RESEARCH ARTICLE

Evidence for locally adaptive metabolic rates among ant populations along an elevational gradient

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Abstract

1. As global temperatures rise, the mechanistic links between temperature, physiology and behaviour will increasingly define predictions of ecological change. However, for many taxa, we currently lack consensus about how thermal performance traits vary within and across populations, and whether and how locally adaptive trait plasticity can buffer warming effects.

2. The metabolic cold adaptation hypothesis posits that cold environments (e.g. high elevations and latitudes) select for high metabolic rates (MR), even after controlling for body size differences, and that this enables high activity levels when an organism is near its cold lower thermal limits. Steep MR reaction norms are further predicted at cold temperatures to enable rapid behavioural activation with rising temperatures needed to exploit brief thermal windows suitable for performing eco-evolutionary tasks.

3. We tested these predictions by performing common garden experiments comparing thermal reaction norms of MR (from 15 to 32°C) and behaviour (from 10 to 40°C) across populations of the ant *Aphaenogaster iberica* sampled from a 2 km elevation gradient in the Sierra Nevada Mountains of southern Spain.

4. As predicted, high-elevation ants had higher MR and steeper MR-temperature reaction norms. However, higher rates of energy use did not yield the predicted benefits of steeper activity-level reaction norms.

5. The evidence for locally adaptive metabolic physiology only became apparent at intermediate temperatures, highlighting the importance of testing thermal performance hypotheses across thermal gradients, rather than focusing only on performance at thermal limits (i.e. critical thermal values).

6. The partial support for the metabolic cold adaptation hypothesis highlights that while organisms likely show a wealth of unexplored metabolic temperature plasticity, the physiological mechanisms and eco-evolutionary trade-offs underlying such local adaptation remain obscure.

KEYWORDS
climate change, common garden experiment, metabolic cold adaptation hypothesis, phenotypic plasticity, thermal performance

1 | INTRODUCTION

Physiology mediates how organisms respond to their environment (Somero, 2010). As such, the distribution of species and biological diversity on our planet depends on physiological adaptations to abiotic factors (McGill, Enquist, Weiher, & Westoby, 2006). With rapid climate change, physiology will thus play an increasingly important role in the spatial reshuffling of species. And yet, accurate predictions about the future are often limited by our limited understanding of eco-physiological mechanisms and their interactions with evolutionary processes (Hoffmann & Sgrò, 2011). If overlooked, these complex links between physiological traits and the abiotic environment can reduce the accuracy of models predicting species responses to climate change (Mitchell et al., 2017).

A major challenge when linking thermal tolerance to the distribution of species is to select biologically meaningful and ecologically relevant thermal performance traits (Sunday et al., 2014). For example, measures of thermal tolerance (i.e. critical thermal maximum (CT_{max}) and minimum (CT_{min})) have been used to understand and predict species distributions among populations (Diamond, Chick, Perez, Strickler, & Martin, 2017), within diverse communities (Kaspari, Clay, Lucas, Yanoviak, & Kay, 2015) and across continents (Deutsch et al., 2008). However, although CT values can respond rapidly under selection (Huey, Crill, Kingsolver, & Weber, 1992), they also reflect acute physiological responses at extreme temperatures and may thus miss important physiological responses to the fluctuating thermal environments typically experienced by organisms (Sunday et al., 2014). Moreover, since CT values are typically defined as temperatures inducing knockdown (e.g. loss of righting response or onset of spasms; Lutterschmidt & Hutchinson, 1997), they depend strongly on subtle details of how they are measured (Lighton & Turner, 2004).

Thermal performance curves provide broader context to interpret CT values, as their shapes and heights highlight variation in dynamic physiological responses across temperature gradients (i.e. between CT endpoints; Kingsolver, 2009). By comparing thermal performance curves across populations in common garden experiments, thermal reaction norms can be generated and used to test hypotheses about phenotypic plasticity or the capacity of a single genotype to express phenotypic variation across a thermal gradient (Kawecki & Ebert, 2004; Sgrò, Terblanche, & Hoffmann, 2015). Tests for such plasticity are typically expressed as Q_{10} values that describe the rate of change in a physiological trait across 10°C temperature intervals (Chown & Nicolson, 2004; Lighton & Bartholomew, 1988). The Q_{10} approach for studying thermal performance is especially useful for studying the factors regulating distributions of insects, small poikilothermic ectotherms that are generally unable to regulate their internal body temperature (Angilletta, 2009).

In the present study, we used Q_{10} values of metabolic rate (MR) to test the metabolic cold adaptation hypothesis (MCAH), which has provided a useful framework for explaining physiological trait variation across thermal gradients (e.g. with increasing elevation

and latitude; Chown & Gaston, 1999). MR provides a powerful physiological trait, since it is mediated by temperature, governs ecological rates of resource demand, expenditure, and population growth (Brown, Gillooly, Allen, Savage, & West, 2004), and also closely tracks global thermal gradients among ectotherms (Dillon, Wang, & Huey, 2010; Irlich, Terblanche, Blackburn, & Chown, 2009; Nielsen, 1986). The MCAH has been supported across diverse insect taxa at biogeographic scales, with cold-habitat-adapted ectotherms tending to have higher MR and steeper MR Q_{10} curves (Seebacher, White, & Franklin, 2015; Terblanche, Clusella-Trullas, Deere, Vuuren, & Chown, 2009). This is thought to enable rapidly increasing activity levels as temperatures rise from lower limits of thermal performance curves so brief warm periods can be exploited to perform eco-evolutionary tasks (e.g. foraging, mating; Addo-Bediako, Chown, & Gaston, 2002). To date, however, fewer studies have tested MCAH predictions among populations of single ectotherm species arrayed across thermal gradients (but see Buckley, Nufio, & Kingsolver, 2014, Schaefer & Walters, 2010).

In ants, locally adaptive physiological temperature sensitivities likely have outsized ecological impacts since these colonial insects typically rank among the most numerically abundant and behaviourally dominant ectothermic consumers where they occur (Del Toro, Ribbons, & Pelini, 2012). However, studies comparing thermal performance of ants across environmental gradients have typically relied on CT measurements (e.g. Bishop, Robertson, Rensburg, & Parr, 2017; Chick, Perez, & Diamond, 2017) and we thus lack consensus about how metabolic temperature sensitivity shapes species distributions. For instance, while Kennington (1957) found evidence of steeper MR Q_{10} values in a high-elevation population of *Camponotus* ants, this study tested only two temperatures across the ant's thermal performance curve and could not determine whether Q_{10} values were best fit by linear (constant change across thermal intervals) or polynomial curves (accelerating or decelerating changes across thermal intervals). Additionally, MacKay (1982) detected steeper MR Q_{10} values at higher elevations in *Pogonomyrmex* ants, although this study examined different species at each elevation and could thus not test for locally adaptive plasticity. Studies are thus needed that provide sufficient resolution across experimental temperatures and populations to test predictions about locally adaptive plasticity in thermal performance.

We tested the MCAH using the ant *Aphaenogaster iberica* collected from populations spanning a 2 km elevation gradient in southern Spain. Mountains provide laboratories to test MCAH predictions because narrow elevation bands expose populations to relatively stable thermal regimes that become colder moving upslope (Janzen, 1967; Polato et al., 2018; Sheldon, Huey, Kaspari, & Sanders, 2018). We used a variety of approaches (phenotypic, metabolic, and behavioural) to describe trait values within and across *A. iberica* populations, testing first whether high-elevation ant populations have higher MR and higher activity levels, regardless of their body size. We then compared MR and behavioural Q_{10} curves across populations to test the MCAH prediction that steeper MR Q_{10} curves in
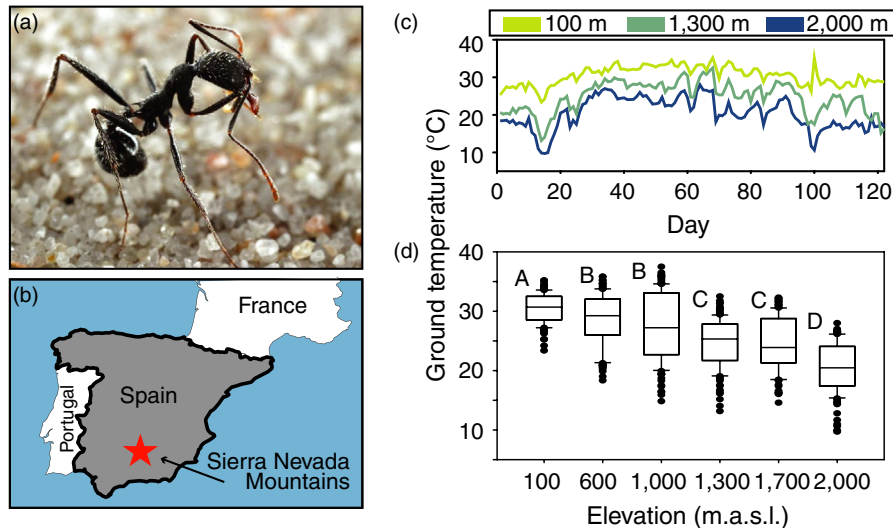
FIGURE 1 (a, b) The ant *Aphaenogaster iberica* was studied along a 2,000 m elevation gradient in the Sierra Nevada Mountains of southern Spain. Both (c) daily and (d) seasonal ground temperatures decreased systematically with increasing elevation. Ground temperatures were measured over 122 days (from June 1 (Day 1) to September 30 (Day 122) in 2015) at six sites (100, 600, 1,000, 1,300, 1,700, 2,000 m) representing a 2 km elevation gradient. In panel c, only the three elevations used for MR trials (100, 1,300, 2,000 m) are presented for clarity. In panel d, each box plot presents the median temperature value (central bar), the first (bottom line of box) and third percentiles (top line of box), and the black data points represent temperature values outside the 5th (bottom error bar) and 95th (top error bar) percentiles. Temperatures decreased systematically with increasing elevation ($F_{5,726} = 82.04$; $p = 0.0001$), and letters next to boxes indicate significant differences ($p < 0.05$) in a post hoc Tukey test exploring this significant result. The unit m.a.s.l. indicates metres above sea level

high-elevation populations correspond to steeper behavioural Q_{10} curves.

2 | MATERIALS AND METHODS

2.1 | Study populations

The ant *A. iberica* (Figure 1a) inhabits arid regions of the Iberian Peninsula of southeast Spain (Figure 1b) where it is a generalist scavenger of seeds and dead insects used to provision monogynous colonies of ≤1,000 workers. We collected *A. iberica* colonies from populations (*hereafter* "elevations") along a 2 km transect on the Southern slope of the Sierra Nevada Mountains (N 36.9162, W 3.4782). The *A. iberica* populations in this study are likely to have low gene flow, since young queens are wingless and disperse from natal colonies on tarsus during colony fission events. For phenotypic and metabolic studies, we sampled ants at 100, 1,300 and 2,000 metres above sea level (m.a.s.l., hereafter m), collecting queenright colonies with abundant brood (one colony in September 2015 and 11 colonies in July 2016) and maintaining them in laboratory conditions until metabolic measurements were performed from 20 to 31 March 2017. For behavioural experiments, we collected 46 additional colonies, including at three additional elevations (600, 1,000 and 1,700 m) in September and October 2015, with adjacent sites across the entire 2 km elevational gradient separated by distances ranging from 1.5 to 30 km. In the laboratory, all collected colonies were housed in rooms at 25°C inside 20 × 15 × 12 cm fluon-coated plastic boxes. The ants nested in 20 × 2 cm test tubes half-filled with

water retained by a cotton plug and were fed three times a week with dead *Tenebrio molitor* worms and pieces of fruit.

We also quantified elevational differences in the thermal environments inhabited by the ants by recording ground surface temperature with two CEM DT171 data loggers. These were placed under small caps on the ground in each study area to prevent overheating while recording temperature three times per hour from June 1 to 30 September 2015. This 122-day sampling period captured the months of peak annual foraging activity when workers would be exposed to environmental temperatures outside the nest.

2.2 | Temperature effects on MR

We estimated the metabolic rates (hereafter, MR) of individual workers across a 17°C range of five ambient temperatures (15, 20, 25, 30, 32°C), performing constant volume respirometry using Sable Systems equipment (Las Vegas, Nevada), and recording MR ($\mu l\ CO_2\ hr^{-1}$) averaged over five hourly measurements following a 1-hr acclimation period that was always excluded from analyses. We manipulated ambient air temperature by placing respirometry chambers (10-ml syringe barrels fitted with rubber stoppers), each with a single ant and a small cotton ball with 200 μl of deionized water, in a dark climate cabinet (Refritherm 200). For each six-hour MR trial, six respirometry chambers were connected to an RM8 multiplexer and a seventh chamber remained empty. Hourly CO_2 values from the empty chamber were subtracted from all experimental chambers to correct for possible system leaks. Respirometry chambers were cleaned with 95% EtOH between trials.

Trials were run using an established plumbing set-up (Shik, 2010). Incurrent air was first scrubbed of H_2O and CO_2 using a drierite/ascarite/drierite column, with a flow rate of 50 ml/min set by a SS-3 subsampler pump that was regulated by a 200 ml/min Sierra valve connected to an MFC unit. This air was further scrubbed of CO_2 using a second drierite/ascarite/drierite column and then sent to the multiplexer, programmed to switch between chambers using SSI Expedata software. Tubing connected incurrent and excurrent ports at the eighth position on the multiplexer, enabling baselining with CO_2 scrubbed air between each hourly ant MR measurement trial. During all trials, ambient temperatures were continuously recorded within the thermal cabinet using a thermistor cable. Equipment was interfaced with a computer using a SSI UI-2 device. At hourly intervals, air was flushed out from each respirometry chamber for 200 s at 50 ml/min, passed through a 10-cc column of magnesium perchlorate (Cl_2MgO_8) to remove any remaining moisture and then to a CO_2 analyser (CA-10, accuracy of 1%, resolution of 0.00001%). We calibrated the CO_2 analyser before each trial, zeroing it with N_2 gas and then spanning it with a gas of known CO_2 concentration (1,000 p.p.m. CO_2 in N_2 ± 1%).

Most CO_2 readings stabilized over 5-hr trials (as per Lighton, 2008) representing 780 total hourly measurements (26 trials × 6 ants per trial × 5 hr per ant). Two trials were removed before analyses: one due to unstable cabinet temperature and one due to incorrect CO_2 baselining, yielding 24 total trials (144 ants). Of the 780 hourly measurements, 17 were slightly negative (i.e. lower than the empty chamber control) and were converted to zero when trial means were calculated for each ant. Measurements for 10 additional ants were excluded from analyses (six died during trials, one had a disconnected air tube, two exhibited signs of thermal stress (i.e. extreme MR outliers) and one was a queen). We thus present MR data for 134 workers (100 m: *n* = 49 ants from four colonies, 1,300 m: *n* = 44 ants from four colonies and 2,000 m: 41 ants from four colonies).

Ants were weighed just prior to MR trials (wet mass), after which they were immediately frozen, dried at 60°C for 24 hr and then weighed to the nearest 10^{-3} mg (dry mass). We used SSI ExpeData software to subtract the empty chamber CO_2 from each experimental measurement, correct for small variations in flow rate (±0.1 ml/min), transform CO_2 measurements from p.p.m. to µl/hr and integrate these values for trial intervals (see Table S1 for ExpeData macro).

2.3 | Temperature effects on behaviour

We examined temperature effects on worker activity levels using a Drosophila Activity Monitoring (DAM) system (Pfeiffenberger, Lear, Keegan, & Allada, 2010), consisting of 5 × 65 mm Pyrex glass tubes (TriKinetics Inc.) enabling simultaneous measurements of individual insect movements over time using a photoelectric cell affixed to each tube. We measured cumulative activity levels for each ant within its tube (i.e. total beam crossing events) within trials that lasted two hours at 10, 20, 30 or 40°C. Ants were measured from six elevations: 100 m (*n* = 256 ants from eight colonies), 600 m (*n* = 160 ants from five colonies), 1,000 m (*n* = 254 ants from eight colonies),

1,300 m (*n* = 288 ants from nine colonies), 1,700 m (*n* = 256 ants from eight colonies) and 2,000 m (*n* = 256 ants from nine colonies). We computed activity means for workers from each colony at each temperature in subsequent analyses.

2.4 | Comparing trait values across elevations

We used the lme function in the nlme package (Pinheiro, Bates, DebRoy, & Sarkar, 2018) in R 3.2.4 (R Development Core Team, 2016) to compare worker wet and dry mass across elevations (100, 1,300, 2,000 m) using mixed model analyses, where colony ID was a random factor. We similarly used a mixed model with colony ID as a random factor to compare mass-specific MR ($Log_{10}(\mu l\ CO_2\ mg^{-1}\ hr^{-1})$) measurements across elevations for each temperature treatment (i.e. this model included elevation, temperature treatment and their interaction as explanatory variables). For all mixed model analyses, we used likelihood ratio tests with chi-square test statistics to examine whether explanatory variables had significant effects on response variables, comparing the model with and without the fixed factors using the R package *car* (Fox & Weisberg, 2011).

2.5 | Comparing Q_{10} curves across elevations

We calculated separate linear and quadratic Q_{10} mixed models for each elevation, regressing the response variables of mass-specific MR ($log_{10}(\mu l\ CO_2\ mg^{-1}\ hr^{-1})$, *as per* Lighton, 2008) or behaviour (log_{10}(activity + 1)) against the explanatory variable of temperature (°C). In both regression approaches, the range of predicted MR Q_{10} values was measured from 15 to 32°C, and the range of predicted activity Q_{10} values was measured from 10 to 40°C. Mass-specific MR is typically used when calculating Q_{10} values Lighton (2008) since body size can strongly influence MR, which is an especially important consideration when testing MCAH predictions since body size may vary across elevations (Chown & Nicolson, 2004).

We used mixed model analyses to test for differences across elevations in the intercepts and slopes of linear and quadratic Q_{10} regressions, including colony ID as a random factor, and compared linear and quadratic models (separately for MR and behaviour datasets) with AIC values. While we performed model fitting by restricted maximum likelihood, models were refitted by maximum likelihood for model comparison purposes (Luke, 2017; Zuur, Ieno, Walker, Saveliev, & Smith, 2009). For linear models, we used the equation ($10^{(slope \times 10)}$) to calculate a MR Q_{10} value or activity Q_{10} value for each elevation (as per Lighton, 2008). For quadratic models, we generated predictive functions of the instantaneous Q_{10} values for MR and behaviour across the range of measured temperatures using the first derivative of their respective quadratic Q_{10} equations (*as per* Lighton & Bartholomew, 1988). As we develop in the results, model comparison using AIC values indicated that quadratic functions for both MR and activity levels performed better than linear functions, and we thus use quadratic functions to assess Q_{10} and test MCAH predictions. This is consistent with physiological assumptions, since there is no a priori physiological reason to expect the temperature

sensitivity of MR (or behavioural activity) to be constant across a range of biologically meaningful temperatures (Lighton, 2008). However, we also provide Q_{10} values and supporting statistics based on linear functions for both MR and behavioural data to facilitate comparison with other studies.

3 | RESULTS

Ants from higher elevations were consistently exposed to lower temperatures upon leaving their nests over the foraging season, both on a daily basis (Figure 1c) and averaged across sampling days (Figure 1d). Higher elevation populations also had larger *A. iberica* ants ($\chi^2_2 = 8.16$, $p = 0.017$), with workers from both 1,300 and 2,000 m being larger than those from 100 m (Figure 2; Table S2). These high-elevation ants (from 2,000 m) also tended to have higher mass-specific MR than ants from 100 m ($\chi^2_2 = 12.45$, $p = 0.002$, Figure S1a). Additionally, even as mass-specific MR generally increased with temperature across all ants ($\chi^2_4 = 395.22$, $p < 0.0001$; Figure S1b), we detected interactive differences among elevations driven by higher mass-specific MR values at higher elevations (1,300 and 2,000 m) at 20 °C ($\chi^2_8 = 23.38$, $p = 0.003$; Figure S1c).

Across all the MR data, global AIC values indicated that the quadratic model is better at predicting new data than the linear model (Table 1). This pattern was consistent for both high elevations tested (1,300 and 2,000 m), although linear and quadratic regressions were similarly good for low-elevation (100 m) ants (i.e. AIC values were within 2 AIC units; Table 2). Quadratic functions showed a significant interaction between elevation and the quadratic temperature variable (Table 1) that was driven by a shift from a concave regression among 100 m ants to convex regressions for the two higher

FIGURE 2 The body size of *Aphaenogaster iberica* workers increased with elevation, as ants in the two higher elevation populations (1,300 m and 2,000 m) were significantly larger (dry mass, mg) than the 100 m ants. Different letters indicate significantly different groupings ($p < 0.05$) based on a post hoc Tukey test. Analysis of worker wet mass (Table 1) yielded the same statistical results ($\chi^2_2 = 7.90$, $p = 0.019$) and post hoc elevation differences. Box plot statistical details are as described in Figure 1

elevation populations (Figure 3a). Instantaneous MR Q_{10} values, computed from derivatives of the quadratic MR-temperature curves for each elevation, highlighted a broad range of possible MR Q_{10} values observed across a realistic thermal gradient (Figure 3b) and supported the MCAH prediction that high-elevation ants have higher MR Q_{10} values at cold temperatures.

As with the MR data, the quadratic analysis of all the activity data had a lower global AIC value than the linear analysis (Table 1), and selection of the quadratic model was further supported when each elevation was analysed separately (Table 2). Quadratic functions indicated that activity levels increased with increasing temperatures (Table 2), but did not differ among elevations (Table 1). Moreover, the slopes of the quadratic regressions of activity levels against temperature did not vary across elevations (Table 1), and we thus plotted a single quadratic regression line through the data (Figure 4a). The instantaneous Q_{10} values predicted from the quadratic regression indicated that while activity rates generally accelerated faster as temperatures increased, these dynamics were not systematically more rapid at higher elevations as predicted by the MCAH (Figure 4b).

4 | DISCUSSION

The potential for locally adaptive MR-temperature plasticity can strongly impact evolutionary responses to climate change (Kawecki & Ebert, 2004), placing importance on defining MR Q_{10} curves using common garden experiments (Hoffmann & Sgrò, 2011). By performing such experiments, we provide the first evidence of locally adaptive steeper metabolic rate (MR)-temperature reaction norms among ants inhabiting high elevations, highlighting that MR-temperature plasticity should be integrated into models forecasting the structure of future ectotherm communities. However, we also note that these population-level differences in energy use did not translate into steeper activity-level reaction norms, as predicted by the metabolic cold adaptation hypothesis (MCAH). Thus, even as high-elevation populations expressed the physiological traits assumed to reflect cold-habitat adaptations, we were unable to detect the behavioural benefits predicted to accompany this physiological trait variation. This partial support for the MCAH implies opportunities to explore the eco-physiological consequences of locally adaptive metabolic temperature sensitivity, filling in the gaps between whole-organism CO_2 respiration measurements and the complex underlying metabolic processes powering behavioural dynamics.

The gap between the temperature sensitivity of MR and activity levels also raises intriguing questions about the extent to which colony life may relax selection on worker-level metabolic traits related to climate variability. On the one hand, thermal performance traits of workers shape ant ecology and distributions at local (Cerdá, Retana, & Cros, 1998; Kaspari et al., 2015; Talbot, 1934) and biogeographic scales (Arnan & Blüthgen, 2015; Arnan, Blüthgen, Molowny-Horas, & Retana, 2015; Diamond et al., 2012). However, colony-level performance is also governed by a capacity
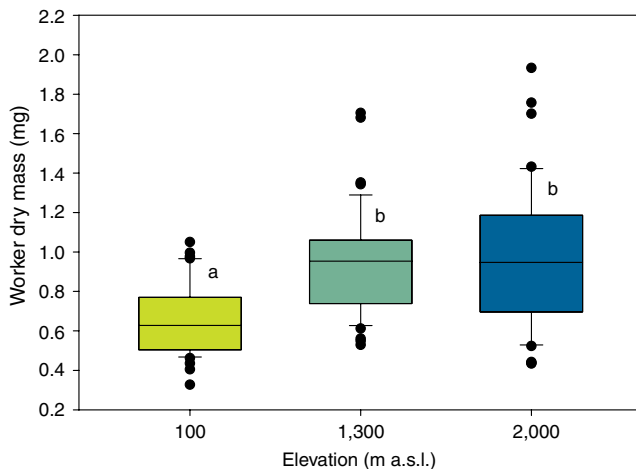
TABLE 1 Mixed model analyses of the interactive effects of temperature and elevation on the MR (mass-specific MR), and behaviour (activity level) of *Aphaenogaster iberica* ants. *Metabolic rate (MR)*: We analysed MR-temperature reaction norms (Q_{10} curves), performing linear and quadratic regression mixed models, where the response variable $\log_{10}$(mass-specific MR) was regressed against the explanatory variable temperature, and the intercepts and slopes were compared across elevations (100, 1,300, 2,000 m), with colony ID included as a random factor. *Behaviour:* We used the same statistical approach to regress the response variable $\log_{10}$ (activity + 1) against the explanatory variable temperature and compare the intercepts and slopes across elevations (100, 600, 1,000, 1,300, 1,700, 2,000 m)

Trait	Test	Factor	df	χ^2	p Value	Global AIC
MR	Linear Q_{10}	Elevation	2	4.83	0.089	−22.72
		Temperature	1	320.04	<0.0001	
		Elevation × Temperature	2	10.21	0.006	
	Quadratic Q_{10}	Elevation	2	8.88	0.012	−32.56
		Temperature	1	21.12	<0.0001	
		Elevation × Temperature	2	6.83	0.033	
		Temperature^2	1	7.47	0.006	
		Elevation × Temperature^2	2	7.69	0.021	
Behaviour	Linear Q_{10}	Elevation	5	10.77	0.056	−136.36
		Temperature	1	382.13	0.0001	
		Elevation × Temperature	5	8.01	0.156	
	Quadratic Q_{10}	Elevation	5	10.77	0.056	−200.40
		Temperature	1	23.85	0.0001	
		Elevation × Temperature	5	4.43	0.490	
		Temperature^2	1	89.07	0.0001	
		Elevation × Temperature^2	5	3.15	0.677	

TABLE 2 Linear and quadratic regressions quantifying temperature sensitivity of MR and behaviour (Q_{10} curves), based on relationships between temperature (MR trials: 15–32°C; behavioural trials: 10–40°C) and either $\log_{10}$(mass-specific MR) or $\log_{10}$(activity level + 1). *N* denotes number of workers in MR analyses and the number of colonies in behavioural analyses. Linear regression coefficients indicate the intercept (a) and the slope (b), with Q_{10} calculated as $10^{(b \times 10)}$. Quadratic regression coefficients include a, b, and b × b

|	Elevation	N	AIC	Adjusted R^2	a	SE of a	b	SE of b	bb	SE of bb	Q_{10}
Linear regression coefficients											
MR	100	49	1.3	0.69	−1.67	0.14	0.064	0.006	—	—	4.37
	1,300	44	−10.9	0.77	−1.46	0.13	0.058	0.005	—	—	3.80
	2,000	41	−9.9	0.71	−0.99	0.13	0.041	0.005	—	—	2.57
Behaviour	100	8	−25.38	0.72	1.45	0.06	0.023	0.002	—	—	1.70
	600	5	4.62	0.43	1.40	0.13	0.023	0.005	—	—	1.69
	1,000	8	−25.90	0.51	1.61	0.06	0.017	0.002	—	—	1.47
	1,300	9	−23.78	0.42	1.67	0.07	0.016	0.002	—	—	1.45
	1,700	8	−23.05	0.63	1.47	0.07	0.021	0.002	—	—	1.61
	2,000	9	−31.95	0.67	1.54	0.06	0.022	0.002	—	—	1.67
Quadratic regression coefficients											
MR	100	49	2.7	0.68	−1.18	0.66	0.020	0.059	0.001	0.001	—
	1,300	44	−21.0	0.81	−3.23	0.51	0.221	0.046	−0.003	0.001	—
	2,000	41	−13.6	0.72	−2.10	0.49	0.142	0.043	−0.002	0.001	—
Behaviour	100	8	−31.89	0.79	1.77	0.14	−0.009	0.012	0.001	0.000	—
	600	5	1.19	0.55	1.93	0.26	−0.030	0.024	0.001	0.000	—
	1,000	8	−60.54	0.88	2.14	0.10	−0.037	0.009	0.001	0.000	—
	1,300	9	−33.79	0.60	2.07	0.13	−0.024	0.012	0.001	0.000	—
	1,700	8	−37.15	0.79	1.90	0.13	−0.023	0.011	0.001	0.000	—
	2,000	9	−41.40	0.77	1.90	0.12	−0.013	0.011	0.001	0.000	—
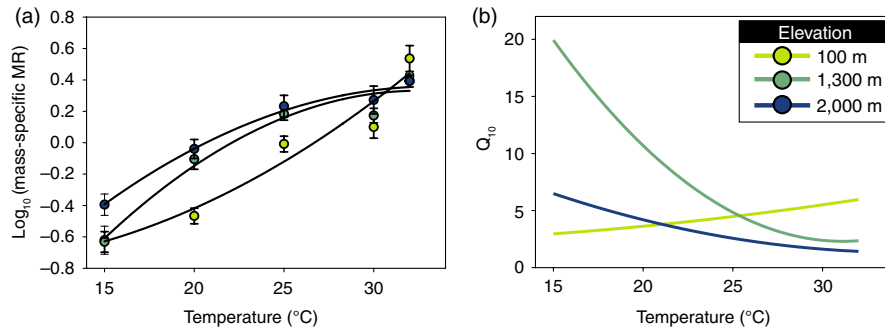
FIGURE 3 Evidence for locally adaptive variation in MR-temperature reaction norms (Q_{10}) supporting the metabolic cold adaptation hypothesis. (a) High-elevation ants (1,300 and 2,000 m) increased their MR ($\log_{10}(CO_2\ \mu l^{-1}\ mg^{-1}\ hr^{-1})$) faster at lower temperatures, with significantly higher MR at 20°C relative to low-elevation (100 m) ants (see Figure S1c for Tukey test comparison). Means are presented ± vertical *SE* bars for MR data, but horizontal *SE* bars are not provided for means of temperature data, as the bars were too narrow to be visualized under data point symbols. (b) Instantaneous Q_{10} values based on derivatives of quadratic MR-temperature curves show higher Q_{10} values among high-elevation ants at low temperatures, and the opposite relationship among low-elevation ants

for thermoregulation (Baudier & O'Donnell, 2016) since colonies can use nest architecture to thermally manipulate larval development rates (Penick & Tschinkel, 2008) and shift colony growth rates (Penick, Diamond, Sanders, & Dunn, 2017). The buffering effects of colony-level thermoregulation may be especially important to understanding our results, since high-elevation habitats can have high diurnal variance in air temperatures that may select against high MR Q_{10} values in ectotherms according to Jensen's inequality (Ruel & Ayres, 1999). It will thus be important to explore how MR Q_{10} curves and activity Q_{10} curves vary across ant species whose colonies span gradients of colony size and capacity for nest thermoregulation. Additionally, since ant colonies may shunt major fractions of assimilated energy (> 80%) towards metabolic maintenance costs (MacKay, 1985), such variation in the temperature sensitivity of metabolic resource requirements and CO_2 emissions could have important ecosystem impacts under climate warming (Dillon et al., 2010).

The finding of larger ants in higher elevation populations adds to a somewhat complicated literature showing a range of insect body size trends along elevation gradients (Chown & Nicolson, 2004). While further study will thus be needed to understand

the mechanisms governing *A. iberica* body size variation, the following hypotheses provide a reasonable starting point. First, cold temperatures during development may simply increase body size by shifting the energetic balance of juveniles from anabolism to catabolism (Atkinson, 1994). Second, cold and low-productivity habitats may favour larger insects through life-history trade-offs where longer life spans (Calabi & Porter, 1989; Peters, 1983) enable individuals to capitalize on greater numbers of shorter growing seasons, at the cost of longer developmental timelines and higher demands for parental resource allocation (Blanckenhorn, 2000; Stearns, 1992). Third, maintenance energy requirements may provide a useful lens to view such body size trade-offs, since larger insects also generally have lower mass-specific MR, as these requirements scale allometrically with insect body mass (i.e. MR $\sim Mass^{0.80}$; Chown et al., 2007; Shik, Hou, Kay, Kaspari, & Gillooly, 2012). With this in mind, larger ants in cold, low-productivity habitats would benefit from low energy costs needed to support each milligram of body tissue (Shik, 2010). However, such MR maintenance cost savings are not supported in the present study, since larger high-elevation ants from 2,000 m actually had higher mass-specific MR than those from 100 m. Instead, this result supports
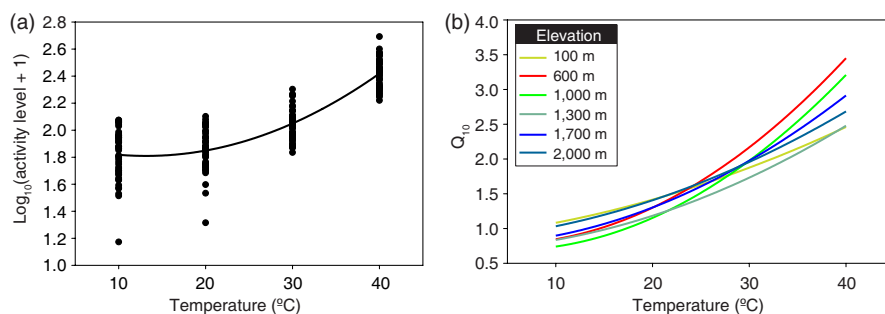

FIGURE 4 Behavioural Q_{10} curves increased with temperature in statistically similar ways across elevations. (a) We plotted a single quadratic function through the activity data ($\log_{10}$(activity level + 1)), while also providing quadratic (and linear) regression coefficients and statistics from each elevation in Table 2. (b) Instantaneous Q_{10} values calculated from quadratic Q_{10} equations showed similar accelerating activity levels with rising temperature across all elevations.

the MCAH prediction that elevated MR rates allow cold-habitat ectotherms to meet higher energetic costs of growth and development (Chown & Nicolson, 2004).

Many macroecological models assume linear MR Q_{10} values (i.e. constant rate changes across thermal gradients; Seebacher et al., 2015; Irlich et al., 2009), although the nonlinearity of temperature-performance dynamics has long been appreciated (reviewed by Angilletta, 2009). While this approach is understandable, given the limited number of empirically measured Q_{10} curves, quadratic Q_{10} values appear ubiquitous across ectotherms and can greatly intensify physiological responses to extreme temperatures (e.g. Q_{10} values range from <2 to 7 in Collembola; Hodkinson, 2003; and Figure 3b in the present study). In ants, empirically derived MR Q_{10} values exist for only ca. 30 of the planet's >14,000 extant species, with widely varying results. A linear $Q_{10} = 2$ (i.e. consistent doubling of MR with each 10°C increase in temperature) is typically assumed (Lighton, 2008), but published Q_{10} values for ants actually range from 1.73 (*Linepithema humile*; Schilman, Lighton, & Holway, 2005) to 3.92 (*Tetramorium* cf. *caespitum*; Jensen & Nielsen, 1975). The shapes of Q_{10} curves are also unclear, as most studies have measured MR at ≤3 temperatures, limiting tests of constant or variable MR-temperature sensitivity across thermal gradients. In fact, published Q_{10} functions range from linear (Lighton, 1989; Schilman et al., 2005), to quadratic (Duncan & Crewe, 1993; Hebling-Beraldo & Mendes, 1982; Lighton & Bartholomew, 1988), to cubic (Vogt & Appel, 1999). Care should thus be taken when performing meta-analyses of ectotherm MR that include respirometry data collected at different temperatures, since MR-temperature sensitivity varies not only across thermal intervals and across species, but as we show here can also vary intraspecifically across environmental gradients.

Our results combined with existing interspecific Q_{10} variation implies that the potential for thermal MR adaptation in insects has been underestimated. More generally, these data also highlight the importance of measuring performance across thermal gradients (i.e. Q_{10} values) when studying cold adaptation in animals, rather than focusing only on extreme temperatures (i.e. CT values), since elevation differences in MR only became apparent at intermediate temperatures (i.e. significant differences in MR across elevations at 20°C, but not at 15°C or 32°C). Measurements of thermal performance across thermal gradients will thus be critical to reach the ultimate goal of quantifying variation in the strength of thermal selective pressures within populations over time and the fitness costs of and constraints on plasticity (Sgrò et al., 2015).

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AUTHORS' CONTRIBUTIONS

DATA ACCESSIBILITY

Data used in this manuscript are available at Dryad Digital Repository: https://doi.org/10.5061/dryad.3hv2826 (Shik, Arnan, Oms, Cerdá, & Boulay, 2019).

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