not with altitude or average monthly rainfall (Table 1). Separate linear regressions

of colour bias on these physical variables were largely supportive (rainfall not significant: $t_{22} = -1.66$, $P = 0.11$, $r^2 = 0.11$; minimum temperature highly significant: $t_{22} = 12.53$, $P < 0.001$, $r^2 = 0.88$) although altitude also had a limited significant

Table 1. Results for multiple regression of $\log_{10}$ transformed physical factors on mean estimated wavelength of reflectance from the exoskeleton of populations of *Gymnopleurus humanus* MacLeay at 24 study sites.

| Physical factors	t_{20}	Probability (P)
Altitude	0.87	0.392
Average annual rainfall	0.80	0.432
Average annual minimum		
Monthly temperature	11.10	0.000*

*Significant $P < 0.001$. Coefficient of determination (r^2) for regression model = 0.89.

influence ($t_{22} = -2.12$, $P < 0.05$, $r^2 = 0.17$). A further linear regression of colour bias on latitude also showed a strong correlation ($t_{22} = -6.34$, $P < 0.001$, $r^2 = 0.65$). However, lower minimum temperatures are strongly interdependent with progression towards higher latitude ($t_{22} = -5.56$, $P < 0.001$, $r^2 = 0.58$).

As annual temperature was strongly correlated with geographical colour polymorphism in *G. humanus*, mean colour bias at the 24 sites was further tested for correlation with seasonal temperature variation. Strongest correlations were with cool dry season temperatures from May until September and with minimum temperatures in general (Fig. 6).

Discussion

The results show a strong correlation between dry season temperatures and exoskeleton colour bias in *G. humanus*. They sug-
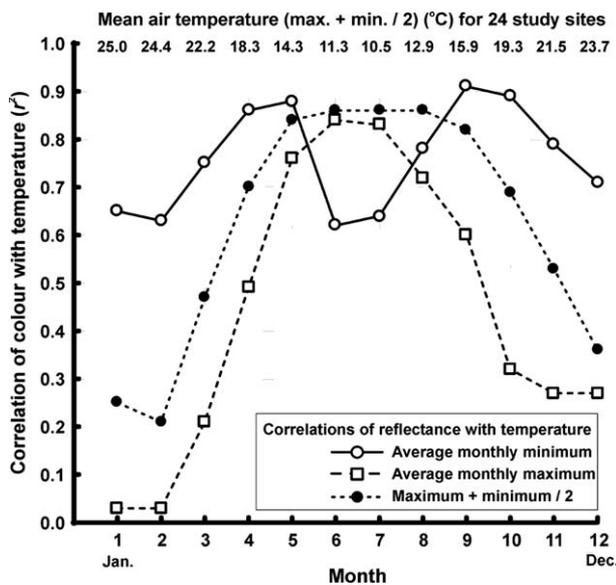
Fig. 6. Seasonal variation in patterns of correlation [coefficient of determination (r^2)] between $\log_{10}$ transformed mean colour reflectance values and $\log_{10}$ transformed average monthly temperatures at 24 study sites. Overall mean seasonal temperatures (max. + min./2) for the sites are also provided.

gest that colour polymorphism might be at least partly driven by environmental factors. Therefore, the discussion considers the mechanics of colour generation and the possible role of differing thermal conditions as drivers of ultrastructural variation during the development of new and differently coloured adults. It also considers the relative contribution of genetic versus environmental factors, and the possible functional advantages of cross-climatic colour variation.

In dung beetles, iridescent colour is generated by multilayer interference sited within the exocuticle (Neville & Caveney, 1969). Within each layer, the ultrastructure comprises parallel microfibrillae that are offset to the left by a standard distance at each successive level to ultimately form 360 degree helicoidal stacks, that define the thickness of the layer or its pitch. This is responsible for the reflected colour wavelength. The optical properties of these microfibrillae are shared with cholesteric liquid crystals (Neville & Caveney, 1969). Such crystals are inherently self-organising with regard to the offset in alignment of successive levels, and some classes of crystals vary in pitch with temperature (Kosho *et al.*, 1999). It is necessary to determine if the microfibrillae in the exocuticle of *G. humanus* might also be both self-organising and temperature sensitive during development of the new adult, as this could, conceivably, be a mechanism for environmentally-driven variation in exoskeleton colour.

Given current limitations in the data, it is not possible to determine the relative roles of environmental versus genetic influences in colour polymorphism. Although Favila *et al.* (2000) consider that colour variation is under genetic control, having obtained mendelian ratios for colour morphs resulting from laboratory cross-breeding of a dung beetle (*Canthon cyanellus*), this evidence is countered by the present results that show a strong correlation between environmental factors and colour variation in the field (*Gymnopleurus humanus*). Then again, recent cross-breeding experiments between violet, black, blue, green, and cupreous individuals of a polymorphic, European, carabid beetle species (*Poecilus lepidus*) also suggest genetic involvement in the generation of colour in filial beetles according to Paarmann *et al.* (2007). As patterns in iridescent exoskeleton colour vary at both geographical and local scales as well as between taxa (A. L. V. Davis, pers. obs.), the relative importance of factors could also possibly vary with scale or taxon. However, further research is necessary to resolve the apparent contradictions.

Observations suggest that patterns shown by African taxa could be related to a complex of environmental, genetic or behavioural factors. For instance, iridescent ball rolling genera or subgenera that are able to select nesting sites (*Anachalcos*, *Kheper*, *Gymnopleurus*, *Allogymnopleurus*, *Garreta*) are frequently observed to show local dominance of a single colour morph. However, multicolour populations are known in some species, including *G. humanus*. In tunnelling genera (*Onthophagus*, *Proagoderus*, *Phalops*), in which nesting sites are dictated by the site of dung deposition, multicolour populations seem to be more frequent. Furthermore, it appears that the patterns may be more complex in less arid regions, as greater heterogeneity in rainfall and plant cover may combine as possible further macroclimatic and microclimatic influences on

colour polymorphism. However, a great deal of quantitative work is required to support or disprove the various observations.

Given the apparent contradictions in findings and interpretation of results, future cross-breeding experiments clearly need to be conducted under several different temperature regimes appropriate for the species under study. This is the only way that the proportional influences of temperature and genotype might be determined. Although genetic factors cannot be ruled out in *G. humanus*, the strong correlations between geographical variation in exoskeleton colour and cool mid-dry season temperatures, suggest that they may exercise a relatively strong influence compared with genotype. Furthermore, the lower dry season correlations between colour and minimum temperatures may indicate a greater influence from the relative magnitude of dry season maximum temperatures. Alternatively, higher correlations with minimum temperatures in spring might be significant if they coincided with pupation and development of nascent adults, particularly, as overwintering in immature dung beetles is, generally, as dormant third instar larvae (Tyndale-Biscoe, 1988; Bang *et al.*, 2004).

Differences in cross-climatic colour polymorphism shown by the largely sympatric dung beetle species, *G. humanus* and *G. andreaei* Ferreira may be related to different seasonal breeding strategies to survive unfavourably cold or dry conditions in southern Africa. There are two principal strategies. Both involve dormancy in the soil, either as adults or as immatures (Edwards, 1988; Davis, 1993). The seasonal chronologies of development in *G. humanus* and *G. andreaei* are unknown. Such paucity of information compared with other regions is frequently the case in Africa (Pimm, 2007). However, *G. andreaei* may complete its breeding cycle under warm summer conditions and overwinter as adults. On the other hand, *G. humanus* may overwinter mostly as immatures, as only two callow specimens of *G. humanus* were noted among the several thousand examined for this study, in contrast to a greater proportion of teneral individuals recorded for smaller numbers of *G. andreaei* from the same traps. Furthermore, the normally deep violet *G. andreaei* shows little cross-climatic colour variation other than the uncommon occurrence of some greenish individuals in the cooler south of its range where the species is comparatively rare (A. L. V. Davis, pers. obs.). By contrast, *G. humanus* is relatively abundant across the entire study area and shows marked cross-climatic colour polymorphism that is strongly temperature correlated.

If maturation of *G. humanus* were staggered over a number of months with completion of development occurring under a range of different temperatures, it could explain how a range of colour morphs is found locally. This scenario would suggest that cool southern and warmer northern temperature ranges might fall either side of the transition points from blue to green to cupreous exoskeletons, that are experienced in intermediate localities. Precise temperature cues cannot currently be ascertained as the immatures develop within brood balls of dung in subterranean nests at unknown depths, and are subject to unknown soil temperatures that differ from air temperatures through the insulating effects of soil. However, air temperatures act as a good surrogate for soil temperatures for purposes of macro-scale correlation. As colour polymorphism of *G. humanus* is highly correlated to temperature, whether or not the correlation reflects a direct physical response or a variable bias in genetic structure within different populations, it might be expected that changes in the colour morph balance of populations would result from changing conditions. This could be used as a sensitive and easily interpreted indicator of global climatic change, particularly in the south.

Various possible functions may be attributable to iridescent colour in dung beetles (Vulinec, 1997). These fall into three categories. Anti-predation may be conferred through crypsis, distraction, or aposematism signifying distastefulness. Alternatively, the colouration may be involved in thermoregulation or in social signalling for male advertisement and female mate choice. Although female preference for brightly coloured males has now been demonstrated in one butterfly species (Kemp, 2007b), behavioural use of colour remains largely speculative for both Lepidoptera (Kemp, 2007a) and dung beetles (Vulinec, 1997). This is particularly the case for signals produced by purposeful movement that changes the angle of incidence and emitted spectra, thus drawing attention towards an individual. However, one possible advantage conferred by iridescence and colour polymorphism in *G. humanus* might relate to thermoregulation, as a result of the different thermal properties of different colours. This is because absorbed light converts to heat energy, different colours absorb different wavelengths of light, and different colour wavelengths produce different amounts of heat. In particular, blue reflecting exoskeletons absorb red wavelengths that generate more heat energy than red reflecting exoskeletons that absorb blue wavelengths. Thus, the colour polymorphism from blue to red shown by *G. humanus* could be an advantage across the temperature gradient from cool to warmer regions. However, the thermal properties of the beetle exoskeletons need to be tested.

Acknowledgements

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