

## REVIEW

# Physiological adaptation to cities as a proxy to forecast global-scale responses to climate change

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## ABSTRACT

Cities are emerging as a new venue to overcome the challenges of obtaining data on compensatory responses to climatic warming through phenotypic plasticity and evolutionary change. In this Review, we highlight how cities can be used to explore physiological trait responses to experimental warming, and also how cities can be used as human-made space-for-time substitutions. We assessed the current literature and found evidence for significant plasticity and evolution in thermal tolerance trait responses to urban heat islands. For those studies that reported both plastic and evolved components of thermal tolerance, we found evidence that both mechanisms contributed to phenotypic shifts in thermal tolerance, rather than plastic responses precluding or limiting evolved responses. Interestingly though, for a broader range of studies, we found that the magnitude of evolved shifts in thermal tolerance was not significantly different from the magnitude of shift in those studies that only reported phenotypic results, which could be a product of evolution, plasticity, or both. Regardless, the magnitude of shifts in urban thermal tolerance phenotypes was comparable to more traditional space-for-time substitutions across latitudinal and altitudinal clines in environmental temperature. We conclude by considering how urban-derived estimates of plasticity and evolution of thermal tolerance traits can be used to improve forecasting methods, including macrophysiological models and species distribution modelling approaches. Finally, we consider areas for further exploration including sub-lethal performance traits and thermal performance curves, assessing the adaptive nature of trait shifts, and taking full advantage of the environmental thermal variation that cities generate.

**KEY WORDS:** Contemporary evolution, Global climate change, Space-for-time substitution, Thermal physiology, Urban heat island

## Introduction: using cities to forecast responses to climate change

Determining whether populations will persist or perish in response to recent and continuing climate change is a critical but challenging area of study. The ability to withstand increases in environmental temperature has proven to be a key trait in forecasting population persistence as the climate warms (Sinervo et al., 2010). However, two of the most commonly used forecasting methods each bear unique limitations. In particular, experimental climate warming can be limited by access to infrastructure and by the biological realism of so-called ‘press’ experiments where warming is applied instantaneously and across relatively brief time scales (Wolkovich et al., 2012).


Furthermore, space-for-time substitutions, in which populations experiencing naturally warmer climatic conditions at lower latitude or lower elevation sites serve as proxies for future climate warming occurring over time, and can suffer from confounding variables that obscure the causal relationship between climate, thermal physiology and population persistence (Damgaard, 2019).

By contrast, cities and their associated urban heat island effects are emerging as a new venue to develop forecasts of responses to climate warming (Youngsteadt et al., 2015). On the one hand, cities represent a globally replicated series of accidental warming experiments. Cities impose warming on biological systems simultaneously across the globe. With careful site selection to mitigate non-temperature confounding variables (Szulkin et al., 2020), and quantification of environmental temperature to account for heterogeneity within different parts of cities and between cities (Zhao et al., 2014), urbanized landscapes can be used in a replicated design to understand the relationship between temperature, physiology and population persistence (Diamond and Martin, 2020a). Indeed, the magnitude of localized warming in many cities can approximate global changes in climate anticipated by 2100 (Imhoff et al., 2010; Stocker et al., 2013). Such ready-made infrastructure can relax constraints on conducting warming experiments. Additionally, while cities differ in rates of development, they generally suffer less from the issue of instantaneous warming over short time scales as traditional warming experiments. On the other hand, cities can serve as human-made space-for-time substitutions by comparing urbanized and nearby non-urban sites (Verheyen et al., 2019). In this case, warming often occurs over compressed spatiotemporal scales compared with traditional space-for-time substitutions that rely on natural climatic variation across latitudinal and altitudinal gradients (Blois et al., 2013). Close proximity of warmed and unwarmed sites might therefore limit confounding variables, such as seasonality, that are present across larger biogeographical gradients. The benefits of using cities as tools to predict responses to climate change would appear to be high, and indeed while the fields of urban ecology and urban evolution are growing at an accelerating rate (Rivkin et al., 2019), their explicit use as forecasting tools is still nascent.

In this Review, we aim to highlight the ways in which cities could be used to forecast responses to global change. We consider cities as replicated experiments that allow us to quantify capacities for compensatory responses in thermal tolerance traits of populations experiencing urban warming. Specifically, we quantify capacities for shifts in trait values via phenotypic plasticity and evolutionary change. In addition, we consider the degree to which urbanization gradient space-for-time substitutions concur with more typically used naturally occurring biogeographical gradient space-for-time substitutions. Finally, we consider how the outcomes of these studies could be used to predict persistence in the face of ongoing climate warming. Throughout, we generally focus on heat and cold tolerance traits, as these are the most well replicated across different studies, although we return to sub-lethal thermal performance traits

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at the end of our review when we consider emerging research areas. Importantly, although interspecific variation in physiological trait means are often used to make inferences about species-level responses to global change (Sunday et al., 2012), here we focus on population-level responses and organismal capacities for coping with change via plastic and evolved shifts in tolerance phenotypes.

### Plasticity and evolution of thermal tolerance across urban versus biogeographical gradients: a case study with a common woodland ant

Because many of the comparisons we make in our review have few, if any, direct antecedents in the scientific literature, we begin with a single case study to introduce key concepts and approaches before expanding our scope in a series of multi-taxon meta-analyses. In this section, we explore the patterns and mechanisms underlying thermal tolerance trait responses to warming in *Lasius americanus*, a common woodland ant across North America (Schär et al., 2018). We first quantify the contributions of phenotypic plasticity and contemporary evolution to shifts in heat and cold tolerance traits in response to urban heat islands. Secondly, because *L. americanus* is one of the few species for which we have data on both shifts in thermal tolerance traits in response to urban heat islands and warming across a latitudinal biogeographical cline in temperature, we directly compare the magnitude and direction of an urban space-for-time substitution with a biogeographical space-for-time substitution.

In addition to undisturbed forests, *L. americanus* also inhabits small islands of forest habitat embedded within urban matrices. Unlike mature colonies that inhabit soil, leaf litter and rotting logs (Ellison et al., 2012), we found incipient colonies, including a newly mated queen and a small number of workers ( $\leq 16$ , on average), inhabiting tree nuts (oak and hickory) in both natural forest habitats (hereafter ‘rural’) and urbanized forest habitats (hereafter ‘urban’). This particular early-stage nesting habit allowed us to collect and return entire colonies to the laboratory for rearing under common garden conditions. These colonies were reared concurrently with colonies of true acorn ants, *Temnothorax curvispinosus*, that spend their entire colony life cycle within the acorn microhabitat, and whose physiological trait data comprise a previously published study on urban thermal adaptation (Diamond et al., 2018a). Thus, although detailed methods for the laboratory common garden experiment involving *L. americanus* are available in Diamond et al. (2018a), we briefly review these methods here (see also Supplementary information).

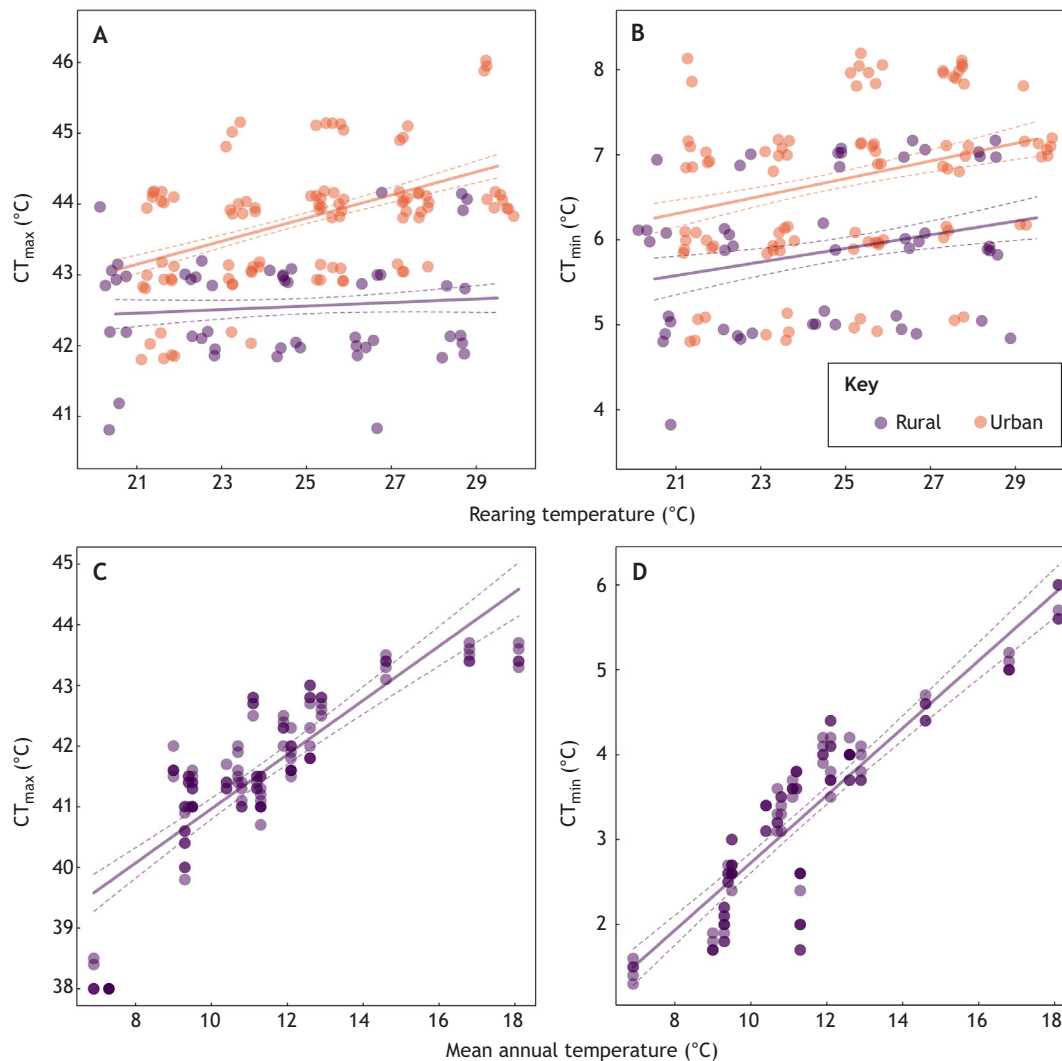
We collected *L. americanus* colonies from urban and rural sites around Knoxville, TN, USA (36°N latitude). Urban sites were 3.64°C warmer than nearby rural sites, as measured within ant microhabitats during the typical growing season. We set laboratory environmental chambers to five developmental acclimation temperatures, from 21 to 29°C. We reared field-collected urban and rural colonies under one of the five temperature regimes for a minimum of 10 weeks until a new cohort of workers was produced, after which time workers were individually tested for heat tolerance and cold tolerance. Our measures of heat and cold tolerance were the critical thermal maximum (CT<sub>max</sub>) and the critical thermal minimum (CT<sub>min</sub>), both in units of °C. Both critical temperatures were assessed with a dynamic ramping temperature protocol until the loss of muscular coordination.

Common garden experiments with laboratory-born organisms, such as the one we performed with *L. americanus*, are critical for being able to disentangle plastic effects of urban heat islands from evolutionary divergence between urban and rural populations. By rearing organisms for at least one generation under the same

conditions, any observed differences in thermal tolerance trait values between urban and rural populations are likely to reflect genetic changes between populations. Even with this experimental design using laboratory-born organisms, it is possible that parental effects might still play a role: for example, in ants, this could occur if the queen’s rearing environment influences her offspring’s thermal tolerance. However, such effects do not appear to explain divergence in thermal tolerance traits of other acorn-dwelling ants (*T. curvispinosus*) across urbanization gradients (Martin et al., 2019). Furthermore, by rearing both populations under several different temperature treatments, we can quantify how thermal tolerance traits are plastically altered by warming within a generation. Thus, the multi-temperature common garden experiment allows us to quantify both plasticity and evolution of thermal tolerance traits. The individual studies that comprise the cross-taxon meta-analyses of evolutionary versus plastic responses to urban heat islands that we perform subsequently are all multi-temperature common garden experiments.

The laboratory common garden study of *L. americanus* revealed evolutionary divergence in heat and cold tolerance traits in response to urban heat island effects (Fig. 1A,B). At the middle rearing temperature (25°C, a non-stressful temperature for many woodland ant species; Penick et al., 2017), urban population ants exhibited a significant increase in heat tolerance (higher CT<sub>max</sub> value) of 1.24°C and a loss in cold tolerance (higher CT<sub>min</sub> value) of 0.821°C compared with rural population ants (Table S1). These differences represent substantial evolutionary change in thermal tolerance trait values over only a century of urbanization in the city of Knoxville. Expressed in terms of haldanes, a standardized measure of evolutionary rate, the estimated rate of trait change in heat tolerance of *L. americanus*, 0.0116–0.0581, falls near the mean for other studies of contemporary evolution (Hendry and Kinnison, 1999). Relative to those studies specifically focused on urbanization, the estimate of the haldane numerator, 1.16, falls near the high end of the distribution (Alberti et al., 2017). For our calculations, we assumed a century of urban warming and an ant generation time, i.e. time from colony foundation to formation of reproductive alates, of 1–5 years (Hölldobler and Wilson, 1990); note that the maximum reported queen lifespan is 9 years for this species (Keller, 1998), leading to an estimate of *g* between 20 and 100 generations.

For heat tolerance, the magnitude of the divergence between populations depended on the laboratory rearing temperature. The populations exhibited different plastic responses to rearing temperature: urban population ants exhibited significant gains in heat tolerance at a rate of 0.163±0.0298°C (mean±s.e.m.) per every 1°C change in rearing temperature, whereas rural population ants gained heat tolerance at a rate of 0.025±0.0382°C per every 1°C; however, this rate was statistically indistinguishable from zero. By contrast, for cold tolerance, plastic responses to rearing temperature were not significantly different between urban and rural population ants. Considering the populations together, there was a significant loss in cold tolerance at a rate of 0.08±0.0463°C per every 1°C change in rearing temperature (Table S1). These results join a growing number of studies demonstrating plastic and evolved shifts in thermal tolerance traits in response to urban heat islands (Diamond and Martin, 2020a), including in water fleas (Brans et al., 2017), acorn ants (Martin et al., 2019), terrestrial isopods (Yilmaz et al., 2020) and *Anolis* lizards (Campbell-Staton et al., 2020). Given the diversity of taxa and geographical locations over which these patterns are found, cities appear to be quite useful as ready-made experiments to explore within-generation (plastic) and between-generation (evolved) responses to temperature rise.



**Fig. 1. North American woodland ant (*Lasius americanus*) thermal tolerance responses to warming.** Thermal tolerances of ants collected from urban and rural populations and reared under laboratory common garden conditions. (A) Heat tolerance (the critical thermal maximum,  $CT_{max}$ ); (B) cold tolerance (the critical thermal minimum,  $CT_{min}$ ). Urban populations are indicated by orange symbols, and rural populations are indicated by purple symbols. (C) Field-caught heat tolerance and (D) field-caught cold tolerance responses across a latitudinal gradient in environmental temperature, expressed as the mean annual temperature. In all panels, each point indicates the thermal tolerance of an individual ant. Continuous lines indicate the predicted relationships from linear mixed effects models of heat or cold tolerance as functions of population (urban or rural) and five laboratory rearing temperature treatments (A,B) or as functions of mean annual temperature (C,D). Dashed lines indicate  $\pm 1$  s.e.m.

Furthermore, cities can also be considered as space-for-time substitutions. With the *L. americanus* system, we aimed to evaluate whether the urban space-for-time substitution pattern resembled the more commonly used biogeographical space-for-time substitution pattern across a latitudinal cline in temperature. The *L. americanus* biogeographical data include heat and cold tolerances of field-caught worker ants across a latitudinal cline in the eastern USA, from 33.1 to 42.6°N latitude, representing a change in mean annual temperature of 6.9 to 18.1°C. This cline encompasses Knoxville, for which we have the data on urbanization effects on thermal tolerance in *L. americanus*, near the midpoint latitude. The field-caught, biogeographical thermal tolerances probably include both plastic and evolved responses to temperature across the latitudinal gradient. To be able to compare these data with the common garden urbanization thermal tolerances, we combined estimates of plastic and evolved responses to urban warming. Specifically, we calculated the change in thermal tolerance between the estimate

for the rural population ants in the coldest temperature treatment (21°C) and the urban population ants in the warmest temperature treatment (29°C). For the urbanization-driven shift in thermal tolerance, this amounts to a gain in heat tolerance of 2°C and a loss of cold tolerance of 1.56°C in context of the urban heat island effect of 3.64°C. By comparison, there was a gain in heat tolerance of 5°C and a loss of cold tolerance of 4.44°C in context of the 11.2°C temperature change across the latitudinal gradient (Fig. 1C,D). Focusing on heat tolerance responses, these shifts translate to a rate of 0.55°C per 1°C across the urbanization gradient versus 0.446°C per 1°C across the biogeographical gradient.

These values are remarkably similar considering the strikingly different gradients across which they were obtained. Although the general patterns for biogeographical and urbanization gradients are the same, there are probably different forces driving the responses. In particular, the relatively large number of generations might drive the biogeographical patterns, providing sufficient time for

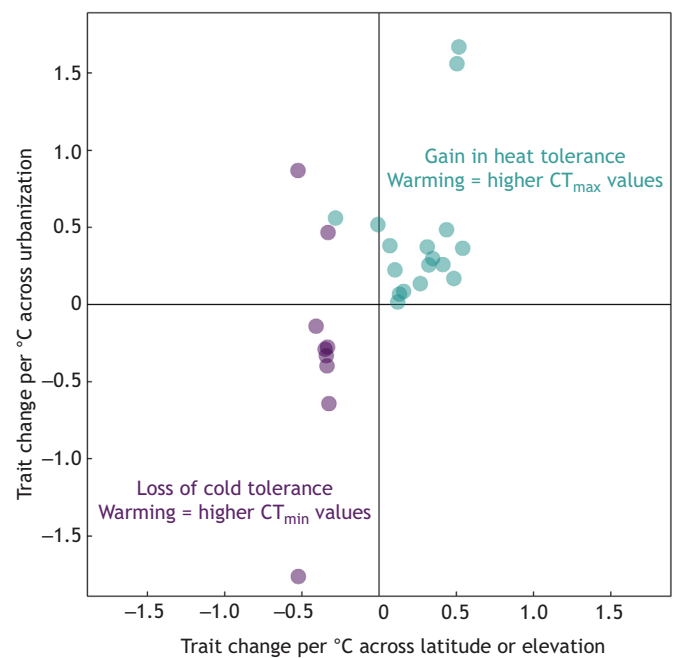
adaptation to occur, whereas the strong selection pressure of urban heat island effects in cities might be relatively more important in driving the urbanization patterns. Nonetheless, it is perhaps encouraging that cities not only appear to be useful as experimental warming venues, but can also provide results that match natural space-for-time substitution patterns.

### Urban versus biogeographical space-for-time: cross-system patterns

A major question, then, is whether the results showing congruence between the biogeographical and urbanization space-for-time substitution patterns for *L. americanus* generalize to other taxa? We performed a literature search to identify studies that contained data on shifts in thermal tolerance traits in response to urban heat islands. We then paired these studies with studies on trait change across biogeographical clines in temperature, either based on latitudinal or altitudinal variation. In most cases, we were able to find urbanization and biogeographical studies on the same species in the same geographical region, but for a small subset we had to use biogeographical data of close relatives and/or more distant geographical regions (Table S2; Supplementary information). Given the limited number of studies that met these criteria, we did not exclude studies on the basis of whether they were able to disentangle plastic from evolved responses to temperature, either across the urbanization gradients or across the biogeographical gradients. For the purposes of standardization, we computed the heat or cold tolerance trait change per °C of environmental temperature change across urbanization, and latitude or elevation. This metric is equivalent to the procedure we performed for the space-for-time substitution analysis of *L. americanus* across biogeographical and urbanization clines. Because this analysis relies on having estimates of thermal tolerance trait change in units of °C, we excluded studies that quantified tolerance in terms of recovery or resistance time to thermal stress.

Our analysis revealed a significant, positive association between the direction of thermal tolerance trait responses across urbanization and biogeographical gradients (chi-squared contingency table analysis:  $\chi^2=12.462$ , d.f.=1,  $P=0.000415$ ). This result indicates that thermal tolerance trait responses to biogeographical gradients and to urbanization gradients in temperature might be broadly predictive of one another (Fig. 2). Two cases for heat tolerance and two cases for cold tolerance each showed opposite responses to urbanization and biogeographical gradients, although as more total studies were available for heat tolerance as for cold tolerance (17 versus 9), the rate of discrepancy between the two gradients was higher for cold tolerance than for heat tolerance. However, given the relatively sparse number of data points available at this time, it is difficult to say whether heat or cold tolerance responses might be more or equally likely to have similar direction of responses to urbanization and geographical warming gradients. In addition, we found that the magnitude of trait change across urbanization versus biogeographical gradients was not significantly different between the two clines (simple linear model of the tolerance trait change as a function of urbanization versus biogeographical cline: heat tolerance,  $F_{1,32}=1.54$ ,  $P=0.223$ ; cold tolerance,  $F_{1,16}=0.224$ ,  $P=0.642$ ).

In natural space-for-time substitutions over geographical clines, lower latitude or elevation populations are intended to simulate what is expected for higher latitude or elevation populations under expected temporal changes in global climate. However, these approaches have been criticized for both environmental confounds and also the time scale of climatic change (reviewed in Wogan and Wang, 2018). These criticisms led some researchers to propose that

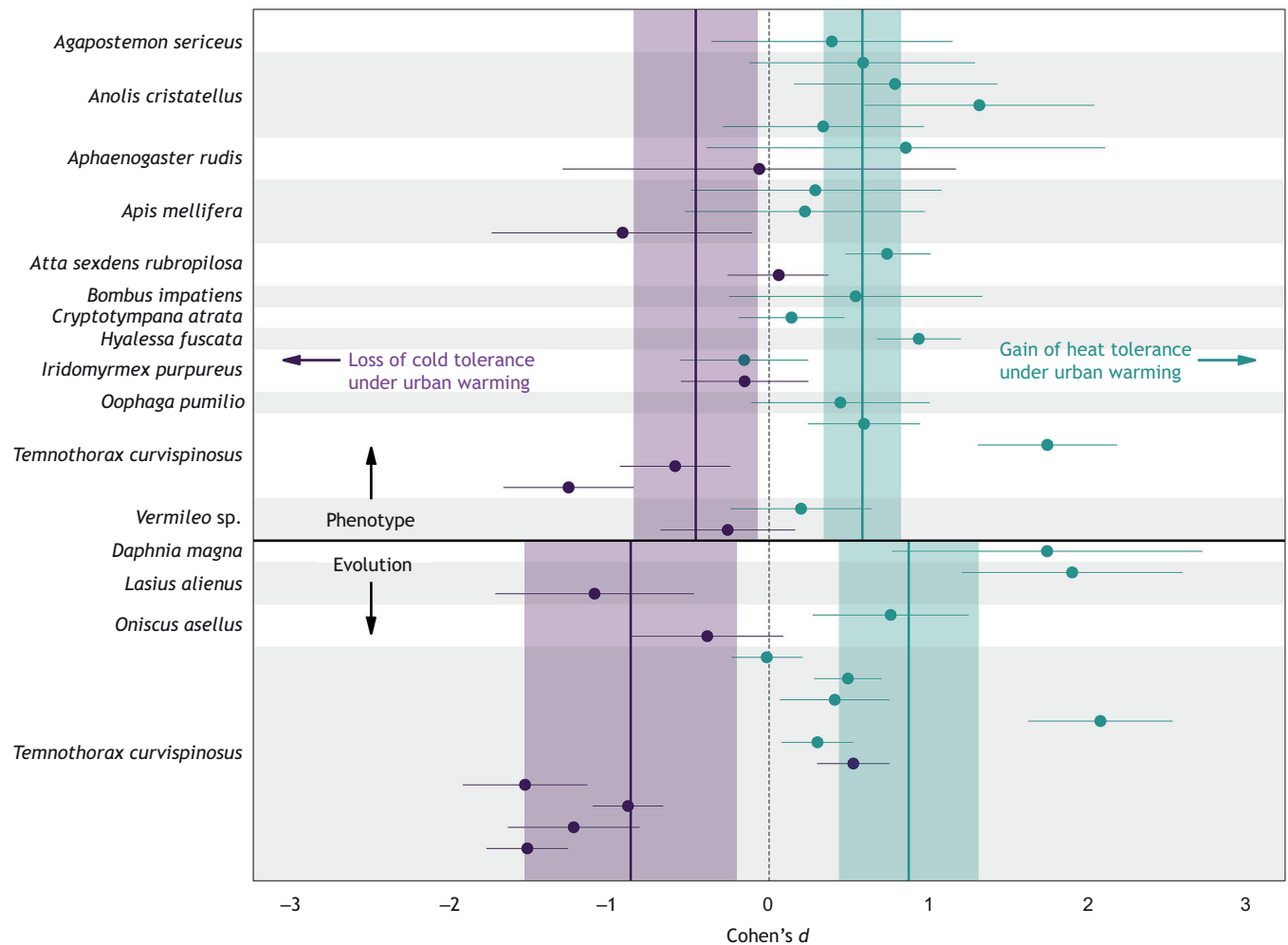


**Fig. 2. Magnitude and direction of the trait change in heat and cold tolerance per 1°C change in environmental temperature across urban heat island gradients versus across biogeographical gradients in latitude or elevation.** Each point corresponds to one species' shift in thermal tolerance across urbanization and biogeographical gradients. Heat tolerance responses are represented by green symbols, and cold tolerance responses are presented such that the loss of ability to withstand cold temperature [or alternatively, higher critical thermal minimum ( $CT_{min}$ ) values] is indicated by negative trait values.

space-for-time substitutions be verified with experiments and other lines of inference (Damgaard, 2019). For example, Elmendorf and colleagues (2015) showed that changes in community abundance-weighted mean thermal tolerances of plants were consistent across natural space-for-time substitutions of latitude and elevation, experimental warming achieved with passive warming chambers, and shifts in communities over time under recent climate change. Cities could be a unique tool to achieve verification in their capacities as experimental warming venues and as their own space-for-time substitutions. In particular, cities generate localized climatic warming, but do so over a much shorter distance, and could perhaps limit some confounding variables associated with more spatially divergent gradients. Of course, there is still a need to verify these patterns against those for responses to recent climate change over time, though these data tend to be quite rare at present (Diamond and Martin, 2020a). In general, targeting species that are already part of long-term monitoring schemes and comparing their responses to recent climate change with newly collected data on their responses to urban warming (if appropriate for that species), could be a useful way to assess congruence or divergence between responses to urban warming and responses to recent climate change.

### Mechanisms of thermal tolerance trait divergence across urbanization gradients

Above, we considered urbanization-driven phenotypic trait changes via any mechanism (plasticity or evolution) and compared the magnitude of trait change with those across biogeographical gradients. In this section, we aim to take a closer look at the mechanisms underlying phenotypic shifts in response to urban



**Fig. 3. Magnitude and direction of change in heat and cold tolerance traits across urban heat island gradients.** Results are separated according to whether trait changes correspond to field-collected data on phenotypes (above the thick horizontal line) versus laboratory common garden-collected data on evolved responses (below the thick horizontal line). Within the grouping variable of phenotype versus evolutionary change, results belonging to the same species are indicated by light gray shading. Points indicate Cohen's *d* effect sizes; 95% confidence intervals for each estimate are shown in the horizontal bars. Heat tolerance responses are represented by green symbols, and cold tolerance responses by purple symbols. Meta-analytic means and 95% confidence intervals for each combination of phenotype versus evolution and heat versus cold tolerance are shown by the vertical continuous lines and colour-shaded areas. Note that cold tolerance responses are presented such that the loss of ability to withstand cold temperature in response to urban warming is indicated by negative trait values.

warming. This is an important consideration, as plastic and evolutionary mechanisms can operate over different time scales and under different constraints (Merilä and Hendry, 2014). Furthermore, it is less of a question whether organisms exhibit phenotypic plasticity in response to urban heat islands (although the magnitude and efficacy of such responses is still under debate, e.g. for a broad discussion of 'plasticity rescue' from environmental change, see Snell-Rood et al., 2018), and more a question of whether these phenotypic shifts broadly reflect contemporary evolution. Evolutionary change over contemporary time scales has now been documented frequently, although consensus is still building in context of urbanization generally (Donihue and Lambert, 2015; Johnson and Munshi-South, 2017) and urban heat islands specifically (Diamond and Martin, 2020a). In general, the number of urban heat island-focused studies that rely on phenotypic shifts in response to urban warming is much greater than the number of studies that are able to disentangle plastic from evolved responses (Fig. 3). In the latter case, organisms are reared under common garden conditions for a generation or more to be able to tease apart environmental effects from genetic divergence underlying trait

change. The expectation is that phenotype-only studies will contain both plastic and evolved responses to the urban heat island and will exhibit upward bias in the estimates of trait change compared with those studies that are able to isolate and disentangle the plastic from evolved aspects of the phenotypic shift.

#### Phenotypic versus evolutionary divergence in thermal tolerance: cross-system comparisons

To address the question of whether phenotype-only studies exhibit upward bias in the magnitude of trait divergence, we performed a formal, mixed effects meta-analysis of phenotype-only versus known evolved responses to urban heat islands. We focused on thermal tolerance traits, including heat and cold tolerance. Because we computed standardized effect sizes (Cohen's *d*), we were able to consider a fairly broad range of thermal tolerance traits including  $CT_{max}$ ,  $CT_{min}$  and resistance to or recovery from thermal stress; most of our estimates were for the  $CT_{max}$  and  $CT_{min}$  (Table S3, Supplementary information). We found evidence of significant increases in heat tolerance and losses in cold tolerance in response to urban heat islands (Fig. 3; tests of whether the meta-analytic

mean±s.e.m. effect sizes are significantly different from zero, for heat tolerance, phenotype-only: estimate=0.585±0.123, 95% CI [0.344, 0.827],  $z=4.75$ ,  $P<0.0001$ ; heat tolerance, evolutionary divergence: estimate=0.906±0.286, 95% CI [0.346, 1.47],  $z=3.1689$ ,  $P=0.0015$ ; cold tolerance, phenotype-only: estimate=-0.461±0.195, 95% CI [-0.842, 0.0799],  $z=-2.37$ ,  $P=0.0178$ ; cold tolerance, evolutionary divergence: estimate=-0.868±0.286, 95% CI [-1.43, -0.308],  $z=-3.0376$ ,  $P=0.0024$ ). These patterns – specifically gains in heat tolerance and losses in cold tolerance – are typical of insect and other ectotherm phenotypic responses to warming across biogeographical clines (Sunday et al., 2011) and plastic and evolved responses to warming applied via field or laboratory experiments (Hoffmann and Sgrò, 2011; Hoffmann et al., 2013; Sgrò et al., 2016).

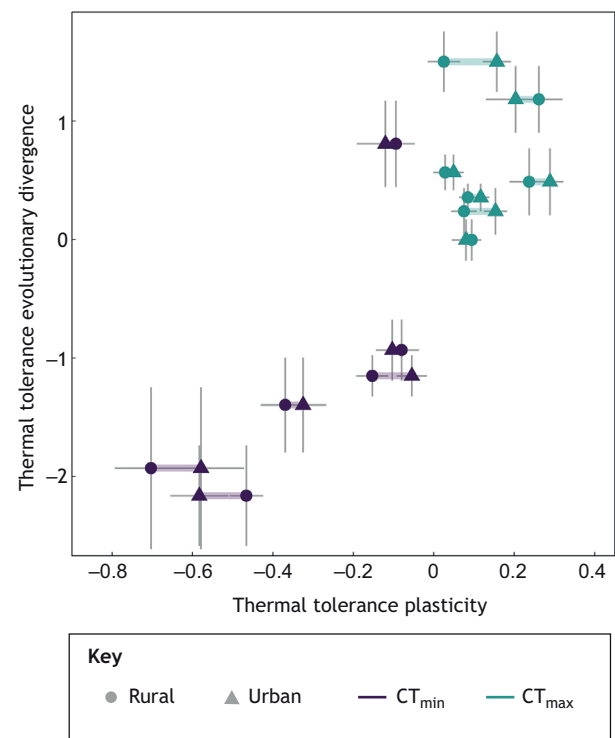
Surprisingly, however, we did not find any significant differences between whether the trait data were from phenotypes only or whether evolved responses were disentangled from plastic responses (test of whether phenotype-only versus known evolutionary divergence is a significant moderator of heat tolerance: estimate=-0.283±0.26, 95% CI [-0.793, 0.227],  $z=-1.09$ ,  $P=0.277$ ; and cold tolerance: estimate=0.402±0.354, 95% CI [-0.292, 1.1],  $z=1.14$ ,  $P=0.256$ ). If anything, the trend was towards evolved responses being larger than the phenotypic divergence studies, although this probably reflects benign laboratory conditions with continuous access to food and water, rather than maladaptive plastic responses dampening the mean evolutionary divergence in the field-caught phenotype-only studies.

Still, there is the question of what exactly produces the equivalence between phenotypic divergence and evolved divergence of thermal tolerance traits in response to urban warming? Are the phenotype-only studies indicative of a very strong evolved, genetic component to the phenotypic trait divergence across urbanization gradients? Or are the phenotype-only versus known evolutionary response studies simply different sub-samples, perhaps with the phenotype-only studies mostly being driven by plastic responses to urban warming, and obviously the latter being driven by genetic changes between urban and rural populations? Ideally, to address these questions, we should compare the magnitude of plasticity versus evolutionary change within the same system.

#### Plasticity versus evolutionary divergence in thermal tolerance: within-system comparisons

We returned to our meta-analytic dataset to develop within-study comparisons of the magnitude and direction of plastic and evolved responses of thermal tolerance traits to urban heat islands. By default, these come from the subset of studies within the known evolutionary category of the meta-analysis performed above, and for which plastic responses to temperature have also been quantified under comparable conditions as the evolutionary divergence component. As a consequence, this data subset is relatively small (Table S4; Supplementary information), and while our analysis does not directly address the question of why the phenotypic and evolutionary studies exhibit the same magnitude of response to urban heat islands, it does provide some indirect insight into this question by addressing the relative contributions of plastic and evolved responses to urban warming.

For each species-city combination in our analysis, we computed three quantities including the magnitude and direction of: the evolutionary divergence (i.e. the difference in thermal tolerance trait means across all temperatures), thermal plasticity of the urban population (i.e. the rate of thermal tolerance trait change per °C of rearing temperature), and thermal plasticity of the rural population. Although others have addressed the question of the relative contributions of plasticity versus evolutionary change in



**Fig. 4. Magnitude and direction of evolutionary divergence and plasticity of heat and cold tolerance responses to urban heat islands.** Rural population responses are indicated by circles. Urban population responses are indicated by triangles. Cold tolerance responses are indicated by purple symbols. Heat tolerance responses are indicated by green symbols. Increased heat tolerance with urbanization and warmer laboratory rearing temperature is indicated by positive values on each axis. Diminished cold tolerance with urbanization and warmer laboratory temperature is indicated by negative values on each axis. Points represent bivariate means±1 s.e.m., as shown by the gray lines. Responses from the same study system, species and city are connected by thick shaded horizontal lines (green: heat tolerance; purple: cold tolerance).

phenotypic responses to urban heat islands by performing variance decomposition (e.g. for life history traits; Brans and De Meester, 2018), which has the advantage of comparing plastic and evolved responses with comparable units (as percentage variance explained), we elected to explore patterns in the trait values themselves. We adopted this approach as we were interested in interpreting changes in both the magnitude and direction of plastic and evolved responses. In addition, not all the necessary data were publicly available to be able to perform the variance decomposition approach for each study in this analysis.

Our analyses revealed several patterns regarding plastic versus evolved responses of thermal tolerance traits in response to urban heat islands (Fig. 4). We reconfirmed significant evolutionary divergence of thermal tolerance traits across the urbanization gradient and additionally found evidence of significant plastic responses to laboratory rearing temperature (test of whether evolutionary divergence is non-zero for heat tolerance: estimate=0.590±0.195, 95% CI [0.208, 0.974],  $z=3.03$ ,  $P=0.0025$ ; and cold tolerance: estimate=-1.09±0.426, 95% CI [-1.92, -0.250],  $z=-2.55$ ,  $P=0.0109$ ; test of whether urban plasticity is non-zero for heat tolerance: estimate=0.145±0.0310, 95% CI [0.0845, 0.206],  $z=4.67$ ,  $P<0.0001$ ; and cold tolerance: estimate=-0.285±0.0965, 95% CI [-0.474, -0.0959],  $z=-2.95$ ,  $P=0.0031$ ; test of whether rural plasticity is non-zero for heat tolerance: estimate=0.107±0.0321, 95% CI [0.0439, 0.170],  $z=3.33$ ,

$P=0.0009$ ; and cold tolerance: estimate= $-0.304\pm 0.0982$ , 95% CI [ $-0.497, -0.112$ ],  $z=-3.10$ ,  $P=0.0019$ ).

Importantly, however, we found divergent responses between heat and cold tolerance traits with respect to the relationship between plasticity and evolution (Fig. 4). For heat tolerance, plastic and evolutionary responses appeared to be independent of one another (test of whether the moderator of plastic versus evolved response is significant for heat tolerance: estimate= $1.12\pm 1.67$ , 95% CI [ $-2.15, 4.38$ ],  $z=0.7672$ ,  $P=0.502$ ). Although the data comprising this particular analysis are quite few in number ( $N=13$  'studies' with three measures each for evolutionary divergence and urban and rural plasticity), we have no evidence at this point to suggest that plasticity either enhances or dampens evolutionary responses in heat tolerance to urban heat islands as it can act in other systems (Muñoz and Losos, 2017). It is therefore perhaps likely that a mixture of plastic and evolved responses underlie the heat tolerance phenotype-only shifts from the earlier meta-analysis, as the known evolutionary divergence cases do not lack plastic responses. By contrast, for cold tolerance, warming led to diminished cold tolerance for both plastic and evolved responses and the magnitude of plastic losses in cold tolerance was positively associated with the magnitude of evolved losses in cold tolerance (test of whether the moderator of plastic versus evolved response is significant for cold tolerance: estimate= $3.10\pm 1.06$ , 95% CI [ $1.03, 5.18$ ],  $z=2.93$ ,  $P=0.0034$ ). At a minimum, this result reconfirms the finding from the analysis of heat tolerance, that plastic responses do not appear to constrain the evolutionary response for cold tolerance, and indeed, could suggest a role for plasticity facilitating evolutionary divergence (West-Eberhard, 2003). In a related analysis, we examined whether urban or rural populations exhibited greater plasticity compared with one another. Our models revealed that urban and rural plasticity were of comparable magnitudes for both heat and cold tolerance (test of whether the moderator of urban versus rural was a significant moderator of plasticity for heat tolerance: estimate= $0.0194\pm 0.0223$ , 95% CI [ $-0.0243, 0.0630$ ],  $z=0.870$ ,  $P=0.384$ ; and cold tolerance: estimate= $0.0094\pm 0.0689$ , 95% CI [ $-0.126, 0.144$ ],  $z=0.135$ ,  $P=0.892$ ).

Many researchers have concluded that plastic responses to temperature rise will be generally insufficient to cope with global climate change (e.g. Sørensen et al., 2016). We do indeed find relatively low plasticity in heat tolerance (although statistically non-zero), similar to other global compilations of heat tolerance plasticity across a diverse range of ectothermic species (Gunderson and Stillman, 2015). As a consequence, a number of recent studies have focused on capacities for rapid evolutionary responses to warming. Although here too, evolution alone might be insufficient to keep pace with climatic warming (Radchuk et al., 2019). Our meta-analyses provide evidence that both mechanisms are contributing to thermal tolerance responses to urban heat islands, and that the plastic responses do not appear to constrain the evolutionary responses. This is encouraging as the two mechanisms on their own might be insufficient to keep pace with climatic warming, but their combined effects might allow for greater population persistence in the face of environmental change.

#### How do we use the data from cities to develop forecasts?

Up to this point, we have provided data to support our position that cities can be used as both warming experiments and human-made space-for-time substitution gradients to explore how thermal tolerance traits shift in response to temperature rise. In this section, we consider the ways in which these data can be used to develop forecasts of responses to warming at local to global scales. First, while

the use of physiological tolerance traits has a now decade-old history of being used to forecast, i.e. applying the discipline of macrophysiology to vulnerability forecasting (Chown and Gaston, 2008; Helmuth, 2009), most often such analyses are conducted at the interspecific level. For example, such analyses typically quantify the difference between environmental temperature and the thermal optimum (the thermal safety margin) or the thermal maximum (the warming tolerance) and compare these values across taxa and across geographical location to establish relative differences in vulnerability to climate change (Huey et al., 2009; Sunday et al., 2014). In general, the broad-scale patterns are that species with low heat tolerance, narrow tolerance breadths and which inhabit already-warm environments such as low latitude and low elevation habitats are at the greatest vulnerability (Huey et al., 2012). However, there have been a number of calls to incorporate population-level trait variation into macrophysiology-based forecasts (Chown and Gaston, 2016; Magozzi and Calosi, 2015; Somero, 2010), as the majority of analyses tend to ignore compensatory responses through phenotypic plasticity and evolutionary change.

Although individual studies demonstrate, for example, a positive association between acclimation capacity and the response to climate change (e.g. Stillman, 2003), it is only relatively recently that trait variation has been employed in macro-scale physiological trait-based approaches. Indeed, a study of thermal plasticity in metabolic rate found surprisingly more plasticity in tropical regions compared with temperate regions, at least suggesting greater compensatory responses in vulnerable tropical locations (Seebacher et al., 2015). In addition, another study found that heat tolerance plasticity dampened geographical range shift responses to recent climate change, potentially by allowing species to remain in their current habitat rather than being forced to track their historical climatic niche (Diamond, 2018).

Thus, a deeper understanding of which species are able to enter and persist in the city, not only on the basis of species-level physiological trait values, but also the capacity of trait values to shift in response to urban warming through plasticity and evolution can ideally enable more realistic vulnerability forecasting assessments (Chown and Duffy, 2015). Indeed, while global data repositories of thermal tolerance traits for individual species are growing (Bennett et al., 2018), there are relatively fewer estimates available for thermal plasticity (Gunderson and Stillman, 2015), and even fewer available for evolutionary responses to climatic warming (Diamond, 2017). The generation of a larger dataset on species mean thermal tolerance traits along urbanization gradients in addition to plastic and evolutionary capacities for coping with warming would allow us to develop a quantitative assessment of species that persist or perish with temperature rise. Such assessments can then be incorporated into conservation and management plans within cities (Lambert and Donihue, 2020) and to non-urban populations as they respond to global climate change. Of course, there could be other non-temperature factors that govern entry and persistence in urban environments, although these can be mitigated with careful site selection to minimize confounding variables (and notably, the same caution would need to be exercised in natural space-for-time comparisons) coupled with laboratory common garden experiments where the temperature effects can be isolated and tested. In any case, the need for careful site selection is arguably going to be outweighed by the benefits of ready-made infrastructure and more rapid rates of warming than for natural space-for-time substitutions.

In a similar vein, thermal tolerance trait variation can also be used in species distribution modeling approaches. For example, heat tolerance can be used to constrain areas of suitable habitat in

environmental niche models (Overgaard et al., 2014). Heat tolerance plasticity and evolutionary potential can also be used in next-generation species distribution models that allow traits to evolve over time and update habitat suitability and vulnerability in light of such compensatory responses. Often, these models tend to show greater persistence than models that do not allow physiological trait values to change over time (Bush et al., 2016; Razgour et al., 2019).

### Caveats and limitations

Whilst cities show great promise for use as climate change proxies, there are a number of caveats and limitations associated with this approach. The major challenge centers on disentangling climate-relevant drivers of phenotypic change from other urbanization-associated changes. This is not a problem new to biology – proper attribution of environmental drivers and agents of selection can be notoriously difficult over natural spatial and temporal gradients (e.g. Caruso et al., 2017; Mitchell-Olds and Shaw, 1987; Wade and Kalisz, 1990) – but one that remains quite relevant for studies performed in cities. In context of our case study with *L. americanus* and meta-analyses of urban-driven physiological trait change, it is possible that temperature might not be the sole driver of thermal tolerance trait shifts. For example, responses to stress imposed by pollutants can also enhance responses to stress imposed by temperature via cross-tolerance, i.e. alterations to non-specific pathways that broadly confer resistance to different types of stress (Sinclair et al., 2013). In this scenario, cities would not provide a suitable climate change proxy. This possibility points to the importance of performing manipulative experiments, either in the laboratory or field setting, to establish a causal link between a putative driver and phenotypic response. Furthermore, the potential for confounding variables to lead to erroneous conclusions when using cities as climate change proxies is unlikely to be equal across all study systems. For example, species with specific habitat requirements such as urban-dwelling acorn ants that are reliant on forest patches embedded within urbanized matrices (Diamond et al., 2018a), might be easier to isolate specific drivers whereas habitat generalists that occur over a range of different urbanized habitat types (e.g. weedy plant species such as ragweed or goldenrod; Gorton et al., 2018; Start et al., 2018) or are quite mobile (e.g. butterflies; Shephard et al., 2020) could be more difficult.

Cities can be studied and used far beyond climate change proxies, in which case the multifarious nature of urban-driven changes might be desirable (Rivkin et al., 2019). However, when cities are used as climate change proxies, we urge caution in their use, particularly so that climatic attributes can be appropriately disentangled from other aspects of urbanization. This requires a deep understanding of the natural history of the organism(s) under study, and how their natural histories shape the ways in which organisms experience urbanized landscapes. In general, we recommend that systems and study sites be selected intentionally to isolate climate drivers, with relevant climatic attributes being measured directly; manipulative experiments should be performed whenever possible; and if multiple drivers are likely to underlie trait divergence (e.g. especially for multiple climate change-relevant stressors such as shifts in temperature and water availability), these should be considered in experimental designs and interpretations (e.g. for urban multi-stressor work, see Brans et al., 2018; Yilmaz et al., 2020).

### Conclusions and future directions

Although we have highlighted the various ways in which cities can be used as warming experiments and human-made space-for-time

substitutions to understand the outcomes and mechanisms of phenotypic shifts in thermal tolerance traits, there are many areas left to explore.

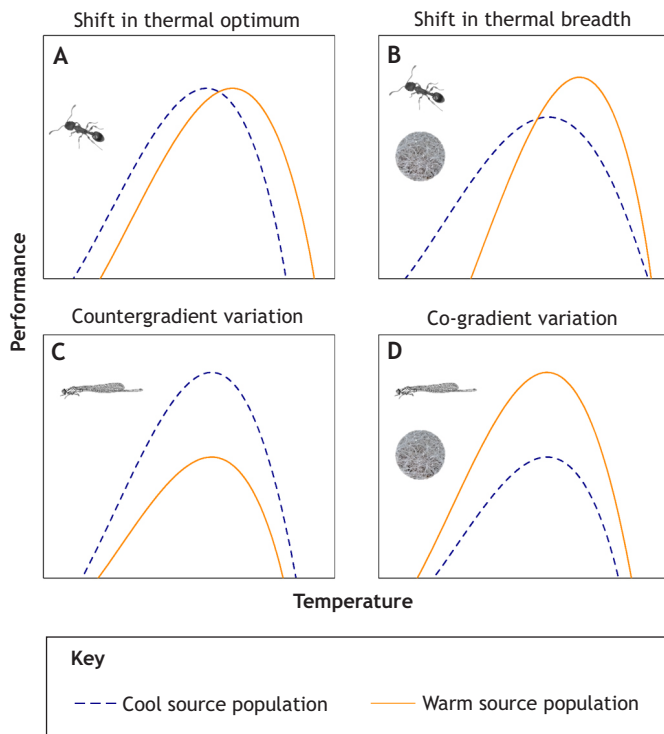
Moving forward, we suggest five areas for future development including the contribution of additional data from more divergent taxa on plastic and evolved responses of thermal tolerance to urban warming; a broader consideration of sub-lethal thermal performance traits; linkages between trait shifts and fitness to assess potential adaptive versus maladaptive responses; considering variation not only in mean temperature differences, but also thermal extremes; and maximizing the use of urban variation to not only consider urban versus rural space-for-time substitutions, but also time-for-time relationships, taking advantage of intra- and inter-city variation in time since urbanization was initiated.

At present, there are strong taxonomic biases towards arthropods in the available data on plastic and evolved responses of thermal tolerance traits to urban heat islands. This pattern remains when we expand the criterion to include any phenotypic thermal tolerance shift in response to urban warming, although here we do obtain additional representatives from amphibians and lizards. Thus, an obvious future direction is to simply increase the diversity of taxa for which we have data on phenotypic, plastic and evolved physiological responses to urban warming.

Moving beyond thermal tolerance traits to consider sub-lethal responses is yet another area for future development. There is some evidence that heat tolerance traits are correlated with sub-lethal performance traits such as development rate (Penick et al., 2017), and theory suggests that heat tolerance and the thermal optimum are co-adapted traits (Huey and Kingsolver, 1993). However, the shape of the thermal performance curve describing a continuous relationship of performance as a function of temperature can be critical for determining the outcomes of responses to temperature rise. For example, in springtails, native and introduced species have similar thermal tolerance values, but have different shapes in their thermal performance curves. Because native species have a ‘cooler-is-better’ shape whereas introduced species have a ‘warmer-is-better’ shape, introduced species are expected to be able to withstand global climate change better than the native species (Chown et al., 2007).

Furthermore, the evolution of thermal performance curves remains enigmatic. While there is substantial theory and empirical studies on the evolution of thermal performance curves, empirical work is still largely disconnected from expectations from theory. For example, specialist-generalist trade-offs are widely anticipated in response to climatic warming, yet are not overwhelmingly found in nature (Angilletta, 2009). Cities could be a largely untapped venue to understand the forces that shape the evolution of thermal performance (Tüzün and Stoks, 2018), and clearly with the springtail example discussed above, curve shape has important influences on the outcome of responses to warming. Although the data are relatively sparse to date, there are at least three study systems for which the evolution of thermal performance have been conducted in cities. Interestingly, across each of these studies, each possible outcome for the evolution of curve shape is represented (Fig. 5). The running speed of acorn ants, an important resource-acquisition trait, shows evidence for evolved shifts towards a higher thermal optimum and a narrower thermal tolerance breadth (specialist-generalist trade-off) in cities (Chick et al., 2020). Damselfly growth rate shows evidence of countergradient variation where rural damselflies grow faster at all temperatures compared with urban damselflies; however, survival shows co-gradient variation where urban damselflies have higher





**Fig. 5. Hypotheses for the evolution of thermal performance curves (following Angilletta, 2009).** (A) Shift in the thermal optimum; (B) shift in thermal breadth; (C) countergradient variation; (D) co-gradient variation. In each panel, the orange line corresponds to a warm source population, and the dashed blue line corresponds to a cool source population. Species silhouettes represent urban evolution of thermal performance curve shape. Note that some species exhibit shifts consistent with multiple hypotheses. Species and trait data shown include damselfly (*Coenagrion puella*) growth rate (C) and survival (D) (Tüzün et al., 2017), acorn ant (*Temnothorax curvispinosus*) running speed (A,B) (Chick et al., 2020) and chitinolytic fungus growth rate (B: *Chrysosporium pannorum* and *Trichoderma koningii*; D: *Torulomyces lagena* and *Penicillium bilaii*) (McLean et al., 2005).

survival across all temperatures compared with the rural population (Tüzün et al., 2017). Finally, two species of chitinolytic fungi show co-gradient growth rate responses to urbanization whereas another two species show evidence of narrower thermal tolerance breadths (McLean et al., 2005). While cities could allow researchers to examine the early stages of contemporary evolution of thermal performance curves in response to rapid urban warming and potentially gain clearer insight into the theory-empirical work disconnect, this research is also important for developing accurate forecasts of species performance and persistence in response to contemporary warming.

Shifts in thermal tolerance traits in response to urban warming are evident, but it is less clear whether these changes in phenotype are adaptive. Demonstrations of adaptive urban evolution are quite rare, although certainly many studies are suggestive of adaptation. For example, the evolution of higher heat tolerance would seem to be advantageous; however, few studies measure fitness across both urban and rural environments, either in laboratory common garden settings or in field reciprocal transplants, and link these responses with physiological trait changes. As one exception, urban acorn ants achieve higher fitness when reared under warm conditions in the laboratory and in their 'home' (urban) environment in the field, and rural acorn ants achieve higher fitness when reared under cool laboratory conditions and in their 'home' (rural) environment in the field, providing support for adaptive evolution underlying the

increase in urban acorn ant heat tolerance (Diamond et al., 2018a; Martin et al., 2020 preprint). A number of urban evolution plant studies (although not on thermal tolerance traits, but rather growth, life history and phenology traits) also measure fitness, but the findings are less straightforward. For example, in ragweed, urban and rural populations appear to be locally adapted with respect to flowering time; however, rural populations had higher lifetime fitness across rural and urban sites, which the authors interpret as an indication of stronger selection in rural habitats (Gorton et al., 2018). Unfortunately, cities do not overcome the long-standing question of how to best measure fitness, although fitness data are necessary for interpreting the adaptive nature of shifts in phenotypes in cities. It is too early to say at this point whether responses to cities are broadly adaptive (but see Martin et al., 2020 preprint), although it is worth bearing in mind that with such drastic changes in the environment including those generated by cities, we must also consider the possibility that responses might also be maladaptive (Brady et al., 2019; Diamond and Martin, 2020b).

Indeed, going a step further, when considering plastic and evolved responses of physiological traits to urban heat islands, typically rather coarse summary measures of the temperature difference between urban and non-urban environments are used, e.g. mean annual temperature. However, thermal physiological traits such as temperature tolerance have repeatedly been shown to be shaped by climatic extremes rather than climatic means (Buckley and Huey, 2016; Clusella-Trullas et al., 2011; Kingsolver and Buckley, 2017). Such distinctions could be especially important in the context of cities and their use as climate change proxies. Urban-driven warming is often not uniform over space and time. For example, in many cities, warming is biased towards the night-time due to impervious surfaces retaining heat accumulated during the daytime hours (Imhoff et al., 2010). As a consequence, cities might relax constraints on performance at low temperatures. This scenario provides an analogue for global climate change which is also associated with reductions in diurnal temperature variance (Stocker et al., 2013). In addition, cities can alter the frequency of extreme temperature events, which again mirrors climate change expectations. For example, snow removal in cities might contribute to the evolution of greater cold tolerance of urban white clover as they are exposed to cooler air temperatures rather than being insulated under snowpack (Thompson et al., 2016). Finally, cities can alter the spatial structure of thermal landscapes. For example, foraging landscapes of acorn ants are more thermally heterogeneous in cities and there has been concomitant evolution of thermal plasticity in response to faster rates of temperature change (Diamond et al., 2018b). As increased habitat fragmentation often accompanies global climate change, altered thermal landscapes in cities could provide useful proxies in this regard. Whilst altered spatiotemporal variation in warming driven by cities might be a potential challenge, especially if not carefully quantified, it also provides an opportunity to gain insight into how temperature extremes and temperature variance shape thermal physiological traits.

Finally, while we focused our review of cities as warming experiments and space-for-time substitutions based on the data that were available, we also acknowledge that cities can be used in other ways to explore the effects of warming on thermal physiological traits. Often, cities are mosaics of different time-since-built areas, even while maintaining comparability between the overall magnitude of development (Szulkin et al., 2020). While many researchers have used the relative variation in how built-up areas are within a city to catalog warming effects on phenotypes, the temporal

element can also be used to track the evolution of phenotypes over time in response to warming across different numbers of generations. Furthermore, many cities differ with respect to build times which can also be another source of temporal variation to allow the tracking of thermal tolerance phenotypes over time. Although cities have generally not been used in this way in context of the evolution of thermal tolerance traits, there seems a great deal of untapped potential for exploration in this area.

#### Acknowledgements

We thank members of the Diamond and Martin laboratory groups, especially Stephanie Strickler, Abe Perez and Lacy Chick, for field work and thermal tolerance assays on the woodland ant system.

#### Competing interests

The authors declare no competing or financial interests.

#### Funding

A National Science Foundation (NSF) CAREER award (DEB-1845126) to S.E.D. and awards from the Oglebay Trust Fund to S.E.D. and R.A.M. provided support for this research.

#### Data availability

Data for the case study on woodland ant responses to urbanization and biogeographical gradients are available from the Dryad digital repository (Diamond, 2020): [dryad.zw3r22871](https://doi.org/10.5061/dryad.zw3r22871)

#### Supplementary information

Supplementary information available online at <https://jeb.biologists.org/lookup/doi/10.1242/jeb.229336.supplemental>

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