

Is phenotypic plasticity a key mechanism for responding to thermal stress in ants?

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Abstract Unlike natural selection, phenotypic plasticity allows organisms to respond quickly to changing environmental conditions. However, plasticity may not always be adaptive. In insects, body size and other morphological measurements have been shown to decrease as temperature increases. This relationship may lead to a physiological conflict in ants, where larger body size and longer legs often confer better thermal resistance. Here, we tested the effect of developmental temperature (20, 24, 28 or 32 °C) on adult thermal resistance in the thermophilic ant species *Aphaenogaster senilis*. We found that no larval development occurred at 20 °C. However, at higher temperatures, developmental speed increased as expected and smaller adults were produced. In thermal resistance tests, we found that ants reared at 28 and 32 °C had half-lethal temperatures that were 2 °C higher than those of ants reared at 24 °C. Thus, although ants reared at higher temperatures were smaller in size, they were nonetheless more thermoresistant. These results show that *A. senilis* can exploit phenotypic plasticity to quickly adjust its thermal resistance to local conditions and that this process is independent of morphological adaptations. This mechanism may be particularly relevant given current rapid climate warming.

Keywords Phenotypic plasticity · Ants · *Aphaenogaster senilis* · Rearing temperature · Thermal resistance · Body size

Introduction

Predicting species' responses to global warming is one of the major challenges in ecology and evolutionary biology. Under climate change, species are expected to either modify their distributions (Parmesan et al. 1999), acclimate (Somero 2010) or adapt to new environmental conditions through natural selection (Hoffmann and Sgrò 2011). If species cannot adopt one of these solutions, they are likely to rapidly go extinct. There are numerous examples in the literature of natural populations responding to global warming with phenotypic changes, but debate exists over whether such changes are caused by natural selection or phenotypic plasticity (Merilä and Hendry 2014). Unlike natural selection, which acts over long time spans, phenotypic plasticity allows an organism to quickly respond to environmental conditions without relying on genotypic modifications. Phenotypic plasticity may therefore be particularly relevant in the context of rapid global warming (Chown et al. 2007; Berg et al. 2010). However, its utilisation by non-model organisms is largely unknown and probably underestimated.

Ants abound in almost all terrestrial ecosystems, where they serve important ecological functions. As ectotherms, their physiological performance is directly dependent on environmental temperatures, and they may be among the species with the greatest sensitivity to global warming. Temperature strongly affects many features of ant behaviour, ontogeny and ecology. For example, when faced with extreme temperatures, ants alter daily and seasonal foraging activity as well as prey choice (Traniello et al. 1984; Marsh 1985; Fernández-Escudero and Tinaut 1998; Amor et al. 2011; Jayatilaka

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et al. 2011). Moreover, in hot habitats, there is a shift towards solitary foraging since high temperatures limit the efficacy of trail pheromones (van Oudenhove et al. 2011, 2012). Temperature is also a major structuring factor at the community level (Andersen 1995; Bestelmeyer 2000; Retana and Cerdá 2000; Wittman et al. 2010). In particular, in Mediterranean regions, behaviourally dominant species are often heat intolerant and risk averse, while behaviourally subordinate species tend to be heat tolerant and risk prone (Cerdá et al. 1998). Thus, species-specific differences in thermotolerance allow local coexistence via temporal niche partitioning: dominant species are active at dawn and dusk, whereas subordinate species are active around midday. In turn, this pattern has major consequences for ant interactions with other taxa, including plants (Boulay et al. 2007a, 2009a).

Thermophilic ants have numerous adaptations that allow them to cope with elevated temperatures (Boulay et al. 2017), including the enhanced synthesis of heat-shock proteins (Gehring and Wehner 1995; Ślipiński et al. 2015), low metabolic rates and limited cuticular transpiration (Gibbs and Pomonis 1995; Cerdá and Retana 2000). In addition, increased body size may greatly improve heat resistance (Cerdá and Retana 1997; Clémencet et al. 2010). First, since larger insects tend to have lower surface area-to-volume ratios, larger workers have lower desiccation rates than do small workers (Lighton and Feener 1989; Hood and Tschinkel 1990; Kaspari 1993). Second, Lighton et al. (1994) reported that larger ants can store more water. Third, body size is positively associated with maximum foraging time (Lighton and Feener 1989) and foraging temperature (Rissing and Pollok 1984). Long-legged ants can distance their bodies from hot surfaces and can run faster, which enhances convective cooling (Hurlbert et al. 2008; Sommer and Wehner 2012).

In this study, we analysed how the temperatures experienced by larvae and pupae affected thermal resistance in adults in the thermophilic species *Aphaenogaster senilis* (Hymenoptera: Formicidae). To date, this question has received little attention in ants. In *Camponotus rufipes*, Weidenmüller et al. (2009) showed that pre-imago exposed to warmer temperatures had higher temperature response thresholds and temperature preferences as adult brood-tending workers. However, insect developmental time is known to be shorter when temperatures are higher and the result is smaller adults (Atkinson 1994; Forster and Hirst 2012). In ants, morphological traits present a high degree of phenotypic plasticity (Purcell et al. 2016) and high temperatures have also been reported to reduce developmental time (Brian 1973; Porter 1988; Southerland 1988; Abril et al. 2010). This situation could lead to a physiological conflict, in which warmer developmental temperatures actually reduce adult thermal resistance by decreasing the average adult body size or by increasing its variance. To test this hypothesis, we examined how temperature affected developmental speed in

larvae and then measured the size and thermal resistance of the resulting adults.

Materials and methods

Model system

Aphaenogaster senilis is a thermophilic ant found in the western Mediterranean basin (Cagniant and Ledoux 1974). It is strictly monogynous (Boulay et al. 2007b), and its nests contain an average of 1260 ± 69 monomorphic workers (Boulay et al. 2010). Fifty-seven colonies were collected in Doñana National Park ($37^{\circ} 1' N$, $6^{\circ} 33' W$, Huelva Province, SW Spain) from November 2009 to March 2014. They were all maintained in the same rearing room at $23 \pm 2^{\circ} C$, given ad libitum access to water and fed twice a week with sliced mealworms (*Tenebrio molitor*).

Experimental design

A total of 72 experimental groups were created; each contained 150 workers and 20 first-instar larvae. Forty-two of the field-collected colonies were used to create one experimental group each. The other 15 colonies were large enough to create two experimental groups each. The experimental groups were kept in artificial nests in glass tubes (diameter \times length 2×20 cm) half-filled with water behind a cotton plug. The nests were connected to foraging arenas (length \times width \times height $18 \times 11.5 \times 7.5$ cm), whose walls were covered in Fluon to prevent the ants from escaping. The experimental groups were kept in environmental chambers at 20, 24, 28 or $32^{\circ} C$ (18 experimental groups per condition). They were maintained in complete darkness and fed a diced mealworm twice weekly. Experimental groups created from the same colonies were assigned to different treatments.

Data measurements

The number of larvae that reached the pupal stage and the number of pupae of each caste (worker, male or queen) were recorded every 3 days for 103 days. A picture of each pupa was taken, and its body length was measured to the nearest 0.005 mm using ImageJ software (Schneider et al. 2012). New pupae were transferred into round plastic containers (diameter \times height 8.5×4 cm); they were cared for by ten workers, which were marked with a dot of paint on their gasters to distinguish them from newly emerged workers. The boxes were connected to a glass tube (diameter \times length 2×20 cm) half-filled with water behind a cotton plug. The number of pupal eclosions and the survival of the callows over their first 2 weeks of life were recorded. Pupae and callows

were kept at the same treatment temperature as larvae (20, 24, 28 or 32 °C).

The thermal resistance of the callows was estimated using half-lethal temperatures (hereafter, LT50). Randomly chosen ants from the same colony and temperature treatment were placed in groups of four on an electric Plactronic Selecta hot plate set to 48 °C; they remained on the hot plate for 10 min. The number of ants that died was recorded. Then, the hot plate temperature was increased by 1 °C, and the test was repeated using another group of ants. The procedure was repeated ten times in total (i.e., until the temperature reached 57 °C). At the end of each test, all the ants, whether dead or alive, were put in 70% alcohol until further morphological measurements could take place. For each worker, two traits were measured using ImageJ software: mesosoma length in profile when the ant was in an extended position and right hindleg length (from femur to tarsus). We then also calculated the ratio between mesosoma length and hindleg length (hereafter, mesosoma/leg length ratio).

Data analysis

The data were analysed using R (RStudio-Team 2016). A series of generalised linear mixed models (GLMMs) were fitted to the data using the LME4 and lmerTest packages (Bates et al. 2015; Kuznetsova et al. 2015). In all the GLMMs, colony identity and the year of colony collection were included as random factors. Pairwise comparisons were performed using the relevel function in the stats package (RCore-Team 2015). First, we tested the effect of developmental temperature on the numbers of worker pupae produced (model 1; Poisson link function) and on the time to pupation (model 2; Gaussian link function). Then, we tested whether developmental temperature affected the probability that worker pupae reached eclosion (model 3; binomial link function) and that callows survived at least 2 weeks (model 4; binomial link function). The homogeneity of variance of pupae and callow worker morphological traits among temperature treatments was tested with the Levene's test. Since the variance of pupae length was significantly different (see below), we tested whether developmental temperature affected pupae length using a Kruskal-Wallis and a Dunn's post hoc test (Bonferroni-adjusted for multiple comparisons). The variance of callow worker morphological traits did not differ among treatments. Thus, we tested whether developmental temperature affected callow mesosoma length, leg length and the mesosoma/leg length ratio (models 5 to 7; Gaussian link function).

The results of the thermal resistance tests (death or survival) were analysed using a model where developmental temperature and hot plate temperature were explanatory variables (model 8; binomial link function). LT50s and their standard errors were estimated for each temperature treatment using the

dose.p function in the MASS package (Venables and Ripley 2002). We also determined, for each developmental temperature, whether the probability of death at a given temperature depended on mesosoma length, leg length or the mesosoma/leg length ratio (models 9 to 11, binomial link function). Unless otherwise mentioned, model-adjusted means were obtained and standard errors were calculated from the parameter estimates (Littell et al. 1996).

Results

The effect of temperature on ant development and survival

Across all treatments, most of the pupae belonged to the worker caste (66, 85, 91 and 98% at 20, 24, 28 and 32 °C, respectively). Significantly more worker pupae were produced at 32 and 28 °C than at 24 °C (model 1: $z_{32-24} = -2.63$, $P = 0.0085$; $z_{28-24} = -2.36$, $P = 0.018$) and 20 °C (model 1: $z_{32-20} = -6.23$, $P < 0.0001$; $z_{28-20} = -6.05$, $P < 0.0001$) and at 24 °C than at 20 °C (model 1: $z_{24-20} = -5.34$, $P < 0.0001$). There was no significant difference between 28 and 32 °C (model 1: $z_{28-32} = 0.55$, $P = 0.5811$) (Fig. 1a). The numbers of males produced were 1 at 20 °C, 16 at 24 °C, 11 at 28 °C, and 2 at 32 °C. Only three queen pupae were produced: 2 at 28 °C and 1 at 32 °C.

Larval developmental times differed significantly among temperature treatments. Worker pupae were produced significantly faster at 32 and 28 °C than at 24 and 20 °C (model 2: $t_{32-24} = 11.26$, $P < 0.0001$; $t_{32-20} = 6.23$, $P < 0.0001$; $t_{28-24} = 11.69$, $P < 0.0001$; $t_{28-20} = 6.05$, $P < 0.0001$) and at 24 °C than at 20 °C (model 2: $t_{20-24} = -2.26$, $P = 0.024$). However, there was no significant difference between 28 and 32 °C (model 2: $t_{32-28} = -0.71$, $P = 0.48$) (Fig. 1b).

Because there was a general failure to develop at 20 °C, this temperature was excluded from further analyses. There was no significant difference in the proportion of worker pupae that reached eclosion among the temperature treatments (model 3: $z_{24-28} = -0.68$, $P = 0.49$; $z_{24-32} = -1.59$, $P = 0.11$; $z_{28-32} = -0.98$, $P = 0.33$). However, the mortality of callow workers was significantly higher at 28 and 32 °C than at 24 °C (model 4: $z_{24-28} = 2.83$, $P = 0.005$; $z_{24-32} = 3.03$, $P = 0.002$) (Fig. 2). Few males and queens eclosed: one male emerged at 24 °C, two males and one queen emerged at 28 °C and one male emerged at 32 °C.

The effect of temperature on pupal and adult morphology

Only worker pupae were measured since almost no males or queens were produced. The variance of pupae length was significantly greater at 28 °C than at 24 and 32 °C (Levene's test: 24–28: $F_{1,1} = 5.29$, $P = 0.022$; 32–28: $F_{1,1} = 8.36$,

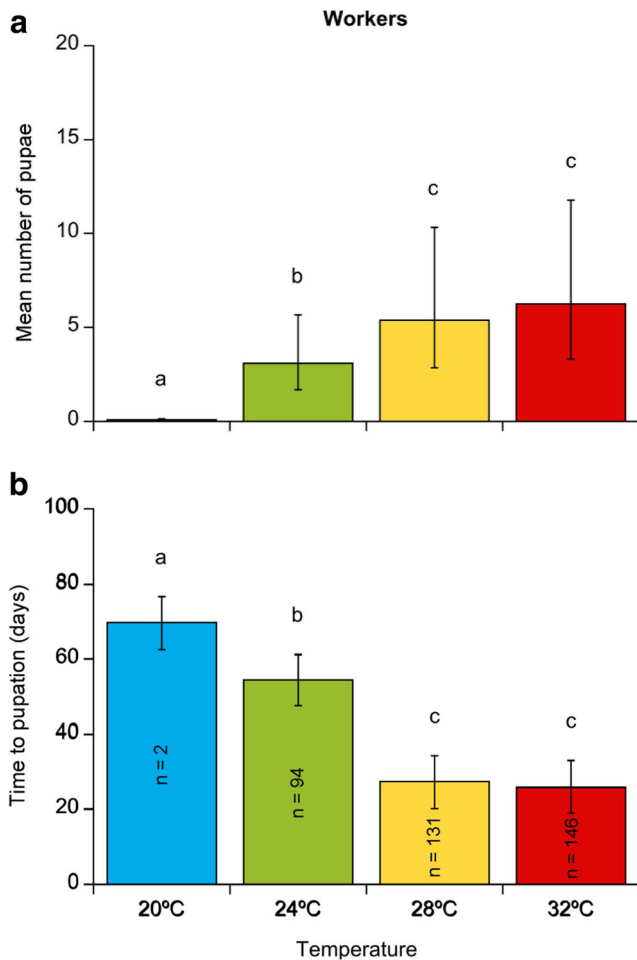


Fig. 1 Number of worker pupae produced (a; mean \pm SE) and time to pupation (in days) for workers (b; mean \pm SE) reared at four different temperatures. The different letters indicate significantly different means ($P < 0.05$)

$P = 0.004$; 24–32: $F_{1,1} = 0.07$, $P = 0.78$). Moreover, pupae length was significantly different among temperature

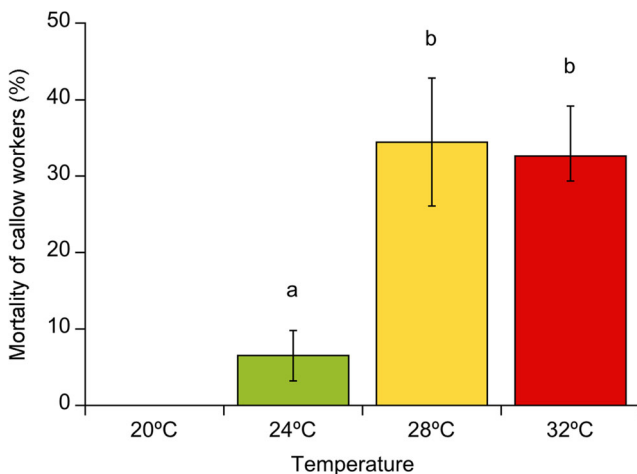


Fig. 2 Mortality (%; mean \pm SE) of callow workers reared at four different temperatures. The different letters indicate significant differences ($P < 0.05$). There was no data for the 20 °C treatment

treatments ($\chi^2 = 44.54$, $df = 2$; $P < 0.0001$). Worker pupae reared at 24 °C were significantly longer than those reared at 28 and 32 °C (Fig. 3; $P_{24-28} < 0.0001$; $P_{24-32} = 0.0001$). The length of worker pupae reared at 28 and 32 °C was not significantly different ($P_{28-32} = 1$).

The variance of callow worker morphological traits did not differ among treatments (mesosoma: $F_{1,2} = 1.20$, $P = 0.3$; leg: $F_{1,2} = 1.04$, $P = 0.35$; mesosoma to leg length ratio: $F_{1,2} = 1.77$, $P = 0.17$). Callow workers reared at 24 °C had longer mesosomas than callow workers reared at 28 and 32 °C (model 5: $t_{24-28} = -2.54$, $P = 0.02$; $t_{24-32} = -2.81$, $P = 0.01$). The mesosomas of callow workers reared at 28 and 32 °C did not significantly differ in length (model 5: $t_{28-32} = -0.368$, $P = 0.71$). Leg length was similar among treatments (model 6: $F_{2,48} = 1.24$, $P = 0.299$). However, callow workers reared at 24 and 28 °C had greater mesosoma/leg length ratios than callow workers reared at 32 °C (Fig. 4; model 7: $t_{24-32} = -3.21$, $P = 0.001$; $t_{28-32} = -2.13$, $P = 0.035$). There was no difference between 24 and 28 °C (model 8: $t_{24-28} = -1.04$, $P = 0.3$).

Thermal resistance

Temperature treatment had a major effect on adult thermal resistance. Ants reared at 24 °C were significantly less resistant to thermal stress than those reared at 28 and 32 °C (Fig. 5; model 8: $z_{24-28} = -3.87$, $P = 0.0001$; $z_{24-32} = -3.59$; $P = 0.0003$). The LT50s for ants reared at 24, 28 and 32 °C were 50.55 ± 0.27 , 52.47 ± 0.21 and 52.22 ± 0.3 °C, respectively. Within treatments, the thermal resistance of callow workers was not significantly related to any of the morphological measurements (models 9–11; Supplementary Material, Tables S1, S2 and S3).

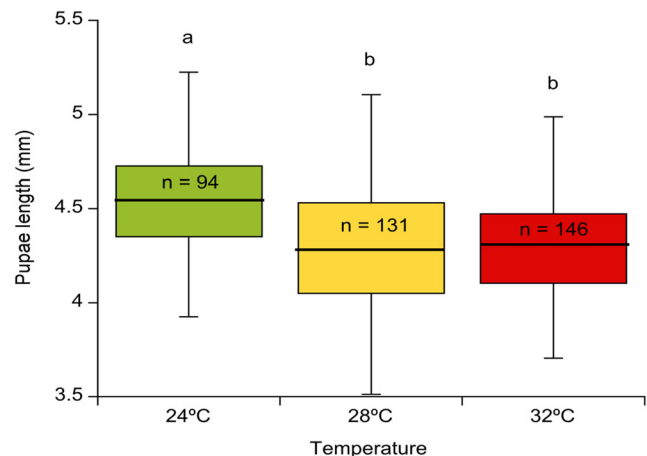


Fig. 3 Length of worker pupae reared at four different temperatures. In the boxplots, the boxes indicate the inter quartiles (Q1, Q3) and central lines within the boxes indicate median. The different letters indicate significant differences between treatments ($P < 0.05$)

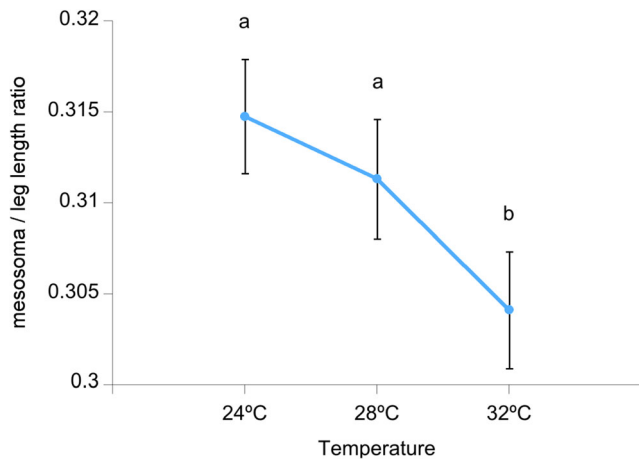


Fig. 4 Mesosoma/leg length ratio (mean ± SE) of callow workers reared at three different temperatures

Discussion

The results of our study show that variation in temperature experienced during development and the early adult life can result in significant plasticity in ant development and thermal resistance. Higher rearing temperatures enhanced developmental success and reduced both developmental duration and callow size. As a consequence, when larvae and pupae were reared at higher temperatures, they produced smaller callows. However, smaller workers were more thermoresistant than larger workers and, within temperature treatments, there was no relationship between body size and thermal resistance. These results show that *A. senilis* has a tremendous capacity to adjust its thermal resistance based on local conditions.

Development was limited at low temperatures: at 20 °C, almost no brood developed, even over a relatively long period of time. Similar temperature constraints have been observed in *Solenopsis invicta* (Porter 1988), *Linepithema humile* (Abril et al. 2010) and *Aphaenogaster rudis* (Southerland 1988). In

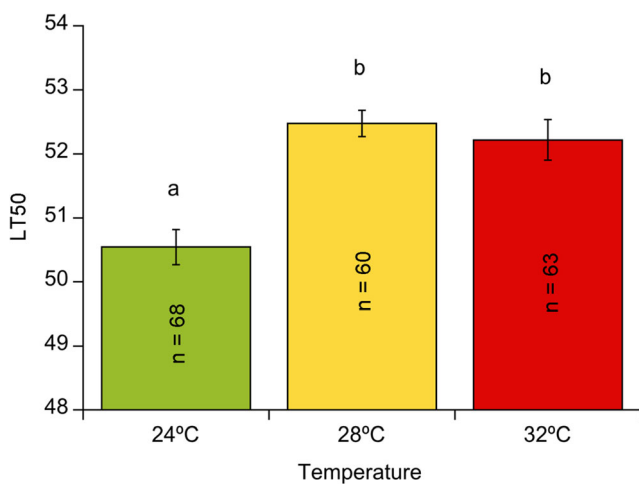


Fig. 5 Half-lethal temperatures (±SE; °C) for callow workers reared at three different temperatures

nature, workers move larvae within the nest, placing them at the temperature that most favours development (Hölldobler and Wilson 1990; Anderson and Munger 2003). For example, in early spring, *A. senilis* workers place the pupae just below the ground surface, where temperatures are warmest. Our results suggest that the optimum developmental temperature for *A. senilis* is approximately 30 °C, which is similar to that of species such as *Camponotus mus* (Roces and Núñez 1989) and *S. invicta* (Porter and Tschinkel 1993). The high rates of pupal survival at all temperatures indicates that this developmental phase was the most resistant to temperature variation.

Most pupae were workers, but a few males and queens were also produced. Some males could be the progeny of workers. Indeed, Boulay et al. (2009b) found that *A. senilis* workers in orphaned groups laid eggs that yielded males and that male production increased about 3 months after orphaning, which is the duration of our study. Villalta et al. (2015) further reported that only a small proportion of individuals laid eggs and that this proportion decreased with group size. In our study, adult workers mortality may have impacted the number of egg-laying workers, especially at high temperature treatments which is known to reduce since worker longevity (Calabi and Porter 1989). Moreover, the higher temperatures could have directly inhibited worker laying capacity or have enhanced egg death, as in *L. humile* (Abril et al. 2010). Regarding queens, only three were produced across all treatments. In *A. senilis*, queens form from young bipotent larvae that, until the second instar, can develop into either queens or workers, depending on their social environment (Boulay et al. 2009b). Low queen production may have been due to a greater mortality rate of the workers that cared for the larvae since queen production increases with group size (Ruel et al. 2012).

Ants reared at higher temperatures had greater thermal resistance. The LT50 of callow workers reared at 24 °C was about 2 °C lower than the LT50 of callow workers reared at 28 and 32 °C. However, higher temperatures speeded up development and, consequently, decreased pupa size, as is usually the case in insects (Atkinson 1994; Forster and Hirst 2012). In callow workers, mesosoma length decreased with rearing temperature, whereas leg length was not significantly affected. Thus, the mesosoma/leg length ratio decreased with temperature: workers reared at higher temperatures had longer legs relative to the length of their mesosomas than did workers reared at cooler temperatures. These results suggest that the greater thermal resistance of workers reared at higher temperatures did not result from morphological differences. This finding contrasts with what was observed in *Cataglyphis* desert ants, where larger workers resisted heat shocks longer than did smaller workers (Cerdá and Retana 1997; Clémencet et al. 2010). Indeed, a non-morphological mechanism or mechanisms may be in play. Traits that could be sufficiently plastic in response to variable rearing temperatures include heat-shock protein synthesis, metabolic rate,

and/or cuticular hydrocarbon synthesis. All three factors are known to be involved in generating thermal resistance in other ants.

Given the advent of global warming, it is crucial to understand how species will respond to increasing temperatures; ectotherms are of particular interest since, unlike endotherms, their physiology is highly dependent on ambient temperatures (Huey and Stevenson 1979; Angilletta et al. 2002). Developmental plasticity could provide a quick and relatively straightforward solution to climate change. As this study highlights, only one generation is necessary to respond to changes in ambient temperatures. This is similar to what has been found in other insects. For example, developmental temperature determines the probability of surviving to a heat shock in fruit flies *Drosophila melanogaster* (Forster and Hirst 2012). It also affects cold tolerance in the butterfly *Lycaena tityrus* (Zeilstra and Fischer 2005). Alternative solutions, such as the colonisation of new geographical regions with favourable habitats (Root et al. 2003; Chen et al. 2011) or the emergence of better performing genotypes via natural selection (Hoffmann and Sgrò 2011), are also possible but would take much longer. Phenotypic plasticity allows species to be more tolerant of changes in environmental conditions (Ghalambor et al. 2007), either by allowing species to deal with new environmental conditions and thus persist long enough to adapt (Pigliucci 2001) or by facilitating evolution through genetic assimilation (Waddington 1953; West-Eberhard 2005).

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