

Thermal Costs and Benefits of Replicated Color Evolution in the White Sands Desert Lizard Community

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ABSTRACT: Traits often contribute to multiple functions, complicating our understanding of the selective pressures that influence trait evolution. In the Chihuahuan Desert, predation is thought to be the primary driver of cryptic light coloration in three White Sands lizard species relative to the darker coloration of populations on adjacent dark soils. However, coloration also influences radiation absorption and thus animal body temperatures. We combined comparative physiological experiments and biophysical models to test for thermal consequences of evolving different color morphs in White Sands across the three species. While light and dark morphs have not evolved different physiological heat limits within species, differences in radiation absorption between morphs lead to body temperature differences that impact relative overheating risk and activity patterns. Moreover, for all three species, an idealized morph that matches the White Sands substrate would have considerably less activity time, by approximately 1 month, than existing light morphs. Overall, there are both benefits and costs to greater substrate matching, the balance of which may prevent the evolution of optimal crypsis. Our work highlights the importance of color in dictating thermal performance and the complexity inherent in understanding the evolution of coloration.

Keywords: adaptation, melanism, thermoregulation, thermal tolerance, preferred temperatures, trade-off.

Introduction

Organisms are complex integrated systems in which a single trait often contributes to multiple aspects of performance. Multifunctionality leads to the potential for phenotypic trade-offs because a trait value that is maximized for one function may not be maximized for another (Stearns 1989; Schluter et al. 1991). Therefore, the expression of

many phenotypes may result from the balance of selection pressures for different functions (Ghalambor et al. 2004; Gómez 2004). A comprehensive understanding of trait evolution is thus dependent on documenting the various ways a trait may impact overall fitness.

Coloration can serve many functions and is therefore expected to be influenced by multiple selective pressures (Endler and Mappes 2017). In addition to signaling, camouflage, and parasite defense, coloration can also impact thermal budgets by dictating radiation absorption (Norris 1967; Porter and Gates 1969; Porter et al. 1973; Gates 1980; Stuart-Fox et al. 2017). For example, studies in insects and reptiles have shown that melanization increases heating rates and/or equilibrium body temperatures (Kingsolver 1983*b*; Stevenson 1985; Luke 1989; Walton and Bennett 1993; Forsman 1995; Bittner et al. 2002; Tanaka 2005; Clusella-Trullas et al. 2009; Geen and Johnston 2014). Indeed, many populations of ectothermic animals in cool habitats have evolved greater melanization than those in warm habitats, a pattern consistent with coloration as a thermoregulatory adaptation (Watt 1968; Kingsolver 1983*a*; Kingsolver 1987; Clusella-Trullas et al. 2008; Xing et al. 2018). These empirical observations have led to the development of the thermal melanism hypothesis, which predicts that greater melanization should evolve more often in relatively cold habitats (Clusella-Trullas et al. 2007).

Trade-offs between thermoregulation and other functions of coloration are not well characterized but are most often investigated with respect to mate choice (Ellers and Boggs 2003) and camouflage (Smith et al. 2016*b*). For example, European wood tiger moths (*Parasemia plantaginis*) from cooler regions (high altitudes and high latitudes) show greater wing melanization, and melanized wings led to warmer body temperatures (Hegna et al. 2013). However, greater wing melanization also increased avian predator

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attack rates. Therefore, thermal benefits of melanized wings were at least partially offset by increased predation risk (Hegna et al. 2013). The role of coloration in camouflage and thermoregulation suggests that trade-offs are likely common, but the strength and generality of the trade-off across taxa remain unknown.

We investigated the thermal consequences of evolving decreased melanization within a community of lizards in the White Sands desert of New Mexico. White Sands is home to three lizard species (*Holbrookia maculata*, *Sceloporus cowlesi*, and *Aspidoscelis inornata*) that have each independently evolved pale blanching coloration (Rosenblum 2006) relative to populations on adjacent dark soils that display the ancestral, more melanized coloration. Natural selection almost certainly plays a role in color divergence, as blanching coloration is maintained in the face of gene flow in all three species (Rosenblum and Harmon 2011) and specific mutations in the melanin-synthesis pathway have been shown to be under selection (Rosenblum et al. 2010). Although definitive experimental evidence is lacking (Hardwick et al. 2015), predation pressure is thought to drive convergence on blanching coloration in White Sands lizards (and other mammals and insects; Rosenblum et al. 2017) because

blanching color increases background matching with the white gypsum substrate (Rosenblum 2006).

The pattern of color evolution in this system is notably opposite of that predicted by the thermal melanism hypothesis. Substrate and air temperatures at White Sands are lower than in nearby dark soil habitats (Fishman et al. 1994), but lizards in this system exhibit less melanization. Indeed, the only thermal study of White Sands lizards found that blanching *H. maculata* have lower body temperatures on White Sands than more melanized *H. maculata* on dark soils (Hager 2000). Therefore, evolving pale coloration could come at a thermal cost by, for example, reducing time available at preferred body temperatures.

Reciprocally, it is possible that thermoregulatory needs constrain crypsis in this system. White Sands lizards are blanching but remain darker than the highly reflective gypsum sand (fig. 1). Indeed, a model of achromatic contrast between lizards and the substrate based on the avian visual system indicates that these lizards are still visible to predators (fig. S1; figs. S1–S4 are available online). Evolving better background matching could be disfavored because it would lead to even greater thermoregulatory costs.

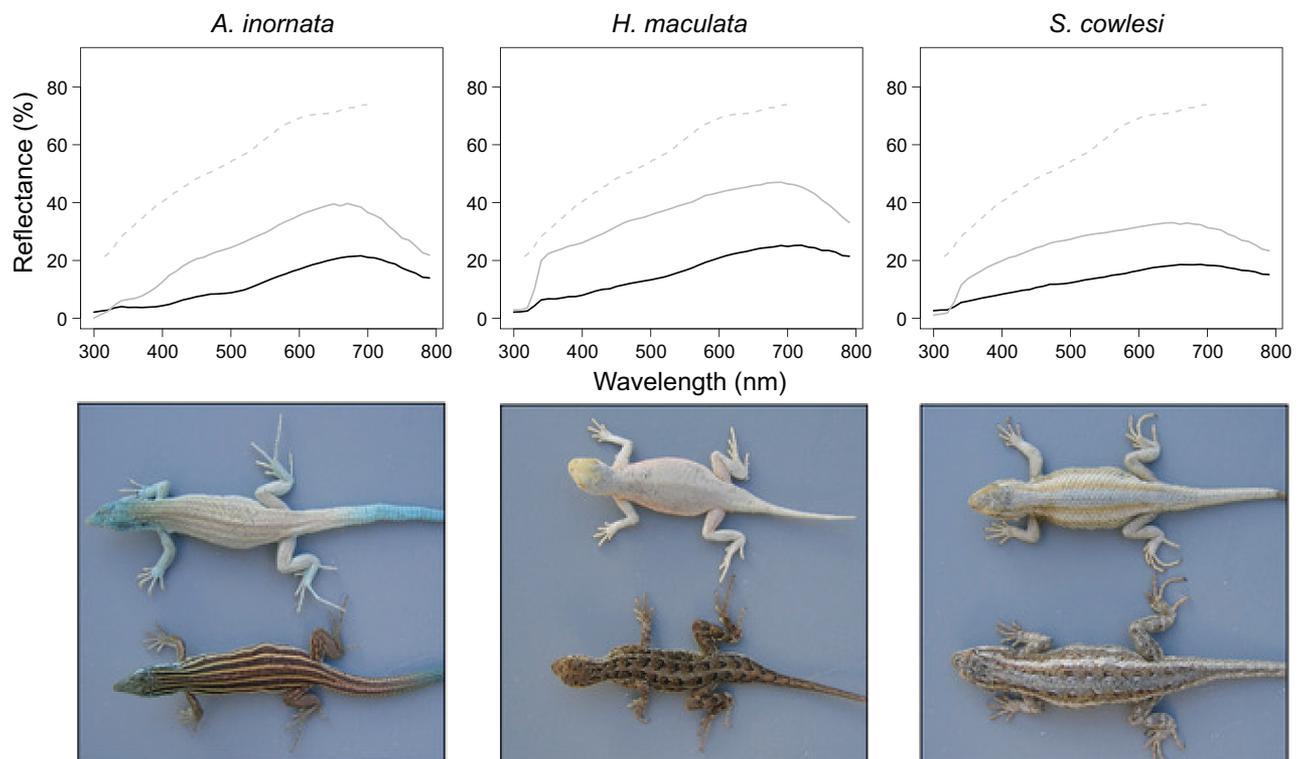


Figure 1: Mean reflectance spectra of lizard color morphs and White Sands substrate. Dashed line = substrate; solid gray line = white sands morph; solid black line = dark soil morph. Data from Rosenblum (2006) and Norris (1967).

To address the thermal consequences of evolving pale coloration, we combined physiological and behavioral experiments with a heat flux model of the White Sands operative thermal environment to address the following questions: First, what are the thermal limits of White Sands lizards and do they differ from dark soil lizards? Second, how does evolving blanched coloration impact activity budgets and overheating risk relative to maintaining darker coloration on the White Sands substrate? And third, is there evidence that the blanched white sands color morph represents a trade-off between camouflage and thermoregulation?

Material and Methods

Field Site and Animal Collection

Lizards with white sands phenotypes were collected within the White Sands National Monument (32.80838N, 106.26444W) from May 16 to 28, 2017. Animals with dark soil phenotypes were collected within the Jornada Ecological Reserve (32.61197N, 106.74167W) and the Carizzozo Valley of Fires Recreation Area (33.74721N, 106.03889W) from May 20 to 31, 2018. Animals were transported to our field laboratory and placed individually in 18 × 11 × 14-cm plastic cages with a sand substrate and a dowel as well as a small rock for perches. Animals were housed for 24–28 h in a temperature-controlled room (between 25°C and 30°C) before experiments.

Physiological and Behavioral Measurements

We measured three temperature-dependent traits: heat tolerance (critical thermal maximum [CT_{max}]), cold tolerance (critical thermal minimum [CT_{min}]), and behavioral thermal preference (T_{pref}). Because of difficulty capturing enough dark soil individuals of all three species during our time in the field, CT_{max} is the only trait for which we have data for both white sand and dark soil morphs. CT_{min} and T_{pref} data were collected only on white sand morphs and were used as reference points in our operative temperature model after confirming that white sand and dark soil animals do not differ in CT_{max} (see below).

CT_{max} and CT_{min} are the upper and lower body temperatures, respectively, at which animals lose neuromuscular coordination, indicated by a loss of righting response (Lutterschmidt and Hutchison 1997). We measured thermal critical limits following the protocol of Leal and Gunderson (2012) and Gunderson et al. (2018). For heat tolerance, the tip of a 36-gauge T-type thermocouple probe was placed approximately 3 mm inside the cloaca and secured to the tail with a piece of surgical tape. The animal was then placed inside a cardboard box and loosely tethered

with dental floss around the waist. The animal was then placed under a 150-W bulb 27 cm above the surface. Lizards were warmed at 2°C min⁻¹, and righting response was tested at 1°C body temperature intervals by flipping animals on their backs and stimulating them to right themselves by gently squeezing their limbs with tweezers for 10 s. CT_{min} experiments were similar, except animals were placed in a chilling incubator to decrease body temperatures. Ten individuals per species, per color morph, were measured for heat and cold tolerance ($N = 5$ for dark soil *Holbrookia maculata*), and different animals were used for each. All animals were released at their capture location after experiments ended. We tested for differences in thermal tolerance among species using ANOVA in the R statistical programming language (R Core Team 2017).

Thermal preference was measured in a photothermal gradient. The gradient was a rectangular cardboard box (101 cm × 31 cm × 31 cm) with a 150-W bulb placed over one end. The box was in a room with an air temperature of 26°C. We used a copper lizard model to determine that operative temperatures in the gradient spanned from 26°C at the cold end to 50°C at the warm end. The copper model was originally designed for operative temperature measurement of *Anolis cristatellus* (Hertz 1992), but White Sands lizards are of similar size. For each trial, a lizard was initially placed at the cold end of the gradient (26°C), an intermediate location (~33°C), or near the warm end of the gradient (~40°C, remaining below the CT_{max} of all three species). Body temperature was measured every 15 min over 45 min, totaling three temperatures per individual per species. Five individuals of each species were run. The preferred temperature range was taken as the central 50% of all observations for a species (Hertz et al. 1993). We tested for significant differences in preferred temperatures among species using a mixed effects linear model with individual as a random factor. Data have been deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.cz8w9gj3c>; Gunderson et al. 2021).

Model of the Operative Thermal Environment

We employed a biophysical modeling approach to estimate overheating risk and activity budgets of different color morphs throughout the year at White Sands, integrating empirical lizard thermal thresholds and preferences. We calculated operative temperatures (T_e), an estimate of equilibrium lizard body temperature under a given set of physical conditions (defined here as the equivalent temperature of a black body cavity that integrates heat loads from complex thermal environments). Our biophysical model is a modified version of that developed by Sears et al. (2011), based on the methods in Campbell and Norman (2012).

We provide the Python script used to generate operative temperature estimates in the supplemental PDF, available online, and a description of the most important equations below.¹

We used NicheMapR to estimate relevant air and soil temperatures in White Sands (Smith et al. 2016b; Kearney and Porter 2017). We parameterized NicheMapR by adjusting the albedo and emissivity of the sand surface based on empirically derived values. We used values of 0.6 albedo and 0.85 emissivity for White Sands substrate (Fishman et al. 1994; Schmutge et al. 2002). We used NicheMapR to estimate air temperature at a height of 0.5 cm from the surface to simulate relevant air temperatures that lizards experience. We specifically extracted monthly minimum and maximum temperatures to estimate annual and daily cycles of relevant temperatures using various methods to determine which method provided the most realistic estimates of operative environmental temperatures (see below). We estimated hourly air temperatures from equations (2.2) and (2.3) in Campbell and Norman (2012).

We validated our soil temperature estimates with soil temperature data from White Sands National Monument collected over a 3-day period in June and July 2000 using iButton temperature loggers (Maxim Integrated) placed 1–5 mm under the sand. We compared hourly empirical soil surface temperature data to estimates from three different quantitative methods: the published routines in NicheMapR and two sets of equations from Campbell and Norman (2012, eq. [2.2], [2.3], and [2.4]). Following Kearney et al. (2014), we compared empirical and estimated hourly temperatures using coefficients of determination to identify the best approach. Equations that estimated air temperatures at ground level best predicted empirical soil temperatures, explaining the most variation in the empirical data set (fig. S2; table S1, available online). Nonetheless, all estimates were approximately equivalent (fig. S2) and did not qualitatively influence our results.

Our simulation-based approach integrates variation in the environmental conditions with biophysical properties of lizards to predict T_e . We predicted T_e using

$$T_e = T_a + \frac{R_{\text{abs}} - \epsilon_s \sigma T_a^4}{c_p (g_r + g_{\text{Ha}})},$$

where T_a is the air temperature at the height of the animal, R_{abs} is the radiation absorbed, ϵ_s is the emissivity of the lizard (0.97), σ is the Stefan-Boltzmann constant ($5.67 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$), c_p is the specific heat of air at constant pressure ($29.3 \text{ J mol}^{-1} \text{ C}^{-1}$), g_r is the radiative conductance, and g_{Ha} is the boundary layer conductance.

1. Code that appears in *The American Naturalist* is provided as a convenience to readers. It has not necessarily been tested as part of peer review.

Lizard solar radiation absorbance was based on reflectance spectra data. We modeled radiation for three different color morphs for each species: dark soil morphs from dark soils, white sand morphs from White Sands, and a hypothetical morph that matches the White Sands background. For *H. maculata*, we used reflectance measurements that spanned from 300 to 1,800 nm (Norris 1967). Available reflectance data for *Sceloporus cowlesi* and *Aspidoscelis inornata* were not as complete and ranged from 300 to 800 nm (Rosenblum 2006; fig. 1). Approximately 65% of incident solar radiation is between 300 and 800 nm. Therefore, our absorbance estimates for *S. cowlesi* and *A. inornata* cover the majority of incoming light, and our calculations should represent relative differences between morphs. Still, we acknowledge that full-spectrum estimates for *S. cowlesi* and *A. inornata* would be preferable (Stuart-Fox et al. 2017). For the hypothetical substrate-matched morph of all species, we assume the lizard absorbs the same percentage of radiation as the White Sands substrate (Norris 1967). Based on these absorbance spectra, we calculated percent radiation absorbed based on the sunlight irradiance spectrum (Gates 1980). We estimated g_r and g_{Ha} using equations (12.7) and (7.3), respectively, from Campbell and Norman (2012), using characteristic dimensions of a cylinder. We assumed a lizard of mass 6 g, a length of 5 cm, and a radius of 0.5 cm. We estimated R_{abs} using

$$R_{\text{abs}} = \alpha_s (F_p S_p + F_d S_d + F_r S_r) + \alpha_L (F_a L_a + F_g L_g),$$

where α_s and α_L are the absorptances of the shortwave and longwave thermal radiation; S_p , S_d , and S_r are the flux density of solar radiation, diffuse radiation, and reflected radiation, respectively; L_a and L_g refer to longwave flux densities, which we estimated from ground temperature; and F_d , F_r , F_a , and F_g are view factors that we assumed were 0.5 on the basis of the methods of Campbell and Norman (2012). For F_p , we estimated the view factor assuming the dimensions of a cylinder using

$$F_p = \frac{\cos \theta + 4h \sin \theta / \pi d}{2 + 4h/d},$$

where θ is the angle between the solar beam and normal to the plane of the soil surface, h is the length of the lizard, and d is the diameter. We refer readers to our Python script (supplemental PDF) and the equations in chapter 11 of Campbell and Norman (2012) for further calculations on simulating the daily and annual variation in solar radiation at specific locations. We conducted simulations assuming that animals were exposed to either full sunlight or 50% of full sunlight in filtered shade. The simulated shade accounts for thermoregulatory potential whereby animals shuttle between sun and shade while active. To assess

whether our model yielded reasonable animal temperature estimates, we compared empirical body temperatures of white sand morph *H. maculata* to T_e predicted by our simulation-based biophysical model for the same species on the same days (fig. S3).

We used our simulation to estimate overheating risk and activity over the course of the year for dark soil, white sand, and hypothetical substrate-matched morphs in the White Sands habitat. We estimated activity by calculating the total amount of time that T_e was within the T_{pref} range based on our measurements of this trait, assuming that animals can access body temperatures between estimates of T_e assuming 0% (i.e., in the open) and 50% shade at a given time. We report calculations assuming four different T_{pref} ranges to assess the generality of our conclusions. One is the classic T_{pref} range that represents the central 50% of body temperatures in a thermal gradient for each species (Hertz et al. 1993). We focus primarily on these calculations because of the association between this metric of T_{pref} and activity rates in lizards (Gunderson and Leal 2015, 2016). As a sensitivity analysis, we also conducted calculations assuming that the T_{pref} range for each species is shifted either 1°C higher or 1°C lower. Finally, we assumed broader T_{pref} windows of 5°C (*H. maculata* and *A. inornata*) or 6°C (*S. cowlesi*; wider because of greater variation in measured T_{pref} values; see “Results”). Results using all T_{pref} ranges are qualitatively similar and yield similar inferences. Overheating risk was calculated as the number of hours that T_e exceeded CT_{max} .

Results

Thermal Physiology and Behavior

We found no difference in heat tolerance ($F_{1,49} = 1.04$, $P = .313$) between color morphs (mean \pm SE; *Aspidoscelis inornata*: 46.7°C \pm 0.2°C for dark soil, 46.1°C \pm 0.3°C for white sand; *Holbrookia maculata*: 46.3°C \pm 0.7°C for dark soil, 45.8°C \pm 0.3°C for white sand; *Sceloporus cowlesi*: 42.0°C \pm 1.0°C for dark soil, 42.2°C \pm 0.7°C for white sand; fig. 2A). There was also no species \times color morph interaction ($F_{2,49} = 0.94$, $P = .397$). However, species differed in heat tolerance ($F_{2,49} = 111.95$, $P < .001$): the grand mean CT_{max} of *S. cowlesi* (42.1°C \pm 0.2°C) was 4.3°C and 3.9°C lower than that of *A. inornata* (46.4 \pm 0.2°C) and *H. maculata* (46.0°C \pm 0.3°C), respectively (fig. 2A).

Species differed significantly in cold tolerance (ANOVA; $F_{2,27} = 41.4$, $P < .001$). *Sceloporus cowlesi* was the most cold tolerant (7.5°C \pm 0.6°C), followed by *H. maculata* (9.9°C \pm 0.5°C) and *A. inornata* (14.1°C \pm 0.3°C; fig. 2B). Differences in behavioral thermal preferences among species followed a similar pattern to heat tolerance. *Sceloporus*

cowlesi had the lowest T_{pref} (34.9°C \pm 1.3°C; central 50% of observations, 33.7°C–36.7°C), while *H. maculata* (37.7°C \pm 1.3°C; central 50% of observations, 37.6°C–38.8°C) and *A. inornata* (37.4°C \pm 0.5°C; central 50% of observations, 36.8°C–38.6°C) had higher and similar values (Wald test; $\chi^2 = 17.2$, $P < .001$; fig. 2C).

Our heat tolerance data do not provide evidence of physiological divergence between color morphs. Previously published CT_{max} estimates of dark soil *H. maculata* (46.5°C \pm 0.3°C; Sena 1978) and *S. cowlesi* (42.7°C \pm 0.7°C; Crowley 1985) in northern New Mexico are also similar to our white sand morph values for these species (45.8°C \pm 0.3°C and 42.2°C \pm 0.7°C, respectively; fig. 2A). In addition, the 7.5°C \pm 0.6°C CT_{min} we measured for white sand *S. cowlesi* is only 1.2°C higher (indicating lesser cold tolerance) than that published for *S. cowlesi* from a dark soil population further north and at higher elevation (Sena 1978). In addition, our mean white sand preferred temperature for *A. inornata* (37.4°C) was only 0.6°C different from that reported for the closely related *A. sexlineatus* that lives on dark soils (Paulissen 1988). Given the weight of evidence, we assumed no differences in physiological and behavioral limits between morphs when estimating performance below.

Coloration and Operative Thermal Environments in White Sands

Based on our model, white sand morphs experience cooler operative temperatures in the White Sands than dark soil morphs. In all species, the annual mean daily maximum temperature in full sun was approximately 3°C lower for a white sand relative to a dark soil morph (table 1). Differences between morphs were smaller (~1.5°C) assuming animals in 50% shade (table 1). Temperature differences between morphs were greatest in summer and least in winter when incident solar radiation is reduced (fig. 3). Mean daily minimum temperatures were predicted to be similar (differing by less than 0.1°C) for both morphs across species because minima occur at night with no solar radiation.

Threat of Exceeding Thermal Limits

Relative to dark soil morphs, white sand morphs experienced fewer hours at which operative temperatures exceed their heat tolerance limits (fig. 4; table 1). Annually and assuming full sun, white sand morphs have 158–179 (14%–16%) fewer hours of overheating risk, depending on the species. The ability to find perch sites with 50% shade reduces overheating risk considerably, but white sand morphs still experienced reduced overheating risk across all species (fig. 4; table 1). At the cold end of thermal

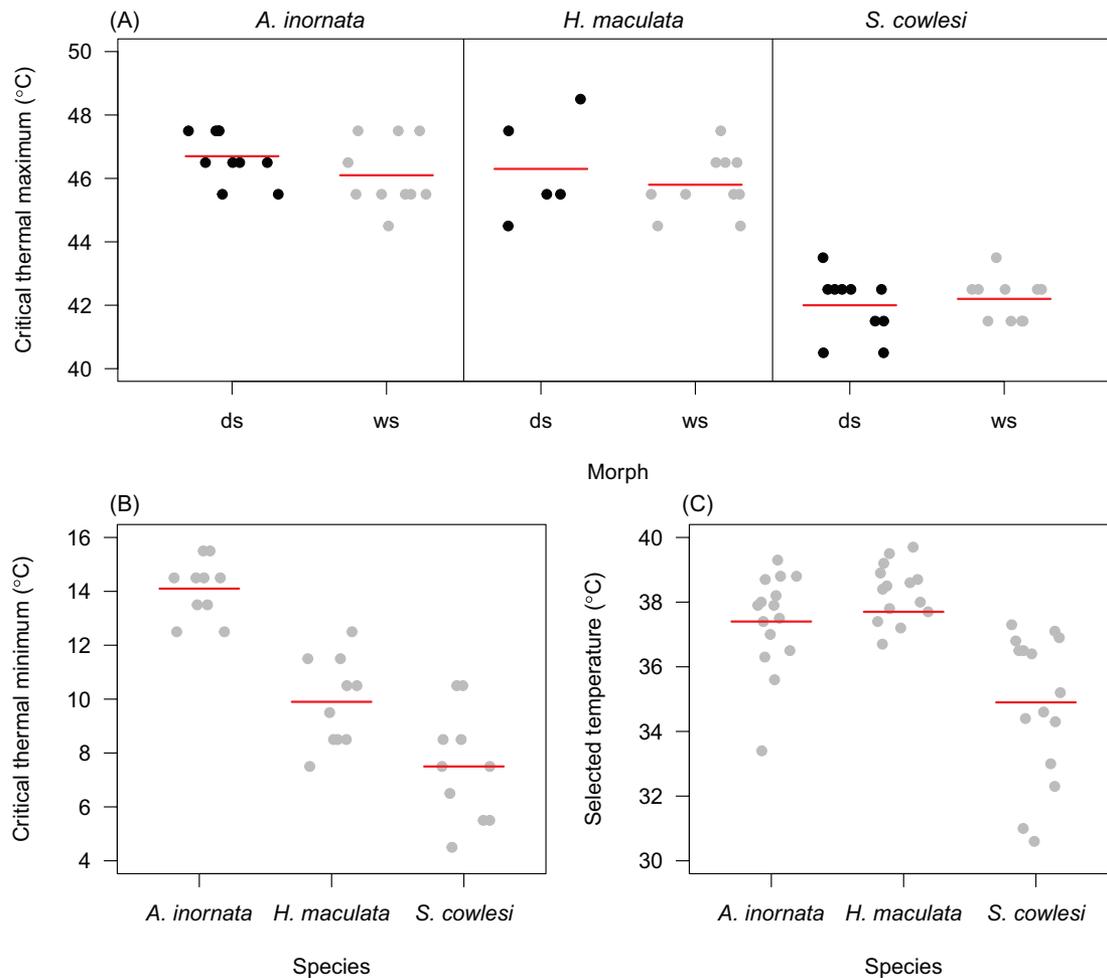


Figure 2: A, Heat tolerance limits (CT_{max}) of lizards of the dark soil (ds) and white sands (ws) color morphs. B, C, Cold tolerance limits (CT_{min}; smaller values reflect greater cold tolerance; B) and body temperatures (C) selected in a thermal gradient of white sand color morphs.

limits, *A. inornata* would experience nightly temperatures below their cold tolerance threshold in the absence of seeking warmer microhabitats, while cold tolerance limits would be exceeded on most, but not all, nights for *H. maculata* (275 nights) and *S. cowlesi* (247 nights; fig. 3).

Activity at Preferred Temperatures

Evolving less melanized coloration is predicted to decrease access to preferred temperatures. On a seasonal basis, white sand morphs would experience a delay in ability to attain preferred temperatures in the spring of 9 days (*A. inornata* and *S. cowlesi*) to 10 days (*H. maculata*) relative to dark soil morphs (fig. 5). In addition, white sand morphs would lose the ability to reach preferred temperatures earlier in the fall relative to dark soil morphs by 6–8 days depending

on the species (fig. 5). In sum, white sand morphs would have between 15 and 18 fewer days per year with preferred temperatures available. Analyses with alternate preferred temperature ranges give similar results (table 1).

White sand lizards are also predicted to have fewer total hours of activity at preferred temperatures, although the magnitude is species specific (fig. 5). For *S. cowlesi*, the white sand morph is predicted to have only 1% fewer hours at preferred temperatures than the dark soil morph (1,078 vs. 1,086 h). White sand morph *A. inornata* and *H. maculata* are predicted to have 4% (950 vs. 988 h) and 7% (950 vs. 885 h) fewer hours at preferred temperatures than dark soil morphs, respectively. Again, analyses with alternate preferred temperature ranges give similar results, although the differences between morphs are generally larger (table 1).

Table 1: Summary statistics for operative temperature, overheating risk, and activity time at preferred temperatures modeled for different color morphs in the White Sands habitat

	Full sun		50% shade		T_{pref}		$T_{\text{pref}} + 1^\circ\text{C}$ shift		$T_{\text{pref}} - 1^\circ\text{C}$ shift		Broad T_{pref}	
	Mean daily T_{max}	Hours over CT_{max}	Mean daily T_{max}	Hours over CT_{max}	Days with T_{pref}	Hours with T_{pref}	Days with T_{pref}	Hours with T_{pref}	Days with T_{pref}	Hours with T_{pref}	Days with T_{pref}	Hours with T_{pref}
<i>Aspidoscelis inornata:</i>												
Dark soil	48.9	1,145	35.2	233	264	988	248	998	261	974	276	1,239
White sand	45.8	987	33.7	101	249	950	231	954	244	956	261	1,206
Substrate matched	39.0	524	30.3	0	208	880	202	863	216	827	221	1,079
<i>Holbrookia maculata:</i>												
Dark soil	47.6	1,107	34.6	206	253	950	260	948	272	937	269	1,224
White sand	44.4	930	33.0	84	236	885	244	878	256	893	253	1,192
Substrate matched	39.0	572	30.3	0	203	834	196	816	210	793	221	1,079
<i>Sceloporus cowlesi:</i>												
Dark soil	48.9	1,436	35.2	531	283	1,086	279	1,083	292	1,062	296	1,340
White sand	45.8	1,257	33.7	408	268	1,078	264	1,072	277	1,057	280	1,306
Substrate matched	39.0	820	30.3	112	231	936	224	910	237	970	242	1,248

Note: Dark soil and white sand morphs were modeled from empirical reflectance data, whereas the substrate-matched morph is a hypothetical morph that matches the reflectance of the White Sands substrate. T_{pref} = activity at the estimated preferred temperature range; $T_{\text{pref}} + 1^\circ\text{C}$ shift = activity assuming the estimated T_{pref} range is shifted 1°C higher; $T_{\text{pref}} - 1^\circ\text{C}$ shift = activity assuming the estimated T_{pref} range is shifted 1°C lower; broad T_{pref} = activity assuming the estimated T_{pref} range is wider. See "Material and Methods" for details.

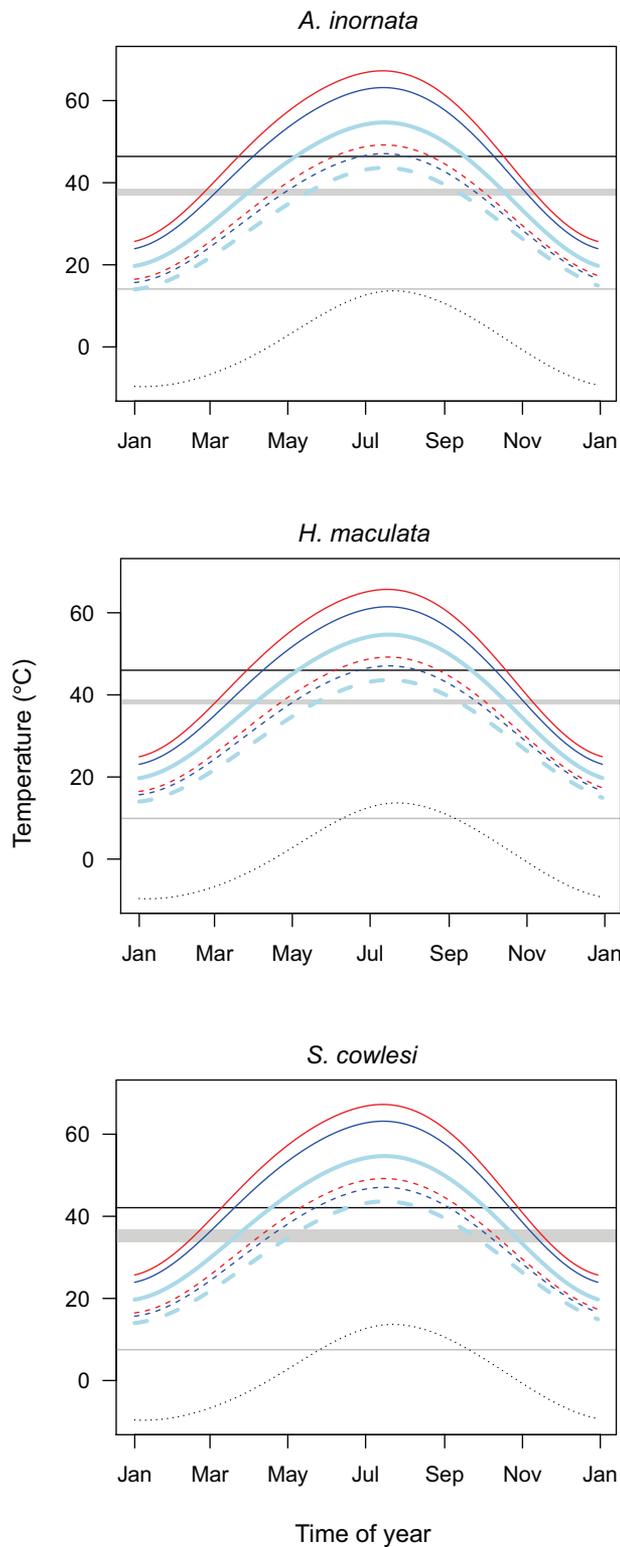


Figure 3: Biophysical model estimates of operative thermal environments for lizards of white sand (blue lines) and dark soil (red lines) color morphs. Solid curved lines = maximum daily temperatures assuming an animal in full sun; dashed curved lines = maximum daily temperatures assuming a lizard in 50% shade; dotted gray line = nightly minimum temperature; straight black line = heat tolerance limit (CT_{max}); straight gray line = cold tolerance limit (CT_{min}); gray rectangle = preferred temperature range (central 50% of body temperatures selected by individuals in a thermal gradient).

The Thermal Consequences of Substrate-Matched Coloration in the White Sands

Assuming full sun, a substrate-matched morph would experience a mean daily maximum temperature (39.0°C) that is 8.6°C – 9.9°C cooler than dark soil morphs and 5.4°C – 6.8°C cooler than white sand morphs (fig. 3; table 1). Assuming 50% shade, a substrate-matched animal would experience a mean maximum temperature (30.3°C) 4.3°C – 4.9°C cooler than dark soil morphs and 2.7°C – 3.4°C cooler than white sand morphs (fig. 3; table 1).

Substrate-matched coloration would have a large effect on overheating risk relative to the current color morphs. Assuming full sun, a substrate-matched individual would experience a 534–621-h (46%–57%) reduction in the time at which they would overheat relative to the dark soil morph and a 358–463-h (53%–65%) reduction relative to the white sand morph, depending on the species (fig. 4; table 1). Assuming 50% shade, a substrate-matched *A. inornata* or *H. maculata* would completely avoid overheating (fig. 4; table 1), while a substrate-matched *S. cowlesi* would have 112 h of overheating risk, several hundred hours fewer than either current morph (fig. 4; table 1).

Annually, substrate-matched lizards would have 50–56 fewer days at preferred temperatures relative to dark soil morphs and 33–41 fewer days relative to white sand morphs (fig. 5; table 1). On a seasonal basis, substrate-matched coloration would delay the day that T_{pref} is available in the spring by a full month (31–33 days) relative to the dark soil morph, and preferred temperatures would become unachievable in the fall 20–23 days sooner (fig. 5). Relative to the white sand morph, a substrate-matched individual would achieve T_{pref} 21–24 days later in the spring and lose the ability to reach T_{pref} 13–17 days earlier in the fall (fig. 5). In addition, a substrate-matched morph would have an 11%–14% reduction in annual activity hours at T_{pref} relative to the ancestral dark soil morph and a 6%–13% reduction relative to the white sand morph, depending on species (fig. 5; table 1). Assuming expanded T_{pref} ranges yields similar results (table 1).

Discussion

Animal coloration serves several functions, setting up the potential for evolutionary trade-offs. In White Sands lizards, reduced melanization has likely evolved because of predation pressure, increasing the color matching of lizards

ratures assuming an animal in full sun; dashed curved lines = maximum daily temperatures assuming a lizard in 50% shade; dotted gray line = nightly minimum temperature; straight black line = heat tolerance limit (CT_{max}); straight gray line = cold tolerance limit (CT_{min}); gray rectangle = preferred temperature range (central 50% of body temperatures selected by individuals in a thermal gradient).

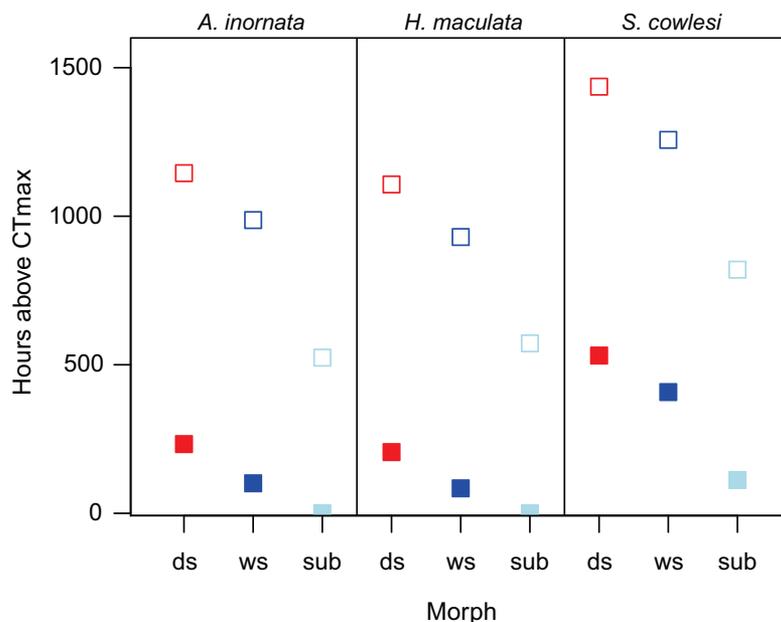


Figure 4: Biophysical model estimates of the annual amount of time operative thermal conditions exceed the heat tolerance limit (CT_{max}) of dark soil (ds; red), blanched white sands (ws; blue), and substrate-matched (sub; light blue) color morphs. Open symbols = estimates assuming an animal in full sun; filled symbols = estimates assuming an animal in 50% shade.

with the substrate. However, melanization also affects radiation absorption and can therefore have thermoregulatory consequences. We investigated these consequences and whether predator avoidance and thermoregulatory needs might trade off in this system. We found that coloration affects overheating risk and thermoregulatory opportunity and that greater color matching may be disfavored in the White Sands system because of thermoregulatory costs. Below, we discuss the implications of our findings.

Temperature-Dependent Performance

We found little evidence that physiological limits diverge with coloration in white sand lizards. Heat tolerance did not differ between color morphs (fig. 2A), and in the lone species for which cold-tolerance data are available for both morphs in New Mexico (*Sceloporus cowlesi*), estimates differ by only 1.2°C (Crowley 1985). The physiological similarity between color morphs is consistent with the evolutionary conservatism in heat tolerance found in other lizards in the southwestern United States (including *Aspidoscelis* and *Sceloporus*; Bogert 1949; Crowley 1985). The lack of divergence is likely due to behavioral thermoregulation reducing (although not necessarily eliminating) body temperature differences between animals in different habitats (i.e., the Bogert effect; Huey et al. 2003; Bodensteiner et al. 2021).

Although color morphs within species did not differ in thermal limits, species differed markedly in thermal physiology, consistent with known differences in their spatial and temporal habitat use. For example, the lower heat tolerance, lower thermal preference, and greater cold tolerance of *S. cowlesi* (fig. 2) predicts that they should occupy cooler microhabitats and be active at cooler times than the other species. Consistent with this prediction, activity in White Sands *S. cowlesi* peaks in the morning and evening, while *Holbrookia maculata* activity peaks closer to midday (Hager 2001); in addition, *S. cowlesi* occupies shade more often than *H. maculata* (Hager 2001) and *Aspidoscelis inornata* (A. R. Gunderson and E. B. Rosenblum, personal observation).

Thermal Consequences of Color Evolution

Melanization can have substantial effects on ectotherm body temperatures (Porter and Gates 1969; Gates 1980; Stevenson 1985; Smith et al. 2016b; Stuart-Fox et al. 2017). As expected, our model predicted that white sands morphs would experience cooler operative thermal environments than dark soil morphs in White Sands (table 1; fig. 3). The next question is how white sand coloration affects thermal performance relative to the ancestral dark soil state on white sand. One might expect white sand coloration to negatively affect thermal performance on cooler white sand, since the pattern is opposite that predicted by the thermal melanism

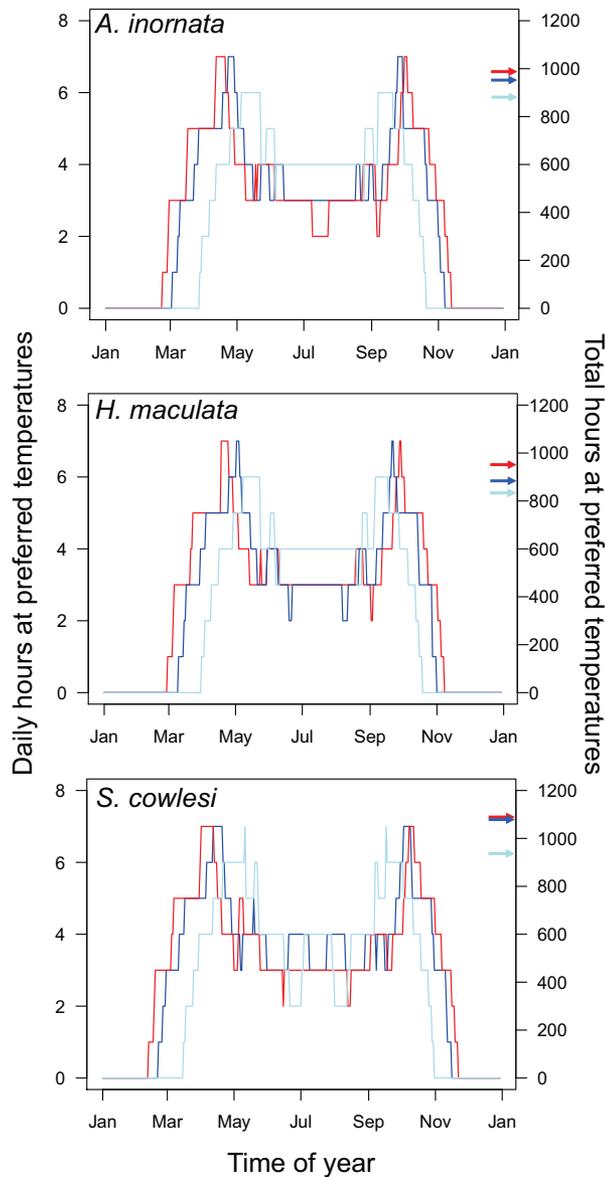


Figure 5: Biophysical model estimates of the number of hours per day that white sand (blue lines), dark soil (red lines), and substrate-matched (light blue lines) color morphs could attain preferred temperatures (y-axis), and total annual hours that preferred temperatures are available for dark soil (red arrows), white sand (blue arrows), and substrate-matched (light blue arrows) color morphs (z-axis).

hypothesis (e.g., less melanization in a cooler environment; Clusella-Trullas et al. 2007).

We found evidence for both benefits and costs of blanched coloration. With respect to overheating, white sand morphs of all three species would experience between 120 and 179 fewer hours (14%–59% reductions) of overheating risk per year than dark soil morphs, whether in full sun or under fil-

tered shade (fig. 4). Reduced overheating risk might be expected to translate into greater activity, as individuals should not have to seek out refuges as often (Sinervo et al. 2010; Gunderson and Leal 2016; Sears et al. 2016). Instead, white sand coloration reduces activity time because of an inability to reach activity temperatures when conditions are cool. With respect to activity timing between morphs, white sand morphs would be delayed in reaching T_{pref} by about 1.5 weeks in the spring and would lose the ability to reach T_{pref} about 1 week earlier in the fall (fig. 5). White sand morphs would also experience a decrease in annual hours at T_{pref} of 1%–7%, depending on the species (fig. 5; table 1). Results are similar assuming different thermal preference ranges, highlighting the robustness of this result (table 1). In sum, evolving from the dark soil to the white sand morph in White Sands habitat has some benefits (lower overheating risk) and some costs (less activity time) with respect to thermal performance. Further empirical studies will be needed to assess whether and how these costs and benefits translate into differences in performance and fitness.

Consequences of Background Matching in the White Sands

If predation pressure selects for greater background matching in White Sands taxa (Rosenblum 2006; Rosenblum and Harmon 2011; Rosenblum et al. 2017), the question remains as to why lizards in this system have not evolved to better match the substrate (fig. 1; fig. S1). There are numerous mechanisms that could contribute, such as intrinsic phenotypic constraints (Futuyma 2010), insufficient time to reach evolutionary equilibrium, and gene flow from dark soil populations (Rosenblum and Harmon 2011). Greater background matching may also have costs with respect to thermal performance, which we investigated with our biophysical model. With respect to overheating, a substrate-matched morph would have fewer hours of risk than either dark soil or white sand morphs. In fact, a substrate-matched morph could completely avoid overheating if it had access to filtered shade during the summer (fig. 4). Therefore, there could be at least some thermal benefit to substrate matching.

As discussed above, a predicted benefit of lower overheating risk is increased activity time. However, this is where the cost of substrate matching appears. A substrate-matched morph would experience a 1–2-month reduction in the days with T_{pref} available relative to dark soil and white sand morphs, reducing the annual window within which physiologically (Martin and Huey 2008) and behaviorally (Gunderson and Leal 2015) optimal temperatures are available (table 1). Indeed, reductions in activity at T_{pref} have been associated with population extirpations in lizards (Sinervo et al. 2010). The effect is most pronounced

in the spring, as the breeding season is ramping up. Then, a substrate-matched morph would be delayed in accessing T_{pref} by several weeks relative to both current morphs (fig. 5). Access to preferred temperatures is critical for seasonally breeding lizards, as they promote gonadal development and increase sperm production (Licht et al. 1969; Licht 1973; Dawson 1975; Van Dyke 2015). Substrate-matched individuals would therefore be at a disadvantage in reproductive development relative to either of the other morphs.

The total annual hours with T_{pref} available would also be lower for a substrate-matched morph relative to both current morphs (by 6%–14% depending on morph and species; table 1). Substrate-matched coloration would be even more detrimental if not for the activity advantage it provides during the warmest summer months, particularly July and August (fig. 5). However, the hot summer months coincide with the lowest net primary productivity in the region (fig. S4), when the activity of desert insect prey is lowest (e.g., Cloudsley-Thompson 2001). Therefore, the thermal advantage of substrate matching may be of little practical import because of seasonal timing. Regardless, our analysis indicates that evolving to better match the White Sands substrate would come with benefits for overheating risk but costs for activity relative to the ancestral dark soil morphs and the current White Sand morphs.

Conclusion

Many phenotypes are likely under conflicting selection pressures, but elucidating performance differences along multiple functional axes can be difficult. Using a biophysical modeling approach to overcome this limitation with respect to color evolution in White Sands desert lizards, we found both costs and benefits of color evolution with respect to thermal performance. At this point, whether color evolution in White Sands lizards provides a net thermal cost or benefit relative to the ancestral color morph is unclear. What is clear is that color evolution has thermal consequences in this system that are likely to interact with other selective pressures, such as predator avoidance (Hegna et al. 2013; Smith et al. 2016a). Our results highlight the complexity in understanding what drives the evolution of traits that affect multiple dimensions of performance, such as coloration.

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Statement of Authorship

A.R.G. and E.B.R. conceived of the project; A.R.G. collected and analyzed empirical data; E.A.R., M.W.S., and A.R.G. developed the biophysical model; and A.R.G. wrote the first draft of the manuscript with subsequent edits by E.A.R. and E.B.R.

Data and Code Availability

Data reported here are available in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.cz8w9gj3c>; Gunderson et al. 2021).

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White Sands *Holbrookia maculata*. Photo: Alex R. Gunderson.