

# Testing the thermal melanism hypothesis: a macrophysiological approach

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## Summary

1. The thermal melanism hypothesis (TMH) predicts that dark (low skin reflectance) individuals are at an advantage in cool climates as they heat faster and reach higher equilibrium temperatures than lighter (higher reflectance) individuals. However, tests of the TMH have yielded mixed support, especially in ectothermic vertebrates.
2. Most studies to date have been undertaken at small spatial scales or using a few, closely related populations or species. Here, we therefore examine the TMH at large scales in heliothermic lizard species, testing two of its major predictions and a corollary thereof, using standard and phylogenetically corrected analyses.
3. First, we test the prediction that skin reflectance and climate variables such as mean annual temperature (MAT) and global solar radiation are positively related across species. Second, we determine whether a positive relationship exists between skin reflectance and body mass. Third, since physiology, behaviour and morphology should be co-adapted, we test the prediction that skin reflectance and traits of thermal biology are positively related.
4. We find strong support for a positive relationship between skin reflectance and mean annual radiation even after adjusting for phylogeny. Moreover, radiation was a better predictor of skin reflectance than MAT. We also find support for a positive relationship of skin reflectance with body size, although this was non-significant after accounting for phylogeny.
5. Skin reflectance was not related to measures of thermal biology, although confounding effects such as methodological differences could not be ruled out.
6. In summary, this study provides novel support for the TMH operating interspecifically at large geographic scales, suggesting adaptive variation of skin reflectance among lizards.

**Key-words:** co-adaptation, colouration, skin absorptance, solar radiation, thermoregulation

## Introduction

Biophysics enables predictions to be made about the thermal balance of ectotherms by quantifying the flow of energy between organisms and their environments (Gates 1962; Helmuth 1998). For example, when two identical ectothermic individuals (i.e. same shape, posture, body size) that differ only in skin reflectance are exposed to the same environmental conditions, the dark (low skin reflectance) individual should heat faster and reach higher equilibrium temperature, by absorbing more solar radiation, than the light (high skin reflectance) individual (Porter & Gates 1969). Studies that compare heating rates and equilibrium temperatures of individuals differing in skin reflectance under the same environmental conditions generally satisfy these predictions and support the hypothesis that melanism plays an adaptive

role in thermoregulation (defined here as the thermal melanism hypothesis (TMH; e.g. Watt 1968; Kingsolver 1987; De Jong, Gussekloo & Brakefield 1996). Since body temperature influences most physiological processes (Hochachka & Somero 2002), the differences in heating rate and equilibrium temperature between low and high skin reflectance individuals should influence performance and reproductive success, and thus evolutionary fitness. Several authors have argued that low reflectance should therefore be beneficial to animals inhabiting cold regions (Watt 1968; Ellers & Boggs 2004), whereas high reflectance should provide an advantage to inhabitants of warm environments by absorbing less solar radiation and precluding lethal temperatures (Gibert *et al.* 1998).

Convincing support for the fitness advantage of thermally beneficial colouration has been found in several invertebrate species (e.g. Kingsolver 1995; De Jong *et al.* 1996; Ellers & Boggs 2004), but explicit investigations thereof in vertebrate

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ectotherms are less common. To date, only a few studies have provided convincing evidence in supporting predictions of the TMH. For example, in their study of the common garter snake *Thamnophis sirtalis*, Gibson & Falls (1979) showed that melanism increased the efficiency of heat absorption, possibly allowing a longer active season for melanistic morphs. Similarly, Capula & Luiselli (1994) and Forsman (1995) found that melanistic females of the adder *Vipera berus* bore offspring more frequently and had higher survival rates than zigzagged (lighter) females. In addition, lizard species with individuals that undergo colour change show a decline in reflectance at lower temperatures in the near-infrared wavelengths, thus enabling faster heating in cold conditions (Norris 1967; Gates 1980; Walton & Bennett 1993). Although these studies suggest that the predictions of the TMH, and therefore the hypothesis itself, are generally supported, two characteristics of the investigations preclude such a conclusion. First, the large majority of investigations tend to have involved either a single species (e.g. Forsman 1995; Tattersall, Eterovick & de Andrade 2006), or perhaps, a few closely related species (e.g. Walton & Bennett 1993). Second, other functions of colouration such as camouflage, UV protection and behavioural adjustments may confound existing melanism–temperature relationships in ectotherms (see review in True 2003) and their detection, especially given the relatively small magnitude in body temperature differences found between individuals varying in skin reflectance (e.g. Bittner, King & Kerfin 2002). As a consequence, the extent to which the TMH is generally supported in ectotherms is difficult to determine.

Whilst additional, small-scale studies would assist in establishing the generality of the TMH, especially if a large number could be subjected to a formal meta-analysis, the case for it would be even stronger if large-scale, multi-species investigations were also unable to falsify its predictions. Indeed, Spicer & Gaston (1999) have argued that among the strongest evidence for adaptive physiological variation is similar findings at several hierarchical levels (e.g. among individuals, among populations and among species). Such an outcome would demonstrate that the signal of selection is detectable despite the confounding influences of other variables at each level, and despite the methodological problems that are inherent to the approaches used to investigate variation within the different levels. Moreover, recent work has emphasized the considerable advantages of multi-faceted approaches to problems in evolutionary physiology (for examples see Gibbs, Fukuzato & Matzkin 2003; Hoffmann *et al.* 2003; McKechnie, Freckleton & Jetz 2006; and for additional discussion see Kingsolver & Huey 1998; Feder, Bennett & Huey 2000; Feder & Mitchell-Olds 2003). Therefore, here we test the predictions of the TMH in vertebrate ectotherms, specifically heliothermic lizards, using a broad-scale, macrophysiological approach (Chown, Gaston & Robinson 2004), to complement and inform the small-scale investigations that are more typical of the field (including work on invertebrates). Specifically, we test two major predictions of the TMH (see Clusella-Trullas, van Wyk & Spotila 2007a for review), and a corollary thereof.

First, we examine the prediction that skin reflectance and climate variables such as mean annual temperature (MAT) and global solar radiation will be positively related across species. It is generally expected that species with low skin reflectance should occupy cool and low radiation areas since melanism favours heat absorption, and that the converse should also apply (Gates 1980). We test for both temperature and solar radiation because their relationship may be non-linear (e.g. tropical mountain peaks are high solar radiation, low temperature environments) and their potential selective roles on skin reflectance may be different depending on whether critical thermal limits or energy budgets are limiting. Thus, at broad spatial scales, positive relationships between skin reflectance and temperature, and between skin reflectance and solar radiation might be expected, but for different reasons.

Second, we determine whether a positive relationship exists between skin reflectance and body mass. It is well established that large animals have lower convection coefficients and higher heat capacity per unit surface area compared with smaller ones (Gates 1980; Stevenson 1985). Therefore, at large scales, we expect that small species will have lower skin reflectance than large species because, although low reflectance enables the former to warm up quickly in cold conditions, they can also dissipate heat relatively rapidly when facing warm conditions. By contrast, large species will likely have higher skin reflectance than small species to avoid reaching high critical temperature thresholds in warm conditions. This pattern has been shown previously in an inter-specific study of 17 lizard species from the deserts and mountains of the southwestern United States and Mexico (Norris 1967).

Finally, we examine the idea that skin reflectance, thermoregulatory behaviour and thermal physiology interactively influence fitness, and are thus co-adapted (Huey & Bennett 1987; Bauwens *et al.* 1995; Angilletta, Niewiarowski & Navas 2002; Angilletta *et al.* 2006). By co-adaptation we mean the correlated evolution of phenotypic traits that results in greater performance and hence, fitness of an organism (Arnold 1983; Huey & Bennett 1987). Previous studies have found either strong or partial associations between temperature preference and optimal temperature for physiological performance (e.g. sprint speed; Huey & Bennett 1987; Bauwens *et al.* 1995) and, generally, support the notion that thermal sensitivity responds to selection (Huey & Kingsolver 1989, 1993). Because of its effect on body temperature, it might therefore be expected that skin reflectance and other aspects of thermal physiology will also evolve in concert. Specifically, we here test whether skin reflectance is positively related to the low temperature activity limit ( $CT_{\min}$ ), high temperature activity limit ( $CT_{\max}$ ) and preferred body temperature ( $T_{\text{sel}}$ ; Huey & Bennett 1987; Bauwens *et al.* 1995).

## Methods

### DATA

To standardize the lizard reflectance data only information from the adult stage of diurnal, totally or seasonally heliothermic species

during their active period was used. We compiled data only from published studies except for reflectance values provided by Warren P. Porter (University of Wisconsin, Madison).

The Anglophone literature (1940–2007) was searched for data on skin reflectance over the wavelength range of 290–2600 nm, which incorporates the ultraviolet, visible and infrared portions of the solar spectrum (nearly 98% of the solar radiation reaching the earth's surface). When data from this range were not available, we included reflectance measurements over a minimum range of 300–1200 nm (seven species) which still represents nearly 75% of the radiant energy absorbed by the organism. For species where individuals are able to change colour, we chose reflectance at a temperature closest to the MAT of the species' sampling location. In cases where several values were found for the same population, the arithmetic mean was calculated.

Body temperatures selected in a thermal gradient ( $T_{sel}$ ; Licht *et al.* 1966) and critical thermal limits (critical thermal minima ( $CT_{min}$ ) and maxima ( $CT_{max}$ ); Cowles & Bogert 1944; Lutterschmidt & Hutchison 1997) were compiled for species for which skin reflectance was available. In the physiological data base compilation, priority was given to field-fresh individuals over short- or long-term acclimated individuals, photothermal over thigmothermal gradients, mean  $T_{sel}$  values (if only the range, central 50% or 75% of  $T_{sel}$  were given, the mean of the set boundaries was calculated assuming a normal distribution of  $T_{sel}$ ; e.g. Hertz, Huey & Stevenson 1993; Clusella-Trullas *et al.* 2007b), the onset of spasms vs. the loss of righting response as the end-point of  $CT_{max}$  (Lutterschmidt & Hutchison 1997), and the loss of righting response as the end-point of  $CT_{min}$ . If several populations with  $T_{sel}$  or critical thermal limit data were found for the same species, we chose the value from the population that matched the population sampled for reflectance. However, if no populations could be matched for geographic location, we selected the study which best fitted the data base criteria given above. In a few cases, when physiological methods were considered equal or if reflectance sampling location was unknown, we calculated the arithmetic mean from all studies. The full list of references used to build our data base is included in Supplementary Appendix S1.

Adult body masses were compiled from the studies that reported skin reflectance or thermal biology or, as the last alternative, from studies that provided these data on the same population or species. The geographic location (latitude and longitude) of the population sampled for skin reflectance was used to determine mean annual air temperature from WorldClim (30 arcseconds spatial resolution; Hijmans *et al.* 2005) using DIVA-GIS software (version 5.2.0.2), and mean annual solar radiation (MRAD; equivalent to incident solar radiation ( $W m^{-2}$ ) estimated from mean monthly sunshine hours (Hulme *et al.* 1995);  $0.5^\circ$  latitude  $\times$   $0.5^\circ$  longitude resolution, IPCC Data Distribution Centre). Radiation data for two species from the Galapagos Islands were not available. If the location of the population sampled for skin reflectance was unknown, the centre of the species' range was determined using species' distribution maps from Cogger (1994) and Behler & King (1996).

## ANALYSES

Ordinary least-square regressions were used (after testing for normality of distributions, homogeneity of variance and independence of error terms; Quinn & Keough 2002) to examine relationships between arcsine square-root transformed skin reflectance (REFL) and MRAD, MAT,  $\log_{10}$ -transformed body mass (MASS),  $T_{sel}$ ,  $CT_{max}$  and  $CT_{min}$ . We refrained from testing these relationships in a

single multivariate model because sample sizes differed among variables (from 68 to 17 species), which would have meant a substantial reduction in the power of the model. To investigate further the likely effects of MRAD on REFL after accounting for the effects of MASS and MAT, a Type I sums of squares general linear model was undertaken with REFL as the dependent variable. MASS, MAT and MRAD were entered sequentially as independent variables in that order.

The regression analyses were repeated using the phylogenetic generalized least-square method (PGLS; Martins & Hansen 1997) to account for statistical non-independence of species data. Since no comprehensive phylogeny was available for the species sampled in this study, a phylogeny was built from multiple sources (available in Supplementary Appendix S2). The adjustment for phylogeny was undertaken using both equal and proportional branch length assumptions (Halsey, Butler & Blackburn 2006). A maximum-likelihood approach was used to estimate  $\lambda$ , which indicates the extent to which trait evolution is correlated with phylogeny, and provides insight into the relative importance of adjusting for phylogeny (for details see Halsey *et al.* 2006;  $\lambda = 1$  indicates strong phylogenetic effect,  $\lambda = 0$  indicates no influence of phylogeny). All data are presented as means  $\pm$  SE and statistical significance accepted at  $P < 0.05$ .

## Results

The heliothermic lizard species investigated here typically had low skin reflectances ( $16.3 \pm 6.4\%$ ,  $n = 68$ , range = 1.7–33.6%, Fig. 1). Ordinary least-square relationships between REFL and MRAD, MAT and MASS, respectively, were all significant (Table 1). In addition, even after accounting for the effect of MASS and MAT, REFL was still significantly related to MRAD (Table 2). However, skin reflectance was not significantly related to  $T_{sel}$ ,  $CT_{max}$  or  $CT_{min}$  (Table 1).

The results of the PGLS analyses revealed that phylogenetic models with equal branch lengths provided better fits to the data than those with a star-shaped phylogeny (i.e. all species equally related) or with proportional branch lengths for the relationships between REFL and MRAD, MAT and

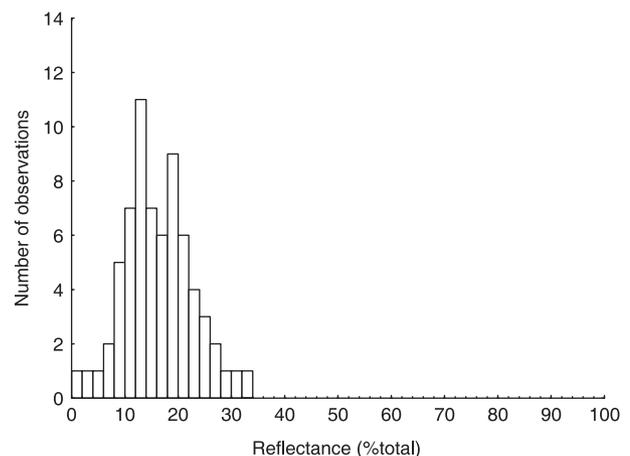


Fig. 1. Frequency distribution of skin reflectance (%) for 68 heliothermic lizard species included in the present study.

**Table 1.** Statistics for ordinary least-square regressions of arcsine-transformed skin reflectance and: mean annual temperature (MAT), mean annual radiation (MRAD),  $\log_{10}$  body mass (MASS), preferred body temperature ( $T_{\text{sel}}$ ), and critical thermal maxima ( $CT_{\text{max}}$ ) and minima ( $CT_{\text{min}}$ ) for heliothermic lizard species

Variable	<i>N</i>	Slope $\pm$ SE	Intercept $\pm$ SE	$r^2$	<i>P</i>	SE estimate
MAT ( $^{\circ}\text{C}$ )	68	0.008 $\pm$ 0.002	0.24 $\pm$ 0.04	0.17	0.0005	0.085
MRAD ( $\text{W m}^{-2}$ )	66	0.002 $\pm$ 0.001	0.08 $\pm$ 5.96	0.26	0.00001	0.080
MASS (g)	68	0.03 $\pm$ 0.01	0.36 $\pm$ 0.02	0.07	0.025	0.089
$T_{\text{sel}}$ ( $^{\circ}\text{C}$ )	42	0.003 $\pm$ 0.004	0.29 $\pm$ 0.13	0.02	0.40	0.083
$CT_{\text{max}}$ ( $^{\circ}\text{C}$ )	28	0.006 $\pm$ 0.006	0.13 $\pm$ 0.24	0.04	0.30	0.092
$CT_{\text{min}}$ ( $^{\circ}\text{C}$ )	17	0.009 $\pm$ 0.007	0.32 $\pm$ 0.06	0.04	0.24	0.108

**Table 2.** Outcome of a general linear model (Type I SS model) examining the residual contributions of  $\log_{10}$  body mass (MASS), mean annual temperature (MAT) and radiation (MRAD) respectively to arcsine-transformed skin reflectance

Variable	d.f.	MS	<i>F</i>	<i>P</i>
Intercept	1	10.89	1708.07	< 0.001
MASS (g)	1	0.038	5.96	0.0175
MAT ( $^{\circ}\text{C}$ )	1	0.060	9.34	0.0033
MRAD ( $\text{W m}^{-2}$ )	1	0.059	9.21	0.0035
Error	62	0.006	–	–

MASS, respectively (lowest value of AIC within each variable, Table 3). In these cases,  $\lambda$  was > 0.8 indicating a strong phylogenetic effect. Overall, MRAD was a good predictor of skin reflectance, whereas the relationship between REFL and

MAT was of marginal significance, and that between REFL and MASS was not formally significant (Table 3). Comparison of model AICs for MAT and MASS suggested that the former was a better fit to the data. The fit of the model for MRAD cannot be compared with these two models because of differences in the data sets. However, comparison is possible if the analyses for MAT and MASS are repeated excluding the two species for which no MRAD data are available. In this case (assuming equal branch lengths), AIC =  $-136.33$  ( $\lambda = 0.95$ ) for MASS and AIC =  $-137.82$  ( $\lambda = 0.94$ ) for MAT, compared to  $-147.00$  for MRAD. The probability that MRAD is the best fit of these three alternative models (its Akaike weight; Burnham & Anderson 2001) is 0.985, vs. 0.01 for MAT and 0.005 for MASS.

For  $CT_{\text{max}}$ ,  $CT_{\text{min}}$  and  $T_{\text{sel}}$ , the best models were those which did not account for phylogeny (Table 3), and thus simply recapitulate the results in Table 1.

**Table 3.** Results of the phylogenetic generalized least-squares (PGLS) analyses adjusting for phylogenetic effects on the univariate relationships of arcsine-square root transformed skin reflectance and several climatic and physiological parameters

Model	Phylogeny	$\lambda$	AIC	Slope $\pm$ SE	d.f.	<i>T</i>	<i>P</i>
MAT	Star	0	-138.93	0.0078 $\pm$ 0.0021	65	3.714	0.0002
	<b>Equal</b>	<b>0.915</b>	<b>-141.3</b>	<b>0.0042 <math>\pm</math> 0.0024</b>		<b>1.750</b>	<b>0.0424</b>
	Proportional	0.026	-137.1	0.0076 $\pm$ 0.0022		3.455	0.0005
MRAD	Star	0	-142.62	0.0022 $\pm$ 0.0005	63	4.400	0.0001
	<b>Equal</b>	<b>0.835</b>	<b>-147</b>	<b>0.0022 <math>\pm</math> 0.0005</b>		<b>4.000</b>	<b>0.0001</b>
	Proportional	0.18	-144	0.0022 $\pm$ 0.0005		4.400	0.0001
MASS	Star	0	-131.44	0.0299 $\pm$ 0.0132	65	2.265	0.0134
	<b>Equal</b>	<b>0.925</b>	<b>-139.79</b>	<b>0.0175 <math>\pm</math> 0.015</b>		<b>1.167</b>	<b>0.1237</b>
	Proportional	0.24	-133.42	0.0294 $\pm$ 0.0138		2.130	0.0185
$T_{\text{sel}}$	<b>Star</b>	<b>0</b>	<b>-86.28</b>	<b>0.0033 <math>\pm</math> 0.0039</b>	<b>39</b>	<b>0.846</b>	<b>0.2014</b>
	Equal	0.48	-85.33	0.0047 $\pm$ 0.0039		1.205	0.1177
	Proportional	0	-84.28	0.0033 $\pm$ 0.0039		0.846	0.2014
$CT_{\text{max}}$	<b>Star</b>	<b>0</b>	<b>-48.17</b>	<b>0.0059 <math>\pm</math> 0.0055</b>	<b>25</b>	<b>1.073</b>	<b>0.1468</b>
	Equal	0	-46.17	0.0059 $\pm$ 0.0055		1.073	0.1468
	Proportional	0	-46.17	0.0059 $\pm$ 0.0055		1.073	0.1468
$CT_{\text{min}}$	<b>Star</b>	<b>0</b>	<b>-23.4</b>	<b>0.009 <math>\pm</math> 0.0074</b>	<b>14</b>	<b>1.216</b>	<b>0.1221</b>
	Equal	0	21.4	0.009 $\pm$ 0.0074		1.216	0.1221
	Proportional	0	21.4	0.009 $\pm$ 0.0074		1.216	0.1221

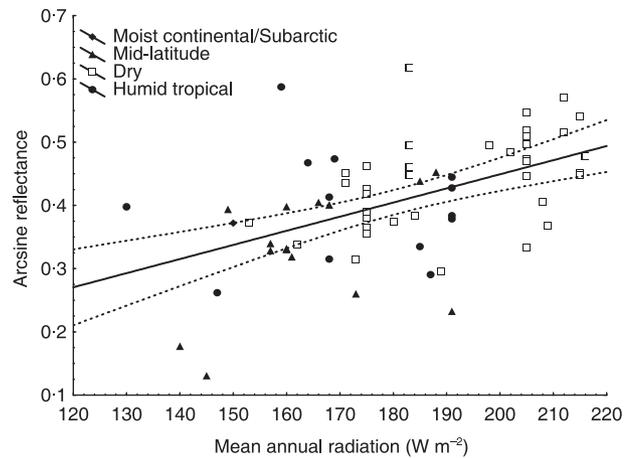
MAT, mean annual temperature ( $^{\circ}\text{C}$ ); MRAD, mean annual radiation ( $\text{W m}^{-2}$ ); MASS,  $\log_{10}$  body mass (g);  $CT_{\text{max}}$ , critical thermal maxima ( $^{\circ}\text{C}$ );  $CT_{\text{min}}$ , critical thermal minima ( $^{\circ}\text{C}$ );  $T_{\text{sel}}$ , preferred body temperature ( $^{\circ}\text{C}$ ). Within one variable (e.g. MAT) the lowest AIC indicates the model with the best fit (highlighted in bold; see Methods and Results sections for details). d.f., degrees of freedom.  $\lambda = 1$  indicates strong phylogenetic effect,  $\lambda = 0$  indicates no influence of phylogeny. Note that AIC values cannot be compared across variables, since sample size and taxonomic composition differ except in the case of MAT and MASS.

## Discussion

One of the primary predictions of the TMH is that skin reflectance should be positively related to solar radiation and/or environmental temperature (Watt 1968; reviewed in Clusella-Trullas *et al.* 2007a). We were unable to falsify this prediction, whether or not we accounted for phylogenetic non-independence. Consequently, this study provides novel, macrophysiological support for the TMH. Indeed, in conjunction with small-scale studies demonstrating the fitness benefits of thermal melanism (Capula & Luiselli 1994; Forsman 1995), this work suggests that, at least in lizards, one explanation for variation in skin reflectance is the benefits that differences therein bring to individuals living in different thermal environments. Whilst skin reflectance and colouration obviously differ among species for various reasons (Majerus 1998; True 2003), the current analysis demonstrates that a long-held, but typically relatively narrowly investigated hypothesis (the TMH) has broad applicability. Moreover, several subtleties emerged from our data that have not previously been documented, mainly because of the typically smaller scope of previous work.

Most heliothermic lizard species have a relatively narrow range of skin reflectance values and are thus dark (Fig. 1). Moreover, the strongest predictor of skin reflectance was MRAD, even after phylogenetic adjustment. By contrast, the relationship with MAT was weaker, and after accounting for phylogeny was only marginally significant, and much less likely than solar radiation to be the best predictor of skin reflectance. Two reasons strike as most plausible for the better predictive value of MRAD than MAT. First, we used MAT as an indication of the environmental temperature experienced by the lizards. Whilst such an approach is reasonable, given the scale of the study (see discussion in Chown, Addo-Bediako & Gaston 2003) and the frequent absence of microclimate data in studies reporting reflectance values (e.g. Hutchison & Larimer 1960), MAT may not always provide an unbiased estimate of the microhabitat temperatures experienced by the lizards (Heath 1964; Bakken 1992; Hertz *et al.* 1993). In consequence, one might expect a weaker relationship between reflectance and MAT than between reflectance and microhabitat temperature. Second, both air temperature and radiation affect the thermal budget of an organism (Gates 1980), but the magnitude of the effect of radiation is dependent on skin reflectance whereas the effect of ambient temperature is mostly independent of skin reflectance (since most organisms reflect < 5% of far-infrared wavelengths, Gates 1980). Therefore, one might expect that levels of solar radiation would have a stronger influence on selection for varying skin reflectance than changes in ambient environmental temperature.

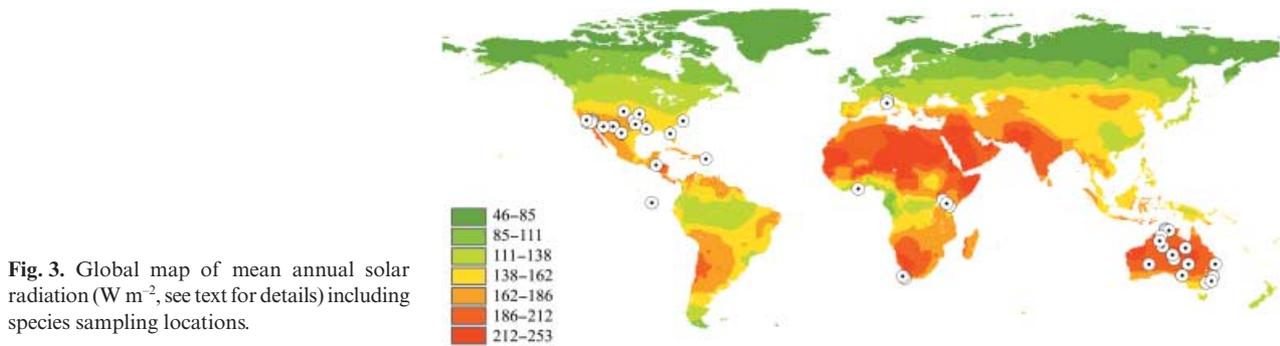
Our data provide an additional way of examining the idea that MRAD may be of more significance in predicting skin reflectance than MAT. The amount of incident solar radiation is influenced predominantly by the incident angle of incoming radiation (i.e. latitude), the state of the atmosphere (e.g. the presence of clouds and dust), the nature of the vegetation in a given area and outside the tropics, by season (Gates 1980; Bonan 2002). In consequence, though solar radi-



**Fig. 2.** Interspecific relationship between mean annual radiation (MRAD;  $\text{W m}^{-2}$ ) and arcsine square-root transformed skin reflectance ( $r$ ;  $n = 66$  species from 12 families). The ordinary least-square relationship (solid line) is described by the equation:  $r = 0.002 + 0.002 \times \text{MRAD}$  ( $r^2 = 0.26$ ,  $F_{(1,64)} = 22.9$ ,  $P < 0.0001$ ). Stippled lines indicate 95% confidence intervals. Major climate zones defined according to Bonan (2002) are represented by different symbols.

ation and air temperature are positively related, much variance exists around the relationship owing especially to atmospheric absorption of incoming radiation, and at ground level to interception by the canopy. Thus, at a given latitude, an area might experience high or low radiation levels depending on average cloud cover. By grouping the species we investigated according to the earth's major climate zones (as given by Bonan 2002; Fig. 2), this effect was immediately apparent. Although tropical species generally live in warmer areas compared with most temperate, mid-latitude species, several of them have low skin reflectances, presumably due to lower average incident radiation (Fig. 2). Low radiation levels in tropical areas such as mesic savannas and rainforests are often caused by frequent cloud cover (Fig. 3). By contrast, most species that live in dry areas (i.e. deserts) experience higher incident radiation than temperate species and generally have higher skin reflectance (Fig. 2). Comprehensive exploration of the subtleties of these patterns requires further sampling across regions and climate zones. For example, our data base only includes one species (*Eumeces obsoletus*) from the humid continental/subarctic climate zone (nearly beyond  $45^\circ$  latitude in the northern hemisphere) and most of our data originated from North- and Central America, and Australia (except for six African and two European species). Additional skin reflectance data for multiple populations of species such as *Lacerta vivipara*, which is distributed from Spain to the Arctic Circle (Grenot 2000), would provide a useful way to examine whether such variation is also typical at the population level.

By contrast with the first prediction, our data provided little support for the idea that body mass has an influence on reflectance. The least-square relationship, though significant, was weak, and once phylogenetic non-independence was accounted for the relationship disappeared entirely. Clearly, from a biophysical perspective, a strong theoretical argument



**Fig. 3.** Global map of mean annual solar radiation ( $\text{W m}^{-2}$ , see text for details) including species sampling locations.

can be made for a positive relationship between body mass and reflectance (Norris 1967; Gates 1980). However, our data failed to support it, or supported it weakly. Why this might be the case is not clear, but perhaps the signal of this relationship is overwhelmed by the multitude of other factors that influence final adult body size (Roff 2002). If this is the case, then only much larger data set will likely have the power to detect the theoretically plausible relationship between reflectance and body mass. Similarly, the influence of other variables on the variance in reflectance remaining after taking the effects of solar radiation into account, such as biologically active UV radiation (see e.g. Porter 1967; Jablonski & Chaplin 2000 for a human example), or differences in behaviour, would require a data set incorporating a much larger number of individuals in which the appropriate traits have been measured, than is presently available.

A lack of correlation between skin reflectance and thermal traits ( $T_{\text{sel}}$ ,  $CT_{\text{max}}$  and  $CT_{\text{min}}$ ) may have several explanations. First, differences in the rates of evolution of thermal physiology and morphology may account for the lack of association between the two (see e.g. Losos, Warheit & Schoener 1997). To our knowledge, no studies have attempted to link rates of physiological evolution with rates of skin reflectance evolution in lizards. For example, behavioural thermoregulation or phenotypic inertia could dampen selection for correlated evolution of skin reflectance and physiology (Huey, Hertz & Sinervo 2003; see also Losos, Schoener & Spiller 2004). Second, differences between studies may have introduced variation into the data set. For example, the same individuals were not necessarily used for both thermal biology and skin reflectance estimates. Moreover, differences in methods among critical thermal limit studies may have further compounded matters (see Terblanche *et al.* 2007). Regardless, the role of skin reflectance in thermal co-adaptation requires further investigation.

In conclusion, our study has provided the first macrophysiological evidence in support of one of the primary predictions of the TMH. In doing so, it has confirmed a wide variety of small-scale studies which have suggested that the variation in reflectance may indeed be a response to the thermal environment. Similar studies of this kind, in other groups, such as the invertebrates, would go a long way towards furthering the generality of the idea. Advances in the portability of spectral measurement devices suggest that this goal may be more readily realized than was previously supposed.

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## Supplementary material

The following supplemental material is available for this article:

**Appendix S1.** Species list and literature sources for skin reflectance, preferred body temperature, critical thermal maxima and minima of heliothermic lizards used in the study

**Appendix S2.** Hypothetical phylogenetic relationships among lizard taxa in the study

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2745.2007.01377.x>  
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