



Behavioural type depends on temperature and body size, but is uncoupled from metabolism, in an African lizard

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The combinations of trait values that occur within individual animals are often constrained, forming syndromes. The pace-of-life syndrome (POLS) theory proposes that physiological, behavioural and life history traits covary in a predictable way, with metabolic rate acting as the underlying driver of phenotypic variation along a ‘slow–fast’ axis. However, in many taxa, support for the association among traits predicted by POLS is either weak or absent. Interindividual variation in thermal sensitivities across traits might explain the lack of support for POLS. Indeed, several studies have found that populations contain ‘thermal types’, with traits falling along a cold–hot continuum, a pattern termed the thermal–behavioural syndrome. Here, we examine the role of temperature in mediating the covariation between physiology, locomotor performance and behaviour in an African lizard, the southern rock agama, *Agama atra*. First, we tested whether individuals vary in suites of behavioural traits (i.e. have behavioural types) and whether these differences persist across temperature. We then explored the relationship between behavioural type and thermal physiology. Lastly, we tested the fundamental prediction of POLS, whether resting metabolic rate underlies variation in behavioural types and thermal performance. We uncovered the presence of behavioural types in rock agamas, and behaviour was predicted by lizard thermal type. However, the direction of the association between these traits was mediated by body size. In large individuals, ‘hot’ and fast lizards were bold, whereas in smaller individuals, hot and fast lizards were shy. Despite theoretical predictions, resting metabolic rate did not drive variation in behavioural type in these lizards but did predict thermal type (i.e. thermal optimum). A general theory of pace of life will likely have to consider relationships between thermal physiology, body size and behaviour independently of the role of resting metabolism.

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According to theory, selection should favour behavioural flexibility in variable environments because behaviour allows individuals to maximize fitness across ecological and social contexts (Sih et al., 2004). Instead, individuals often maintain consistent differences in behavioural traits across time and context (Réale et al., 2010). Individual animals can exhibit a particular ‘behavioural type’, occupying a place along the ‘bold–shy’ axis, where ‘bold’ individuals generally express more active, aggressive and exploratory behaviour, but are less social than ‘shy’ individuals (Réale et al., 2010).

There has been an overwhelming amount of evidence indicating the presence of behavioural types, also often referred to as ‘personality’, in different taxa (e.g. insects: Blight et al., 2016; fish: Huntingford, 1976; lizards: Carter et al., 2012; birds: Bókonyi et al., 2012; mammals: Yuen et al., 2016), with most studies detecting partial or full covariation between different behaviours (activity, exploration, sociability, aggressiveness and boldness).

The most comprehensive paradigm explaining the prevalence of the consistent interindividual variation in behaviour is the pace-of-life syndrome (POLS), which suggests that the covariation between physiological, behavioural and life history traits constrains individuals into predictable behavioural phenotypes that vary along a slow–fast continuum (Careau & Garland, 2012; Réale et al., 2010). On one end of this continuum, ‘fast’ individuals are more aggressive

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and bolder, have high activity rates and levels of exploration and exhibit high resting metabolic rates, growth rates and fecundity (often with earlier reproduction). Conversely, 'slow' individuals are characterized by low levels of aggression and boldness, display low levels of activity and have low metabolic rates. The foundation of this axis of trait covariation is thought to be the 'metabolic machinery' of individuals, whereby higher resting metabolic rates and higher aerobic capacity allow some individuals to follow the more proactive behavioural profile (Biro & Stamps, 2010; Careau & Garland, 2012).

While POLS is apparent in some taxa (e.g. delicate skinks, *Lampropholis delicata*: Goulet, Thompson, Michelangeli, et al., 2017; olive flounder fish, *Paralichthys olivaceus*: Rupia et al., 2016; zebrafish, *Danio rerio*: Yuan et al., 2018; deer mouse: *Peromyscus maniculatus*: Careau et al., 2011), other studies have found weaker support (e.g. house crickets, *Acheta domestica*: Royauté et al., 2015; common lizards, *Zootoca vivipara*: Le Galliard et al., 2013; Mell et al., 2016; great tits, *Parus major*: Bouwhuis et al., 2014; guppies, *Poecilia reticulata*: White et al., 2016). A review by Biro and Stamps (2010) showed that approximately 75% of studies demonstrated a positive relationship between behaviour and resting metabolic rate. In contrast, a meta-analysis by Royauté et al. (2018) found that studies that included both physiological and behavioural traits tended to provide little support for the POLS hypothesis. The lack of correlation between behaviour and metabolic rate in many studies suggests that this relationship might be trait specific (i.e. behaviours with weak associations with net energy gain or loss may not be correlated with maintenance metabolism; Mathot et al., 2019) or obfuscated by additional extrinsic (i.e. environmental) factors (Montiglio et al., 2018; Niemelä & Dingemans, 2018). In ectotherms, increased complexity in the POLS framework might arise from temperature mediating the relationships between physiological, performance and behavioural traits.

While metabolic rate is thought to be a major driver of fast versus slow phenotypes, metabolism is highly thermally sensitive, increasing exponentially with body temperature (Clarke & Fraser, 2004; Schmidt-Nielsen, 1997). Indeed, as body temperature increases, individuals tend to engage in increased activity and become bolder, more aggressive and less social (Biro et al., 2010). Goulet, Thompson, and Chapple (2017) proposed a framework known as the thermal-behavioural syndrome (TBS), stating that individuals have a thermal type, occupying a position along the cold-hot axis. Goulet, Thompson, and Chapple (2017) proposed that thermal physiology (e.g. optimal performance temperature, preferred temperature) should be incorporated within the POLS framework whereby individual position along the cold-hot axis should covary with their position along the shy-bold axis, forming 'extended POLS'. In other words, in addition to having higher metabolic rates, bold individuals should also have higher thermal optima for performance traits and higher preferred temperatures compared to shy individuals. This hypothesis depends on the premise that individuals that have higher thermal optima for fitness-related traits (e.g. locomotor performance) might actively choose to maintain warmer body temperatures and therefore maintain higher metabolic rates, which allow them to adopt a proactive (i.e. bold) behavioural type (Biro & Stamps, 2010; Careau & Garland, 2012). Recent studies in zebrafish (Rey et al., 2015), Nile tilapia, *Oreochromis niloticus* (Cerqueira et al., 2016), mountain log skinks, *Pseudemoia entrecasteauxii* (Stapley, 2006), and delicate skinks (Goulet, Thompson, Michelangeli, et al., 2017; Michelangeli et al., 2018) have generally supported extended POLS, demonstrating clear links between thermal and behavioural types.

While studies on extended POLS have revealed covariation between thermal types and behavioural types, it is currently unclear whether metabolism is the underlying driver of this covariation.

The only study to date to have incorporated metabolism, thermal performance and behaviour showed that while metabolic rate and behaviour were positively correlated, this fast pace of life was negatively correlated with thermal performance in damselfly larvae (*Ischnura elegans*; Tüzün & Stoks, 2022). Moreover, it is unknown whether individual responses to temperature change are consistent across traits and whether covariation between metabolic rate, physiological performance and behavioural traits persists at different body temperatures. Thus, the generality of covariations between thermal and behavioural types, and the extent to which we should integrate TBS with classical POLS remains uncertain (Gopal et al., 2023).

Here, we studied a population of southern rock agama lizards, *Agama atra* (henceforth, 'rock agamas') in South Africa to test (1) whether behavioural types exist and correspond to differences in thermal type following the predictions of extended POLS, (2) whether resting metabolism predicts thermal and behavioural types and (3) whether the covariation between POLS traits depends on temperature. To this end, we measured resting metabolic rate, locomotor performance and a suite of behavioural traits across multiple individuals at different temperatures. We hypothesized that (1) behavioural type and thermal type form a syndrome, where hot individuals are bolder and more active than cold individuals, (2) variation in thermal and behavioural types is driven by underlying metabolism and (3) the strength of integration of POLS traits is mediated by temperature.

METHODS

Ethical Note

Animal collection, maintenance and experiments were approved by CapeNature (permit no. AAA007-00206-0056) and the Research Ethics Committee: Animal Care and Use, protocol SU-ACUD14-00061. This research strictly adhered to standard procedures for minimizing animal stress throughout all stages of the project. Animals were captured using the common lasso technique, a swift procedure that minimizes handling time and stress. For performance trials, a thin thermocouple connected to a temperature reader was inserted in the cloaca and confirmed that individuals had achieved the target temperature. While this practice may induce a small amount of discomfort in lizards, the procedure is common and not harmful and only takes a few seconds to complete. At the end of the study, all lizards were in good body condition and were returned to field sites.

Lizard Collection and Maintenance in Captivity

We collected adult male rock agamas ($N = 21$; body mass range 18.3–30.6 g, mean body mass \pm SEM = 24.9 \pm 2.9 g) from Jonaskop, in the Western Cape Province of South Africa. Lizards were collected using the lasso technique (Losos, 2011) in February 2018 to measure sprint speed. Lizards were marked and released to their original collection sites after 3 weeks in captivity and recaptured in April 2018 to measure behavioural traits and metabolic rate. All collection events fell within the autumn postreproductive season for this population. All animals were brought back to our laboratory facility at Stellenbosch University to measure sprint speed, behavioural traits and metabolic rate. Before the start of experiments, lizards were habituated to laboratory conditions for 5 days. Lizards were individually housed in glass terraria with access to a wooden hide box and a basking rock. Each terrarium was equipped with an infrared heat bulb (75 W) to enable thermoregulation and an ultraviolet light for vitamin D3 synthesis. Each day, lizards were given water and fed medium sized crickets and mealworms

(approximately 5% of their body mass) dusted in vitamin and mineral supplement. Cardboard dividers were placed between lizard terraria to ensure that individuals could not see one another. Lizards were kept in captivity for 6 weeks in total to measure metabolic rate, physiological performance and behavioural traits across different temperatures, and upon the completion of experiments were released back to their original capture location.

Physiological and Behavioural Measurements

Resting metabolic rate

We measured resting metabolic rates (RMRs) using flow-through respirometry at the ecologically relevant temperatures of 25 °C and 35 °C (Alujević et al., 2023; Logan et al., 2019; Van Berkel & Clusella-Trullas, 2018). The RMR of lizards ($N = 21$) was measured once at each of the test temperatures and individual VCO_2 was recorded during two separate 30 min periods. VCO_2 data in ppm were collected using a LI-COR infrared CO_2/H_2O analyser (Li-7000, LI-COR, Lincoln, NE, U.S.A.) plumbed in differential mode and calibrated using a span gas of 0 and 395 ppm CO_2 . We scrubbed the incoming air of water by passing it over silica gel and Drierite (W.A. Hammond Drierite Co Ltd, Xenia, OH, U.S.A.), and we scrubbed it of CO_2 by passing it over soda lime. Scrubbed gas was then pushed through a mass control valve (Side-Track Model 840, Sierra Instruments, Monterey, CA, U.S.A.) at the rate of 400 ml/min. We randomized the order of temperatures for batches of six or seven individuals, each of which were housed in glass cuvettes (180 ml), which were themselves placed inside an incubator (Sanyo Cooled Incubator, MIR-153, Sanyo Electric) and connected to an eight-channel multiplexer (Intelligent Multiplexer V3, Sable Systems, Las Vegas, NV, U.S.A.). All respirometry trials started in the morning (between 0900 and 1000 hours) with a 1 h habituation period at the test temperature. Lizards were fasted for 48 h before measuring metabolic rate, to minimize the effect of digestion on experiments while not compromising their body condition (Plasman et al., 2019). We did not provide light so as to recreate conditions similar to darkened crevices in the field where lizards rest or take refuge during daytime. We separated the cuvettes with pieces of cardboard to prevent interactions between individuals during measurements. The scrubbed air was pushed through each cuvette consecutively (switching cuvettes every 30 min), and we recorded two 30 min periods of individual VCO_2 measurements at a 1 Hz sampling rate per trial. Trials lasted 8 h in total and each lizard recording included 1 h of data. We recorded baseline measurements using an empty cuvette at the beginning and end of each trial to correct for baseline drift. Inactive channels were flushed with dry CO_2 -free air at 400 ml/min to avoid accumulation of CO_2 during nonmeasuring periods. RMRs were analysed using ExpeData software (Sable Systems, Las Vegas, NV). Data were visually examined to ensure they were collected during periods of inactivity (resting). Resting periods were associated with at least 5 min of stable readings, and we validated this approach using pilot trials where we measured lizard RMR while monitoring individuals with a Logitech C920 webcam. Following Basson and Clusella-Trullas (2015), we averaged the lowest set of VCO_2 values taken during any 5 min period of stable measurements for each individual lizard. Transformation of extracted data to ml CO_2 /h was done using standard equations according to Lighton (2008).

Locomotor performance

We measured the sprint speed of lizards ($N = 18$) at 15 °C, 25 °C, 35 °C, 38 °C, 42 °C and 44 °C. For this and all subsequent experiments, lizards were fasted for 24 h prior to the start of the experiment. Lizards were placed in a climate-controlled room until they equilibrated to the target temperature, which we confirmed with a

cloacal thermometer. We randomized the order of temperature exposures, except for the 44 °C trial, which we conducted for all lizards at the end of the experiment to avoid heat-shock effects on subsequent trials. All lizards were given at least 2 h rest between trials at different temperatures and were never measured at more than two temperatures per day. Sprint speed was measured in an enclosed runway ($2 \times 0.15 \times 0.3$ m) constructed out of wood and demarcated every 25 cm (Noble et al., 2014). Lizards ran on a layer of fine sandpaper for traction. We encouraged lizards to run by gently tapping their tails (Noble et al., 2014). Three trials were run at each temperature per individual and in quick succession. We recorded trials with a video camera set to a rate of 60 frames/s (PowerShot G16, Canon Inc., Tokyo, Japan) and determined the maximum sprint speed over any 25 cm section using frame-by-frame analysis in the motion analysis software program Kinovea (version 0.8.15, Kinovea, France). If a lizard failed to run for at least 25 cm, we scored the sprint speed as 0 m/s (Logan et al., 2014). The fastest of the three runs per temperature was used in all subsequent analyses.

We constructed thermal performance curves for each individual by fitting a set of biologically realistic equations (asymmetrical parabolic functions) in TableCurve 2D software (version 5.01, Systat Software Inc., San Jose, CA) to the sprint speed data (Angilletta, 2006; Logan et al., 2014). Following Battles and Kolbe (2019) and Logan et al. (2020), we anchored thermal performance curves with minimum and maximum critical thermal (CT) values taken from literature on agamids ($CT_{min} = 6.3$ °C and $CT_{max} = 44.9$ °C; Clusella-Trullas & Chown, 2014). Because critical thermal limit values typically only vary among individuals by a small percentage of the full breadth of the performance curve, this method can be used when it is necessary to constrain model fits and avoid curves that are biologically unrealistic (e.g. performance increasing infinitely with temperature; also see Telemeco et al., 2022). We selected the best curve fit for each individual using Akaike's information criterion (ΔAIC_c ; Akaike, 1987; Angilletta, 2006), and then extracted maximum performance (P_{max}), thermal optimum (T_{opt} ; body temperature at which performance was maximized) and performance breadth (B_{80} ; the range of temperatures over which the lizard achieved at least 80% of maximal performance, including the lower and upper thresholds, lower B_{80} and upper B_{80} , respectively) from each curve.

Behaviour

For each individual ($N = 21$), we measured two types of behaviour: activity and boldness. We estimated boldness as both the time delay to emerge from a shelter into a novel arena and the response to a predator attack. We focused on these traits because they are ecologically relevant in lizards and were logistically feasible given our study design. We conducted these behavioural experiments in the following order: emergence from a shelter, level of activity and response to a predator. This order was maintained for all individuals to minimize potential carryover effects from one experiment type to another (Bell, 2012). We tested each individual twice, with 3 days between measurements, for each behavioural trait to assess behavioural repeatability as a measure of individual differences in behaviour but minimize a potential learning effect (Bell et al., 2009; Martin & Réale, 2008). Lizards had two rest days between each experiment and the order of individuals across trials was randomized to eliminate a time-of-day confound. All lizards were given at least 2 h of rest between trials at different temperatures and were never measured at more than two temperatures per day. Prior to the start of each experiment, we placed lizards inside a climate-controlled room for 1 h to acclimate. We checked lizard body temperature using a cloacal thermometer before the start of each trial. All trials were recorded with a Logitech C920 webcam (at 60 frames/s) for later playback and data extraction using Kinovea software.

We conducted emergence experiments in an experimental arena (120 × 120 cm; marked with 5 × 5 cm grid squares) at 25 °C and 35 °C. A white plastic box (24 × 16 × 8 cm) with a string-operated door was placed against the arena wall. At the beginning of the trial, we placed each lizard inside the closed box for 5 min to allow it to get used to the new environment (Bajer et al., 2015). After 5 min, the door was opened by pulling a string attached to the box door. This was done from behind a black curtain to ensure that the lizard was not disturbed by human presence. The time until the lizard's head (snout emergence) and full body (including the base of the tail) emerged from the shelter was recorded (Bajer et al., 2015). The total experimental time was 1 h (recorded from the moment we opened the door). Lizards that did not emerge from the shelter by the end of the experiment were scored with a maximum trial time of 60 min.

Activity trials were also conducted at 25 °C and 35 °C. Each animal was first placed in the centre of the arena (120 × 120 cm) and kept covered for a few seconds until it became relaxed (movement under the experimenter's hand ceased). We then allowed the lizard to move freely in the arena (Michelangeli, Chapple, et al., 2016; Michelangeli, Wong, et al., 2016) and recorded its movements for 30 min. We discarded the first 2 min of each trial to reduce the potential influence of handling. The level of activity was estimated as the total distance travelled during the 28 min tested period.

To evaluate the response to a predator attack, we placed each lizard in the centre of a second, larger arena (170 × 170 cm) on a rock positioned under a heat lamp (175 W, Philips Incandescent 240 V PAR 38). The temperature of the basking site was set to match the preferred temperature for this species (~35 °C; Logan et al., 2020; Van Berkel & Clusella-Trullas, 2018) and was substantially higher than the surrounding air temperature (25 °C), creating a desirable basking zone that was about 30 cm in diameter. After the initial placement on the rock, the lizard was left to habituate for 2 min. We then released a model of a bird (ca. 25 cm wing span) that was attached with fishing line to the ceiling above the arena, simulating a strike 10 cm above the basking spot, before immediately retracting it behind a black curtain (Michelangeli, Chapple, et al., 2016). We recorded the time from initial fleeing until the lizard returned to the optimal thermal zone and the time from initial fleeing to its return to the basking posture on the rock as measures of boldness.

Repeatability of behavioural traits

To test whether lizards showed consistent differences in behaviour, we estimated behavioural repeatabilities (R) as ratios of between-individual phenotypic variance to total phenotypic variance (Dingemans & Dochtermann, 2013). Behavioural repeatabilities were calculated separately for each of the tested temperatures. Calculations were done using general linear mixed-effects models fitted by restricted maximum likelihood (REML) with individual identity as a random effect (Nakagawa & Schielzeth, 2010) using the package 'rptR' (Stoffel et al., 2017). We accounted for variation in mass among individuals by adding body mass as a fixed effect. When necessary, traits were log-transformed to conform to model assumptions. All statistical analyses were performed in R Studio (RStudio Team, Boston, MA, U.S.A.) for R (version 3.5.0).

Interindividual variation in thermal sensitivity of POLS traits

To test for the presence of interindividual variation in thermal sensitivity of POLS traits, we calculated thermal repeatability (R_T) for RMR, sprint speed, activity and boldness (emergence from the shelter) across two temperatures (25 °C and 35 °C). Thermal repeatability is an estimate of the extent to which differences between individuals in a trait are maintained across a range of body

temperatures and is computed as a ratio of between-individual phenotypic variance to total phenotypic variance across that temperature range (Dingemans & Dochtermann, 2013). We estimated thermal repeatability using mixed-effects models with temperature and body mass as fixed effects and lizard identity as a random effect (Careau, Biro, et al., 2014; Careau, Gifford, et al., 2014). The statistical significance of fixed effects was determined using likelihood ratio tests with maximum likelihood estimates. We obtained statistical significance of the random intercept using a likelihood-ratio test with REML estimates, comparing log likelihoods of a full model that included lizard identity as a random factor with one in which lizard identity was excluded (Careau, Biro, et al., 2014; Careau, Gifford, et al., 2014). To examine the pattern of interindividual variation of thermal sensitivity of traits, linear mixed models with random intercepts were expanded to include variance in slopes and covariances between intercepts and slopes (Careau, Biro, et al., 2014; Careau, Gifford, et al., 2014). Statistical significance of covariance between intercept and slope was assessed using likelihood-ratio tests by comparing a model that included random slopes and the covariance between intercept and slopes against a model that only included random intercepts. We ran linear mixed-effects models using the 'lme' function ('nlme' package) and 'lmer' function ('lme4' package). We calculated standard errors for thermal repeatability estimates using the 'pin' function ('nadiv' package).

Extended POLS

To test for the presence of a behavioural type, we examined correlations between behavioural traits obtained at 35 °C (the test temperature closely matching the preferred temperature for this species; Van Berkel & Clusella-Trullas, 2018) using the 'cor' function in the R package 'stats'. We illustrated the correlational relationships between paired behavioural traits using the 'ggcorplot' function. We repeated the same process to test for correlations between thermal variables describing the shape of thermal performance curves.

To test for the covariation between behavioural and thermal traits (i.e. extended POLS, $N = 14$; some individuals that were tested for sprint speed were not able to be recaptured to measure behaviour), we carried out a multivariate analysis using the 'brm' function in the R package 'brms' (Bürkner, 2017). The 'brm' function fits generalized linear multivariate multilevel models using Stan for full Bayesian inference. Our multivariate model included five repeatable behavioural traits as response variables and thermal traits as predictors. To avoid issues with collinearity among our predictors, we inspected the variance inflation factor (VIF) and only retained variables with $VIF < 5$ (optimal temperature, maximum performance and performance breadth). We included an interaction term with body mass as a continuous variable for each of our predictors. We used mean values of behavioural traits obtained at 35 °C to avoid pseudoreplication. All variables were mean-centred and scaled to 1 standard deviation to facilitate model fitting and interpretation of the results. Each model was run with four separate chains, each for 4000 iterations with a burn-in phase of 2000. We confirmed model convergence using the Gelman–Rubin (\hat{R}) diagnostic test in package 'brms'. Probability of direction was computed using the 'pd' function in the 'bayestestR' package (Makowski et al., 2019).

Drivers of extended POLS

To test whether resting metabolic rate predicts extended POLS, we fitted separate multivariate models for RMR predicting behavioural and thermal traits following the approach described above. To test whether resting metabolic rate predicts behavioural type at 25 °C (suboptimal temperature for rock agamas), we fitted a multivariate model with three behavioural traits measured at this

temperature as response variables and RMR and body mass as predictors. Following the same approach, we fitted a separate model with five behavioural traits measured at 35 °C as response variables to test for the effect of RMR on behavioural type at species thermal optimum. We also tested if RMR predicts thermal type by fitting a model that included three thermal traits as response variables, and RMR and body mass as predictors. Lastly, we wanted to test whether individuals show similar responses to temperature across traits, i.e. whether individual thermal sensitivity is correlated across traits. Due to our limited sample size, we could not generate a multivariate model with a complex random term that would allow us to estimate the covariance between individual slopes across temperature and pairs of traits. Instead, we extracted individual slopes (i.e. thermal sensitivity) for RMR, activity and boldness traits from mixed-effects models described in the thermal repeatability section above (Interindividual Variation in Thermal Sensitivity of POLS Traits). Finally, we fitted a multivariate model with behavioural trait slopes as response variables and RMR slope and body mass as predictors.

RESULTS

Repeatability of Behavioural Traits

Linear mixed-effects models showed statistically significant adjusted repeatability estimates (R), for all the measures of behavioural traits (i.e. boldness and activity) across both tested temperatures (Table 1). At 25 °C, repeatability estimates ranged between 0.69 and 0.94. Similarly, repeatability estimates were high and significant for all boldness traits (0.73–0.99) and activity (0.64) at 35 °C.

Interindividual Variation in Thermal Sensitivity of POLS Traits

Body mass of adult males did not have a significant effect on thermal repeatability of behavioural traits but had a significant effect on both resting metabolic rate and sprint speed (Table 2). The thermal repeatability of metabolic rate was not significant, meaning that (after accounting for mass) individuals that had higher metabolic rates at the lower test temperature did not have higher metabolic rates at the higher test temperature (i.e. individual rank was not consistent across temperatures; $R_T < 0.01$, $\chi^2 < 0.1$, $P = 0.99$). Conversely, locomotor performance was thermally repeatable as lizards that were faster at the lower temperature were also faster at the higher temperature (maximum sprint speed: $R_T = 0.45$, $\chi^2 = 4.05$, $P = 0.04$; Fig. 1). Similarly, activity ($R_T = 0.33$, $\chi^2 = 10.28$, $P = 0.001$), snout emergence ($R_T = 0.70$, $\chi^2 = 50.02$, $P < 0.001$) and body emergence ($R_T = 0.69$, $\chi^2 = 48.62$, $P < 0.001$; Table 2) were all thermally repeatable (Fig. 1). Adding covariance between random intercepts and slopes significantly improved

model fit for resting metabolic rate ($P < 0.001$), where individuals with higher RMR at lower temperature had lower RMR at higher temperature (the thermal reaction norms crossed). In addition, covariance between intercepts and slopes was also negative and significant for activity ($P = 0.004$) but was not significant for body emergence ($P = 0.06$), snout emergence ($P = 0.53$) or sprint speed ($P = 0.14$; Appendix, Table A1).

Extended POLS

Pairs of behavioural traits were significantly correlated, indicating the presence of behavioural types in our study population (Fig. 2a). Individuals that took longer to emerge from the shelter and return to the basking zone after a simulated predator attack spent less time active, indicating shy individuals. By contrast, lizards that were faster to emerge from the shelter and faster to return to the basking zone after a predator attack were also more active (i.e. bold individuals).

Maximum performance was significantly correlated with performance breadth (Fig. 2b). Individuals that had higher maximum performance had a narrower range of temperatures at which they could maintain optimal performance. While optimal temperature was positively correlated with maximum performance and negatively correlated with performance breadth, this relationship was not significant (Fig. 2b).

We observed a highly credible effect of maximum performance on boldness (probability of direction: snout emergence 94% and body emergence 95%; Appendix, Table A2). However, the effect of maximum performance on boldness was mediated by body mass (probability of direction: snout emergence = 89%; body emergence = 91%; time to optimal zone = 84%; time to basking = 80%; Fig. 3). Similarly, the effect of optimal temperature on boldness was mediated by body mass (probability of direction: snout emergence = 95%; body emergence = 96%; time to optimal zone = 98%; time to basking = 97%; Fig. 3, Appendix, Table A2). In larger adult males, individuals with 'hot' phenotypes (high thermal optimum and maximum performance) were bolder (were faster to emerge from a shelter and return to the optimal basking zone after a simulated predator attack, while in smaller individuals, 'hot' lizards were shy (Fig. 3). Individuals that had a narrower optimal performance range took longer to emerge from a shelter into a novel arena (probability of direction: snout emergence = 98%; body emergence = 99%; Appendix, Table A2).

Drivers of Extended POLS

Resting metabolic rate had a highly credible positive effect on optimal performance (probability of direction = 96%; Fig. 4b, Appendix, Table A3). Body mass, but not RMR, predicted maximum performance (probability of direction = 92%) and performance breadth (92%), where larger individuals had lower maximum performance and broader performance range. However, RMR did not predict any of the tested behavioural traits at either temperature (Fig. 4a, Appendix, Tables A4–A5). In addition, thermal sensitivity of RMR did not predict thermal sensitivity of activity or snout emergence but had a highly credible positive effect on thermal sensitivity of body emergence (probability of direction = 92%; Appendix, Table A6).

DISCUSSION

We integrated physiological, behavioural and performance data on a set of rock agama lizards to examine the potential effects of temperature in modifying the predictions of the extended pace-of-life syndrome theory. Our study lizards showed high consistency in

Table 1

Within-temperature repeatability estimates (R), standard errors (SE) and associated P values for interindividual variation in behavioural traits measured twice at 25 °C and 35 °C

Test	Temperature (°C)	Behaviour	R	SE	P
Activity	25	Distance travelled	0.94	0.03	<0.001
		Snout emergence	0.69	0.13	<0.001
		Body emergence	0.83	0.08	<0.001
Activity	35	Distance travelled	0.64	0.14	<0.001
		Snout emergence	0.96	0.02	<0.001
		Body emergence	0.73	0.11	<0.001
Boldness	35	Time to optimal zone	0.99	0.001	<0.001
		Time to basking spot	0.94	0.03	<0.001

Significant repeatability values are in bold.

Table 2
Parameters from linear mixed-effect models with the fixed effects of body temperature (T_b) and mass (M_b) and a random intercept (lizard identity) with estimates of between- (V_i) and within-individual variance (V_R) and thermal repeatability (R_T) for sprint speed, resting metabolic rate (RMR) and behavioural traits across 25 °C and 35 °C

Trait	Variable	Fixed effects				Random effect				R_T	SE
		T_b		M_b		Intercept			Residual		
		χ^2	P	χ^2	P	V_i	χ^2	P	V_R		
Sprint speed	Maximum speed	5.17	0.02	4.33	0.04	0.06	4.05	0.044	0.07	0.453	0.19
RMR	CO ₂ production	22.84	<0.001	7.95	<0.01	<0.01	<0.1	0.99	0.76	<0.01	0.00
Activity	Distance travelled	23.72	<0.001	1.05	0.31	20.36	10.28	0.001	41.72	0.328	0.12
Boldness	Snout emergence	5.91	0.02	0.94	0.33	0.64	50.02	<0.001	0.28	0.696	0.09
	Body emergence	6.91	0.01	0.93	0.33	0.73	48.62	<0.001	0.33	0.689	0.09

Estimates were calculated based on two measurements per temperature for behavioural traits and one measurement per temperature for RMR and sprint speed. Statistically significant thermal repeatability values are in bold.

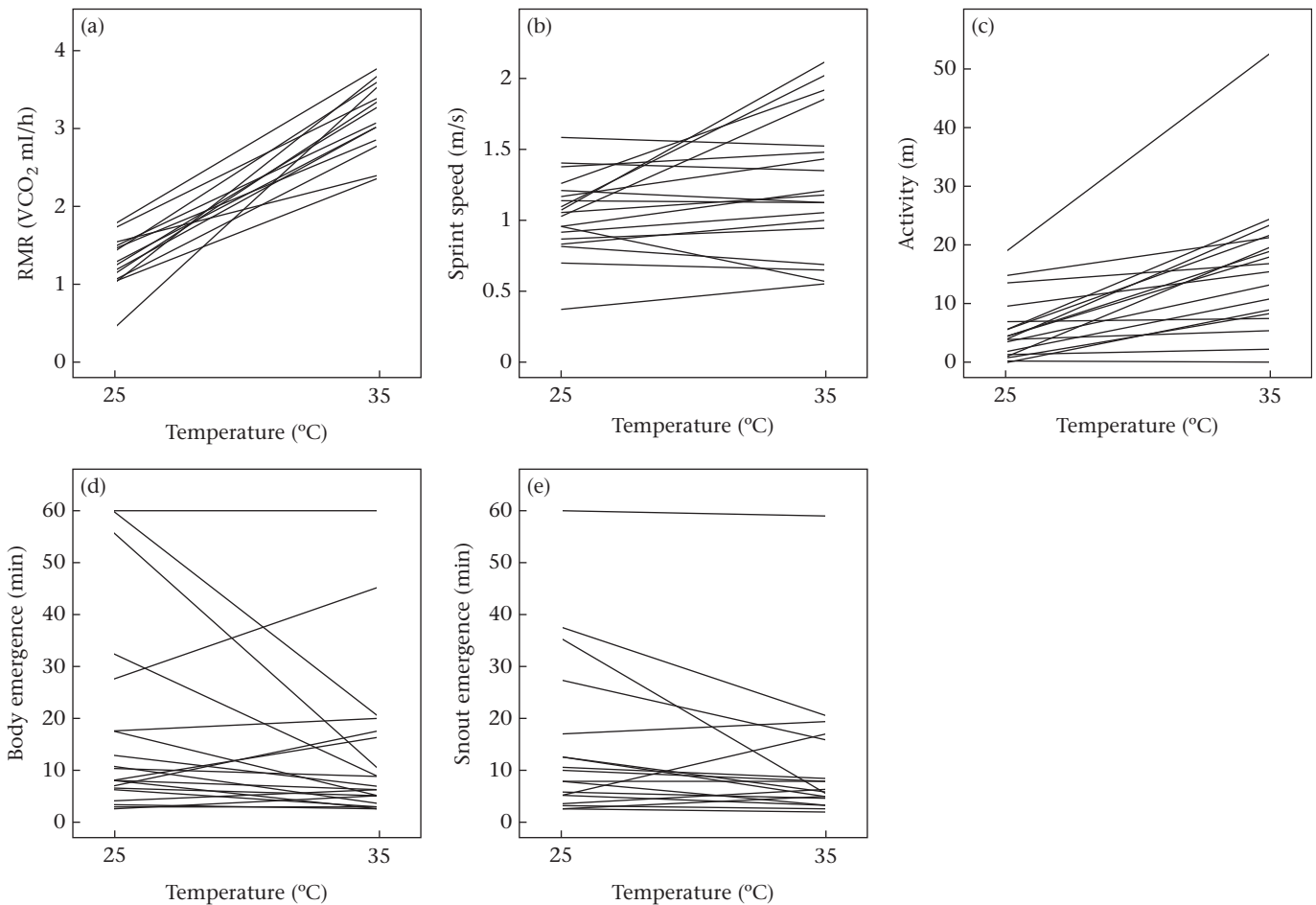


Figure 1. Thermal dependence of (a) resting metabolic rate ($N = 17$), (b) sprint speed ($N = 19$), (c) activity ($N = 21$), (d) snout emergence ($N = 21$) and (e) body emergence ($N = 21$) in rock agamas; individual thermal reaction norms are the result of linear fits to raw data obtained at 25 °C and 35 °C.

behavioural traits, both across time and two temperatures. Our analyses revealed presence of behavioural types in the population, and behavioural type was correlated with thermal type. However, the direction of covariation between these traits was dependent on body size. The prediction of extended POLS (cold = shy; hot = bold) was only evident in larger adults, while in smaller adults, 'hot' lizards were shy. While bolder and hotter individuals remained at the fast end of the POLS spectrum across temperatures, this was not underpinned by resting metabolic rate, which refuted a central prediction of classical POLS theory. Our findings suggest that interindividual variation in the thermal sensitivity of resting metabolism is not strongly associated with differences in the thermal sensitivity of behaviour, at least in some species.

Behavioural traits exhibited high short-term repeatability in rock agamas. We detected strong and significant associations between all behavioural traits, providing evidence for the presence of behavioural types in these lizards. Individuals that were generally more active also displayed higher boldness by being more willing to explore a novel environment and return to an exposed basking spot soon after being attacked by a predator. Lizards that sprinted the fastest at their thermal optimum were also faster than other individuals across the entire thermal range. Maximum performance was negatively correlated with performance breadth, showing that lizards that achieved higher maximum performance were unable to perform well across a broad range of temperatures near their thermal optimum (i.e. generalist–specialist trade-off). The presence of

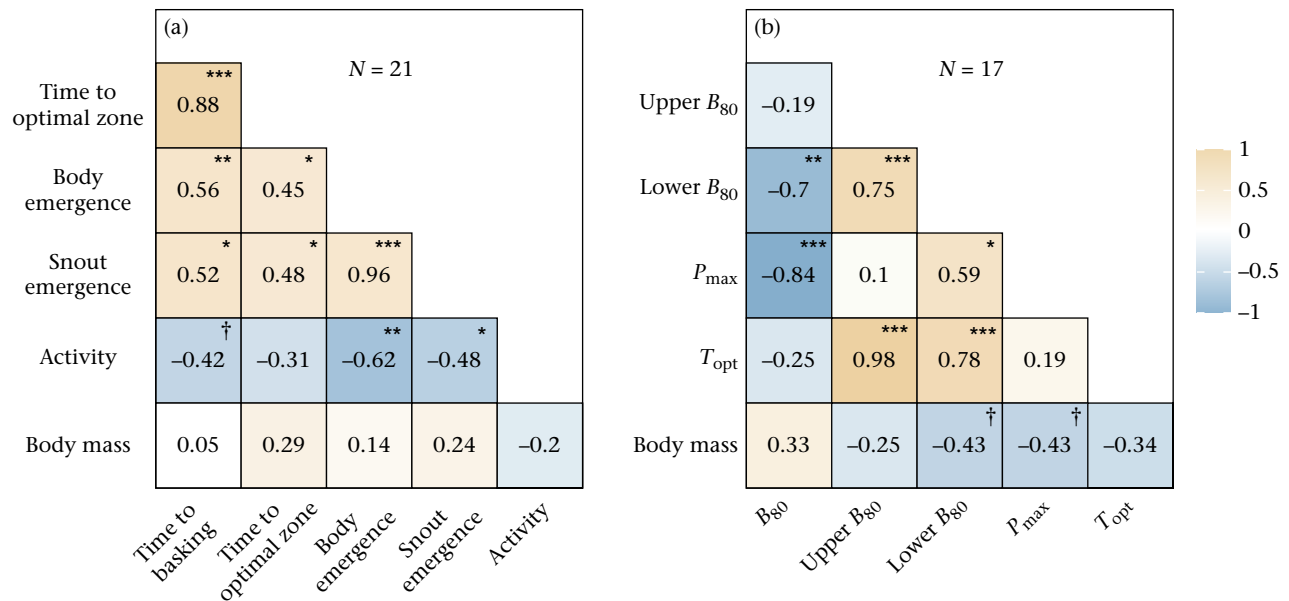


Figure 2. Correlation matrix of (a) behavioural traits tested at 35 °C and (b) thermal traits obtained from individual thermal performance curves. Thermal traits are represented as follows: T_{opt} = optimal temperature; P_{max} = maximum performance; B_{80} = performance breadth; Lower B_{80} = lower temperature threshold for optimal performance; Upper B_{80} = upper temperature threshold for optimal performance. Correlation coefficients are displayed inside the boxes, with colours indicating both the strength and direction of the correlation. Statistical significance of correlations is shown with asterisks as follows: † $P < 0.1$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

generalists and specialists in a population might be maintained due to genetic constraints that correspond to distinct thermal strategies, each of which offers fitness benefits. For example, specialists, despite having narrower performance breadths, might maximize their performance and activity levels to have better access to resources and a higher chance of evading predators while generalist lizards must sacrifice maximal performance capacity but can occupy a wider range of habitat types.

In agreement with the predictions of extended POLS, we found covariation between thermal types and behavioural types in the rock agama. An increasing number of studies have reported associations between thermal traits and behaviour (Cerqueira et al., 2016; Goulet, Thompson, Michelangeli, et al., 2017; Michelangeli et al., 2018; Rey et al., 2015; Stapley, 2006). While the direction of correlations between trait categories reported in the literature suggests that higher optimal temperature and higher maximal performance positively correlate with boldness, our results demonstrate that additional factors, such as body size, can mediate the relationship between thermal performance traits and behaviour. In larger individuals, hot thermal types were bolder, but this relationship changed with body size such that in smaller lizards, bold individuals exhibited the cold thermal type. This interaction might reflect ontogenetic shifts in the relationship between thermal physiology and behaviour (Groothuis & Trillmich, 2011). Individuals in our study were all sexually mature males, but size variation might reflect differences in age. Larger individuals tend to have defined territories and are therefore potentially more established in the social hierarchy compared to smaller adults that spend more time moving around as they compete for an established territory (Alujević et al., 2023; Bruton, 1977). Open habitats such as rock outcrops usually represent desired microhabitats for territorial lizards such as rock agamas and are frequently occupied by large, dominant males (Alujević et al., 2023; Bruton, 1977; Fey et al., 2019). Therefore, the patterns we observed could be explained by differences in the ecological challenges faced by adult lizards of different body sizes. In line with this hypothesis, thermal type correlated with microhabitat use in delicate skinks, where ‘hot’

individuals spent more time in exposed rocky microhabitats with high-quality heat sources for thermoregulation, compared to ‘cold’ individuals found in vegetated microhabitats (Michelangeli et al., 2018). Furthermore, active territory defence likely comes with costs such as higher exposure to predation. Predation-related selection in large males might have resulted in higher optimal temperatures and maximal speed in these individuals that would allow dominant males to compensate for the potential fitness trade-off between social dominance and higher predation risk (Carter et al., 2010). Conversely, smaller adults that are less able to compete for good-quality territories and instead spend a portion of their time in suboptimal thermal zones would benefit from having a generalist thermal type. These lizards are also bold, which may confer a fitness advantage if small but bold individuals have more mating opportunities and an increased chance to successfully compete for better-quality territories. Since thermal physiology and thermoregulatory behaviour can exhibit low heritability in some lizards (Logan et al., 2018; Martins et al., 2019), observed correlations between behavioural type and thermal physiology are potentially influenced by developmental plasticity. While these patterns are intriguing, it is important to recognize that they emerged from a limited sample size. Further investigations are warranted before drawing overarching conclusions about the role of body size in extended POLS. Nevertheless, body mass mediated the relationship between multiple thermal and behavioural traits in the same direction, adding to the robustness of our findings.

A central hypothesis in POLS theory is that variation in higher-level traits is driven by variation in resting metabolism. However, our results do not corroborate these predictions, and several explanations have previously been proposed when studies fail to detect correlations between resting metabolic rate and performance or behavioural traits. These include body size or sex mediating the relationship between traits, lack of underlying genetic variation in traits, or other variables like ontogeny, immune defence, competition, predation risk, resource availability and abiotic variables that might mediate these relationships (Bouwuis et al., 2014; Niemelä & Dingemans, 2018; Royauté et al., 2015; White et al., 2016). Despite

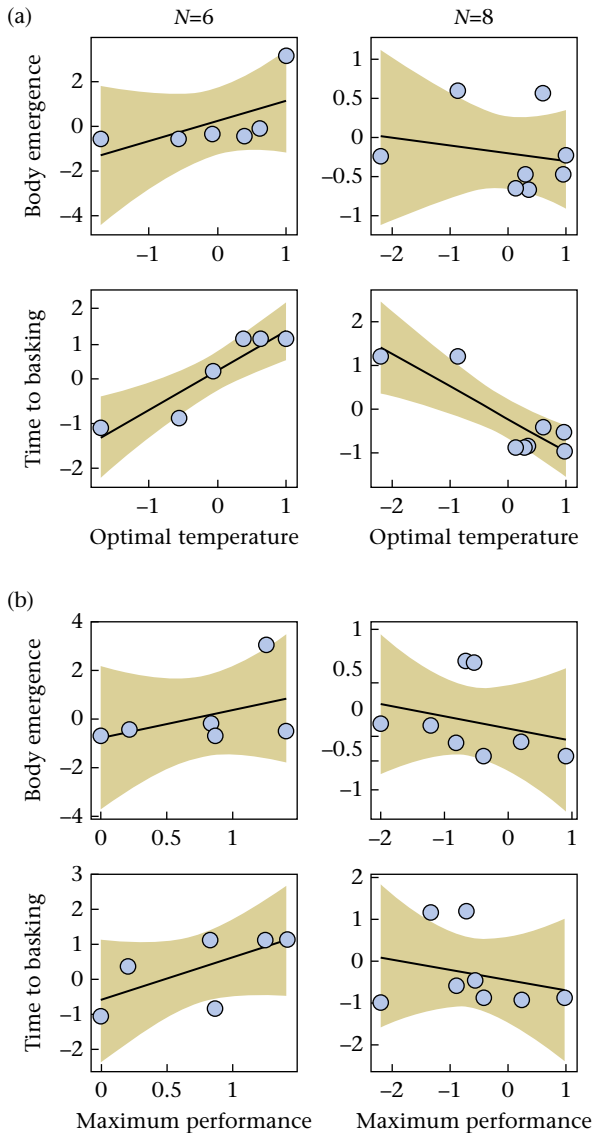


Figure 3. The effect of (a) optimal temperature and (b) maximum performance on boldness (body emergence from the shelter into a novel arena and time to return to the optimal basking spot after a simulated predator attack) mediated by lizard body mass. To illustrate the significant effect of body mass, thermal and behavioural traits for lizards smaller than the mean body mass ($M_b < 25.3$ g) are shown on the left and thermal and behavioural traits for lizards bigger than the mean body mass ($M_b > 25.3$ g) are shown on the right. Note: body mass was included as a continuous variable in the multivariate model ($N = 14$; some individuals that were tested for sprint speed were not able to be recaptured to measure behaviour). All response variables were mean-centred and scaled to 1 SD. Shaded bands represent 95% confidence intervals.

our prediction that temperature could affect the strength of covariation between metabolism and behaviour, and therefore provide an explanation for the absence of POLS in some taxa, resting metabolism and behavioural type were not integrated within a syndrome at either suboptimal (25 °C) or optimal (35 °C) temperatures in our study species. However, both activity and boldness were highly thermally repeatable. Bolder and more active individuals at a lower temperature were also bolder and more active at a higher temperature, but some variation in individual thermal reaction norms for activity was also explained by the negative correlation between intercepts and slopes. This means that the individuals that were less active at lower temperatures increased their performance more with a given increase in temperature (Appendix, Table A1). In fact, we observed two

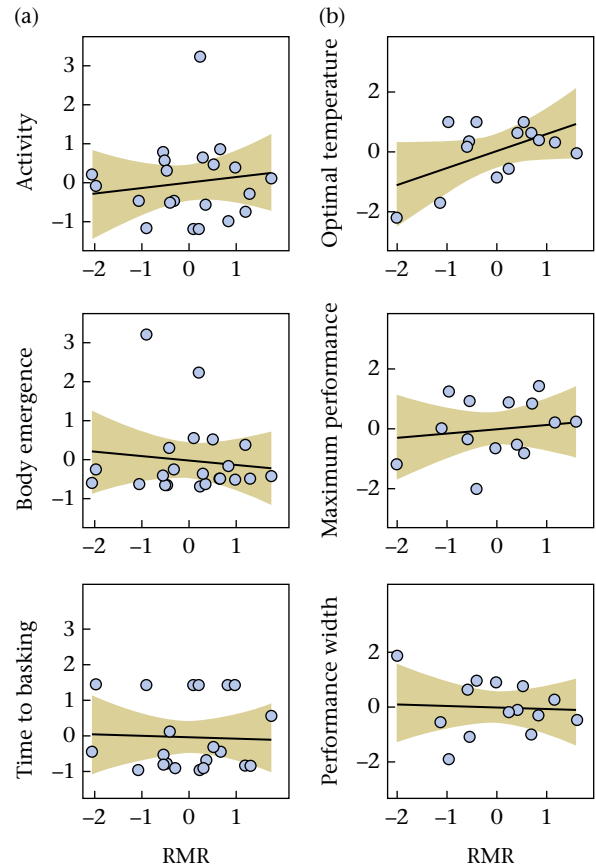


Figure 4. The effect of resting metabolic rate (RMR) on (a) behavioural traits measured at 35 °C ($N = 21$) and (b) thermal performance traits extracted from individual thermal performance curves ($N = 14$). Data are mean-centred and scaled to 1 SD. Shaded bands represent 95% confidence intervals.

distinct patterns of thermal responses: individuals whose activity remained relatively consistent across temperatures and individuals whose activity increased substantially at 35 °C compared to 25 °C (Fig. 1, Appendix, Fig. A1). In contrast, covariation between intercepts and slopes was not significant for boldness proxies, indicating that individuals maintained their rank across temperatures and that there was little variation among individuals in the thermal sensitivity of boldness.

Resting metabolic rate was not thermally repeatable in the rock agama. In other words, individuals that had higher resting metabolic rate at one temperature did not have higher metabolic rate at other temperatures. This pattern may reflect a compensation mechanism allowing lizards to have higher metabolic rates at warm temperatures as a result of energy savings from low-energy expenditure at low temperatures (Mell et al., 2016). This suggests that variation in thermal sensitivities of traits is an important factor that needs to be considered when examining trait covariation in ectothermic organisms. While we hypothesized that interindividual variation in thermal sensitivity might affect the strength of covariation between POLS traits at different temperatures, we found that individual responses to temperature for resting metabolism differed, and thus, is potentially reliant on a separate mechanism compared to higher-level traits such as physiological performance or behaviour.

Compiling a data set encompassing repeated measurements of several behavioural traits, locomotor performance and resting metabolic rate, across a range of temperatures in vertebrates, poses significant challenges. The extent of this challenge is evident in the scarcity of studies that have explicitly examined the extended POLS

hypothesis (Gopal et al., 2023). Although many studies have examined the relationships between pairs of physiological, performance and behavioural traits, we present one of the first examinations of how temperature mediates the integration of these traits into ‘complex phenotypes’. While this study brings novel insights into the complexity and direction of relationships within extended POLS, it is based on low sample sizes, and we therefore urge caution in extrapolating our results to other ecological and taxonomic contexts. Our study’s unbalanced sampling design, which originated from the difficulty of repeating physiological and performance measurements compared to behavioural trials, hindered the application of a single all-encompassing multivariate mixed-effect model incorporating repeated measurements across traits (and temperatures), as suggested by Dingemans and Wright (2020). Despite these challenges, the use of separate Bayesian multivariate models (following Godin et al., 2022) tailored to specific hypotheses facilitated comprehensive examinations of our research questions within the study’s limitations.

Understanding the role of temperature in mediating trait covariation is crucial because many ectotherms experience substantial fluctuations in their daily, seasonal and annual thermal environments, and thermally sensitive traits can undergo selection across the entire range of ecologically relevant temperatures. While the presence of covariation between behavioural and physiological traits has been found in a number of ectothermic taxa, our results suggest that the relationships between traits within the extended POLS are more complex than initially predicted and should be further explored. In particular, studies including more balanced designs and larger sample sizes with repeated measurements across multiple traits (Dingemans & Dochtermann, 2013; Dingemans & Wright, 2020) are needed to increase the robustness of our results. Overall, the understanding of ecological and evolutionary processes underlying POLS is still in its early stages (Dammhahn et al., 2018; Mathot & Frankenhuis, 2018) and there is a need to test these associations across a diversity of taxa and environments. In addition, our knowledge on the potential adaptive functions of these trait associations is mainly theoretical and will remain limited until additional empirical studies and field studies in particular are conducted (Moiron et al., 2020). Additionally, future research should test for the presence of consistent differences in thermal sensitivity (i.e. reaction norm slopes) in multiple ecologically relevant traits and examine whether interindividual variation in thermal sensitivity has an underlying genetic basis that can respond to selection. Further work along these lines will move the field towards a cohesive understanding of the mechanisms that shape the evolution of complex phenotypes in nature.

Author Contributions

Karla Alujević, Jeffrey W. Streicher, Michael L. Logan and Susana Clusella-Trullas conceived and designed the study. Karla Alujević collected the data and performed statistical analyses. All authors participated in data discussion and interpretation. Karla Alujević wrote the first draft of the manuscript. All authors contributed to manuscript editing and revision and gave final approval for publication.

Data Availability

Data that support the findings of this study are available on GitHub (<https://github.com/kalujevic/agamaPOL>).

Declaration of Interest

We declare no conflicts of interest.

Acknowledgments

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Appendix

Table A1

Covariance parameters between random intercept and slope (COV_{I-S}) obtained from linear mixed-effect models for behavioural, physiological and performance traits: activity (Activity), snout emergence (Snout), body emergence (Body), sprint speed (Sprint) and resting metabolic rate (RMR)

	SD	Correlation	χ^2	<i>P</i>
Model: lme(Activity~Temperature, random=~1+Temperature Lizard_ID)				
(Intercept)	10.77	(Intercept)	11.34	0.004
Temperature	0.52	-1		
Residual	5.70			
Model: lme(log(Snout)~Temperature, random=~1+Temperature Lizard_ID)				
(Intercept)	1.36	(Intercept)	1.28	0.526
Temperature	0.04	-0.806		
Residual	0.49			
Model: lme(log(Body)~Temperature, random=~1+Temperature Lizard_ID)				
(Intercept)	2.00	(Intercept)	5.51	0.063
Temperature	0.06	-0.902		
Residual	0.48			
Model: lme(Sprint~M_b+Temperature, random=~1+Temperature Lizard_ID)				
(Intercept)	0.49	(Intercept)	3.91	0.142
Temperature	0.02	-0.97		
Residual	0.21			
Model: lme(RMR~M_b+Temperature, random=~1+Temperature Lizard_ID)				
(Intercept)	3.92	(Intercept)	16.99	<0.001
Temperature	0.11	-1		
Residual	0.40			

Lizard_ID = lizard identity; M_b = body mass. Statistical significance of COV_{I-S} was tested by comparing COV_{I-S} model to a model with random V_I only. Significant *P* values are shown in bold.

Table A2

Coefficient estimates from Bayesian models showing the effect of thermal traits and body mass (M_b) on behavioural traits

Predictor	Response	Estimate (LCI, UCI)	Prob. of direction	\hat{R}	Bulk ESS	Tail ESS
T _{opt}	Activity	0.37 (-0.95, 1.74)	0.74	1.01	1133	553
M _b	Activity	0.26 (-0.93, 1.48)	0.68	1.00	3031	3403
P _{max}	Activity	0.69 (-1.50, 2.84)	0.76	1.01	904	379
B ₈₀	Activity	0.92 (-1.54, 3.19)	0.81	1.01	899	1023
T _{opt} :M _b	Activity	0.53 (-1.73, 2.74)	0.72	1.00	1943	1427
P _{max} :M _b	Activity	0.25 (-2.51, 2.88)	0.59	1.00	1360	1272
B ₈₀ :M _b	Activity	0.16 (-2.67, 2.85)	0.56	1.00	1787	2639
T _{opt}	Snout emergence	-0.05 (-0.64, 0.57)	0.55	1.00	1943	1849
M _b	Snout emergence	0.18 (-0.42, 0.76)	0.76	1.00	3745	4530
P _{max}	Snout emergence	-0.76 (-1.78, 0.27)	0.94	1.00	1920	3230
B ₈₀	Snout emergence	-1.21 (-2.31, -0.04)	0.98	1.00	1803	3305
T _{opt} :M _b	Snout emergence	-0.85 (-1.90, 0.24)	0.95	1.00	2230	2395
P _{max} :M _b	Snout emergence	-0.74 (-2.04, 0.54)	0.89	1.00	2203	2958
B ₈₀ :M _b	Snout emergence	0.03 (-1.27, 1.37)	0.51	1.00	2125	2992
T _{opt}	Body emergence	-0.03 (-0.60, 0.52)	0.54	1.00	2394	3357
M _b	Body emergence	0.16 (-0.41, 0.68)	0.76	1.00	3614	3853
P _{max}	Body emergence	-0.79 (-1.72, 0.18)	0.95	1.00	1682	3175
B ₈₀	Body emergence	-1.25 (-2.26, -0.23)	0.99	1.00	1367	2864
T _{opt} :M _b	Body emergence	-0.87 (-1.84, 0.15)	0.96	1.00	2416	2331
P _{max} :M _b	Body emergence	-0.77 (-1.96, 0.45)	0.91	1.00	2275	3374
B ₈₀ :M _b	Body emergence	0.02 (-1.2, 1.24)	0.51	1.00	2123	3059
T _{opt}	Time to optimal zone	-0.31 (-1.09, 0.47)	0.83	1.00	2908	3307
M _b	Time to optimal zone	0.10 (-0.64, 0.85)	0.62	1.00	2492	3620
P _{max}	Time to optimal zone	-0.08 (-1.35, 1.29)	0.56	1.00	1072	1076
B ₈₀	Time to optimal zone	-0.44 (-1.82, 1.12)	0.77	1.00	1289	995
T _{opt} :M _b	Time to optimal zone	-1.49 (-2.77, -0.15)	0.98	1.00	1709	1482
P _{max} :M _b	Time to optimal zone	-0.69 (-2.32, 1.07)	0.84	1.00	1780	1018
B ₈₀ :M _b	Time to optimal zone	-0.69 (-2.33, 0.96)	0.83	1.00	2174	2944
T _{opt}	Time to basking	-0.23 (-1.06, 0.59)	0.73	1.01	1273	3745
M _b	Time to basking	-0.07 (-0.86, 0.75)	0.57	1.00	1867	2561
P _{max}	Time to basking	-0.14 (-1.53, 1.28)	0.60	1.01	2189	3358
B ₈₀	Time to basking	-0.43 (-1.98, 1.15)	0.74	1.00	1829	2328
T _{opt} :M _b	Time to basking	-1.44 (-2.83, 0.05)	0.97	1.00	2004	2198
P _{max} :M _b	Time to basking	-0.61 (-2.35, 1.30)	0.80	1.00	1929	1035
B ₈₀ :M _b	Time to basking	-0.59 (-2.41, 1.25)	0.77	1.00	2448	3200

B₈₀ = performance breadth; ESS = effective samples sizes; LCI = lower confidence interval; M_b = body mass; P_{max} = maximum performance; \hat{R} = Gelman–Rubin diagnostics; T_{opt} = optimal temperature; UCI = upper confidence interval. Estimates are the median of the posterior distribution, and the 95% credible intervals are shown in parentheses. The probability of direction indicates the area of the posterior that is not overlapping zero. Effects estimated with confidence at or above 80% are shown in bold.

Table A3Coefficient estimates from Bayesian models showing the effect of resting metabolic rate (RMR) and body mass (M_b) on thermal traits

Predictor	Response	Estimate (LCI, UCI)	Prob. of direction	\hat{R}	Bulk ESS	Tail ESS
RMR	T_{opt}	0.57 (–0.10, 1.21)	0.96	1.00	2718	2739
M_b	T_{opt}	0.25 (–0.40, 0.90)	0.78	1.00	3242	2653
RMR	P_{max}	0.15 (–0.49, 0.80)	0.69	1.00	2485	2457
M_b	P_{max}	–0.44 (–1.11, 0.22)	0.92	1.00	2414	2457
RMR	B_{80}	–0.07 (–0.73, 0.56)	0.69	1.00	2094	2271
M_b	B_{80}	0.39 (–0.28, 1.08)	0.92	1.00	2087	2370

Abbreviations as in Table A2. Effects estimated with confidence at or above 80% are shown in bold.

Table A4Coefficient estimates from Bayesian models showing the effect of resting metabolic rate (RMR) and body mass (M_b) on behavioural traits at 35 °C

Predictor	Response	Estimate (LCI, UCI)	Prob. of direction	\hat{R}	Bulk ESS	Tail ESS
RMR	Activity	0.15 (–0.36, 0.66)	0.73	1.00	3214	2557
M_b	Activity	–0.42 (–0.92, 0.11)	0.95	1.00	3658	2705
RMR	Snout emergence	–0.17 (–0.64, 0.33)	0.77	1.00	3492	2546
M_b	Snout emergence	0.21 (–0.25, 0.68)	0.82	1.00	3176	2850
RMR	Body emergence	–0.12 (–0.59, 0.36)	0.70	1.00	2977	2415
M_b	Body emergence	0.20 (–0.26, 0.68)	0.81	1.00	2697	2896
RMR	Time to optimal zone	–0.15 (–0.64, 0.34)	0.75	1.00	2689	2596
M_b	Time to optimal zone	0.22 (–0.26, 0.70)	0.83	1.00	3006	2973
RMR	Time to basking	–0.03 (–0.53, 0.47)	0.56	1.00	2640	2619
M_b	Time to basking	0.09 (–0.40, 0.58)	0.65	1.00	2981	2836

Abbreviations as in Table A2. Effects estimated with confidence at or above 80% are shown in bold.

Table A5Coefficient estimates from Bayesian models showing the effect of resting metabolic rate (RMR) and body mass (M_b) on behavioural traits at 25 °C

Predictor	Response	Estimate (LCI, UCI)	Prob. of direction	\hat{R}	Bulk ESS	Tail ESS
RMR	Activity	–0.09 (–0.34, 0.16)	0.78	1.00	5873	4991
M_b	Activity	–0.17 (–0.42, 0.09)	0.91	1.00	6512	5721
RMR	Snout emergence	–0.03 (–0.56, 0.50)	0.54	1.00	4677	4786
M_b	Snout emergence	0.14 (–0.41, 0.72)	0.69	1.00	4267	4359
RMR	Body emergence	–0.17 (–0.71, 0.37)	0.73	1.00	4606	5046
M_b	Body emergence	0.27 (–0.29, 0.86)	0.83	1.00	4355	4566

Abbreviations as in Table A2. Effects estimated with confidence at or above 80% are shown in bold.

Table A6Coefficient estimates from Bayesian models showing the effect of thermal sensitivity of resting metabolic rate (RMR) and body mass (M_b) on thermal sensitivity of behavioural traits

Predictor	Response	Estimate (LCI, UCI)	Prob. of direction	\hat{R}	Bulk ESS	Tail ESS
RMR	Activity	0.09 (–0.49, 0.68)	0.63	1.00	6445	5234
M_b	Activity	–0.07 (–0.67, 0.52)	0.60	1.00	6701	5978
RMR	Snout emergence	–0.10 (–0.62, 0.45)	0.65	1.00	5079	5286
M_b	Snout emergence	0.13 (–0.41, 0.67)	0.70	1.00	4873	5072
RMR	Body emergence	–0.35 (–0.87, 0.19)	0.92	1.00	4739	5126
M_b	Body emergence	–0.03 (–0.55, 0.49)	0.55	1.00	4847	4716

Abbreviations as in Table A2. Effects estimated with confidence at or above 80% are shown in bold.

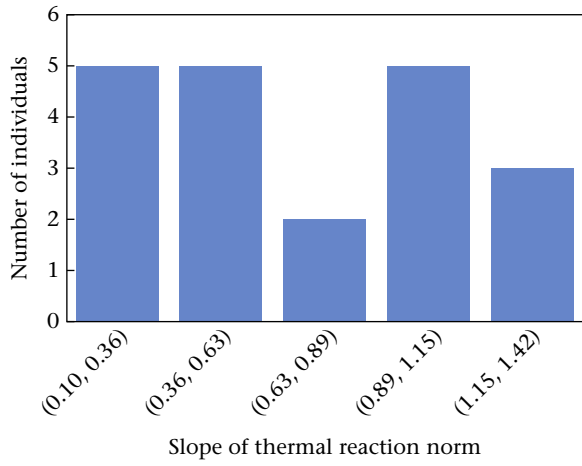


Figure A1. Bimodal distribution of slopes for activity thermal reaction norms.