The effect of skin reflectance on thermal traits in a small heliothermic ectotherm

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ABSTRACT

Variation in colour patterning is prevalent among and within species. A number of theories have been proposed in explaining its evolution. Because solar radiation interacts with the pigmentation of the integument causing light to either be reflected or absorbed into the body, thermoregulation has been considered to be a primary selective agent, particularly among ectotherms. Accordingly, the colour-mediated thermoregulatory hypothesis states that darker individuals will heat faster and reach higher thermal equilibria while paler individuals will have the opposite traits. It was further predicted that dark colouration would promote slower cooling rates and higher thermal performance temperatures. To test these hypotheses we quantified the reflectance, selected body temperatures, performance optima, as well as heating and cooling rates of an ectothermic vertebrate, *Lampropholis delicata*. Our results indicated that colour had no influence on thermal physiology, as all thermal traits were uncorrelated with reflectance. We suggest that crypsis may instead be the stronger selective agent as it may have a more direct impact on fitness. Our study has improved our knowledge of the functional differences among individuals with different colour patterns, and the evolutionary significance of morphological variation within species.

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1. Introduction

Variation in colour patterning is one of the most pervasive and conspicuous outcomes of the selective process. Such diversity is not only prevalent among species but within species as well. Understanding the mechanisms which underlie the emergence and maintenance of colour variants across levels of biological organization has, therefore, become a central topic in evolutionary biology (Galeotti et al., 2003; Mckinnon and Pierotti, 2010; Roulin, 2004). A number of theories have been proposed to explain the evolution of colour pattern variation, with crypsis (Endler, 1984; Stuart-Fox et al., 2004) and communication (Dufva and Allander, 1995; Geen and Johnston, 2014; Joron and Mallet, 1998; Wang and Shaffer, 2008) being the most common. For example, colour can provide critical information used in evaluating the fitness among potential mates (Andersson and Simmons, 2006), it can act as a signal of aggressiveness or toxicity towards competitors and predators (Da Silva et al., 2013; Wang and Shaffer, 2008), and it can serve as camouflage through background matching or disruptive patterning (Endler, 2006; Stuart-Fox et al., 2004).

Alternatively, variation in colour patterning may instead arise in response to physiological selective pressures whereby it serves a thermoregulatory function. Skin colouration influences thermal traits through its mechanistic link with solar radiation, pigmentation, and reflectance (Norris and Kunz, 2012). As incoming visible, ultraviolet, and infrared wavelengths interact with the pigmentation of the integument, the biophysical character of colour producing cells referred to as chromatophores causes the light to either be reflected or absorbed in differing degrees (Bechtel, 1978; Harris et al., 2012). Dark colouration arising from the diffuse distribution of melanin, has low reflectance, allowing more solar radiation to penetrate into the body relative to pale colouration produced by iridophores (Bechtel, 1978). Thus, the type and distribution of colour pigments throughout an organism's skin is expected to directly impact the rate at which solar radiation is absorbed and converted into heat (Ahnesjö and Forsman, 2006; Schultz and Hadley, 1987; Watt, 1968).

This colour-mediated thermoregulatory strategy should be especially important among ectothermic species as they are reliant upon the acquisition of external radiant energy for optimal body temperature regulation. Accordingly, darker individuals are able to heat up faster, reach a higher equilibrium temperature, and cool slower than lighter individuals of the same body size, enabling them to maintain their body temperature within an optimal range for longer (Clusella-Trullas et al., 2008; De Keyser et al., 2015; Harris et al., 2012). Locomotory, digestive, and reproductive performance would then be maximized with minimal energetic or time investment (Zhang et al., 2008). This apparent thermal advantage conferred by dark colouration may, however, only be realized within particular environments such as low levels of solar radiation or cool climates. Pale colouration, on the other hand, may

be more beneficial under extreme radiative conditions or hotter climates in that a higher reflectance would reduce the risk of overheating (Clusella-Trullas et al., 2007).

Convincing support for the association between thermal traits and colour has been found in many invertebrate species (Davis et al., 2008; Forsman, 2000; Forsman et al., 2002; Harris et al., 2012; Jong et al., 1996; Kingsolver and Huey, 1998; Parkash et al., 2010; Telonis-Scott et al., 2011). For instance, in pygmy grasshoppers (*Tetrix subulata*) darker phenotypes were shown to heat up faster and prefer higher body temperatures than paler phenotypes (Forsman, 2000). Similarly, common garden experiments involving six altitudinal populations of *Drosophila melanogaster* indicated that dark coloured flies exhibited positive correlations with cold resistance and negative correlations with heat resistance while pale coloured flies exhibited the opposite pattern (Parkash et al., 2010). These findings suggest that the thermal environment may impose selective pressures on both skin pigmentation and physiology causing the traits to coevolve.

In contrast, empirical evidence investigating the colour-mediated thermoregulation theory among vertebrates remains more equivocal. To date only a few studies have reported that dark colouration influences heating rates and equilibrium temperatures [*Thamnophis sirtalis*: Gibson and Falls (1979); *Vipera berus*: Forsman (1995) and Forsman et al. (2002); and *Tiliqua scincoides*: Geen and Johnston (2014)]. And even fewer have shown that dark colouration is able to confer a thermal advantage under cold conditions [*Rana temporaria*: Alho et al. (2010); *Cordylus niger and Cordylus oelofseni*: Clusella-Trullas et al. (2009)]. However, in other studies, even of the same species, colour patterning was not found to influence heating rates or body temperature preferences [*Liolaemus multiformis*: (Pearson, 1977); *Anolis carolinensis*: Claussen and Art (1981); *T. sirtalis*: Bittner et al. (2002)].

So why do studies of vertebrate taxa provide ambiguous results? One reason could be inconsistency in the methodology employed, particularly with respect to quantifying colour (Geen and Johnston, 2014). The use of standard techniques, such as spectrometry, or natural colour variants were not always utilized by previous authors, but instead, colour was described qualitatively or animals were painted as a means of comparing different phenotypes (Ahnesjö and Forsman, 2006; Alho et al., 2010; Bittner et al., 2002; Broennimann et al., 2014; Forsman, 1995; Forsman et al., 2002). Thus, in order to acquire greater insight into the generality of the colour-mediated thermoregulatory hypothesis we evaluated the relationship between reflectance (a proxy of colour) and four thermal traits, optimal performance temperature (T_{opt}), thermal preferences (Ernst et al., 2014), and heating and cooling rates of untreated (e.g., not painted) delicate skinks (*Lampropholis delicata*) using standard methods. This species was selected as it exhibits a high degree of inter- as well as intra-population variability in terms of its: 1) dorsal colouration and patterning, 2) thermal traits and 3) its microhabitat use (Chapple et al., 2014; Wilson and Swan, 2013). Moreover, this is the only study that we are aware of that has examined the physiological impact of colour on traits beyond temperature equilibria and heating rates. In considering the full suite of thermal traits of a polymorphic habitat generalist, the fitness-related effects of colour can therefore be fully evaluated.

2. Methods and materials

2.1. Study species and field collection

The delicate skink is a small [35–55 mm adult snout-vent length (SVL)], heliothermic, insectivore that is locally abundant and geographically widespread in eastern Australia (Chapple et al., 2011; Wilson and Swan, 2013). Its distributional range spans 26° of latitude from north Queensland to southern Tasmania (Chapple et al., 2011). Common habitat associations include moist habitats,

including rainforests, wet sclerophyll forests, woodlands, and heaths. Adult male lizards with complete tails were collected from the Brisbane region (Queensland, Australia: 27° 38 S 153° 05 E) between October and December 2013. Each were individually marked with a unique Visible Implant Elastomer colour code and transported back to the animal housing facility at Monash University (Clayton, Victoria, Australia). Lizards were held in groups of four and maintained at 20 °C with a 14 h light: 10 h dark cycle (06:00–20:00 h). Basking lamps created a thermal gradient of 20–35 °C to promote natural thermoregulatory behaviour. Lizards were fed crickets (*Acheta domesticus*) three times weekly and provided water *ad libitum*. All procedures were conducted in accordance with our Monash University Animal Ethics Committee approvals (BSCI/2012/17, BSCI/2013/03).

2.2. Physiological measurements

Lizards ($n=22$) were exposed to laboratory assays within three weeks of capture. Assays quantified physiological and morphological traits, including T_{opt}, T_{sel}, heating and cooling rates, and skin reflectance. Seventy-two hours separated test days to avoid interactions among experimental responses. All tests were performed when lizards were in a post-absorptive state (two days without food) (van Berkum et al., 1989). Inter-limb length (ILL), SVL, and mass were measured prior to each test to assess body size effects.

Sprint speed was measured by racing lizards down a 1 L m × 10 W cm racetrack at each of five temperatures (15, 20, 25, 30, and 35 °C) in a randomized order (Cromie and Chapple, 2012). Lizards were tested at a single temperature three times each test day with at least 30 min between successive runs. Prior to the first trial and in between trials, lizards were placed into a thermal chamber set to the race temperature for at least 15 min. Sprint speed was determined by infrared sensors positioned at 25 cm intervals. Each race produced a velocity measurement for each of the four segments between the sensors with the fastest segment being considered its V_{max}. Maximum speed data at each temperature were then used to generate individual performance curves from which T_{opt}, defined as the T_b which maximizes performance, was calculated using OriginPro version 9.1 (Origin Lab, Northampton MA, USA). Critical thermal minima and maxima needed to construct the curves were based upon published data (Greer, 1989).

Lizards were placed into a 40×100 cm thigmo-thermal gradient constructed of aluminium and partitioned into four equal runways. A row of iButton dataloggers spanned the length of each lane to measure surface temperature. Dataloggers were calibrated against a subset of lizards. A near linear gradient ranging from 15 to 36 °C was produced by hanging two 250-W infrared bulbs at one end of the chamber and placing a cold plate beneath the other end. Because the delicate skink is heliothermic, infrared bulbs were used to eliminate the effect of light as a potential confounding factor. At the onset of the test, lizards were placed individually into the cool end. After a 1 h acclimation period the locations of each lizard was monitored from 10:00–16:00 with video cameras positioned over the chamber. In having a short time-constant (1.30 ± 0.338 min; (Fraser and Grigg, 1984)), T_b's were inferred from the selected positions along the gradient. These data were used to calculate individual T_{sel}, defined as the mean selected body temperature.

Heating rates were measured using radiant energy as a heat source. Prior to each trial, lizards were placed into a thermal chamber set to 20 °C for 15 min T_b's were measured with a T-type thermocouple attached to a quick-read digital thermometer (Eutech Instruments model PT100). Thermocouples were inserted ≤ 5 mm into the cloaca of the lizard and secured to the base of the tail using surgical tape (Leucopore©). Lizards were then transferred to a 9 cm³ aluminium chamber fitted with two 28 W bulbs