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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).

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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

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 (≤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_{max}) and the critical thermal minimum (CT_{min}), 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_{max} value) of 1.24°C and a loss in cold tolerance (higher CT_{min} 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.
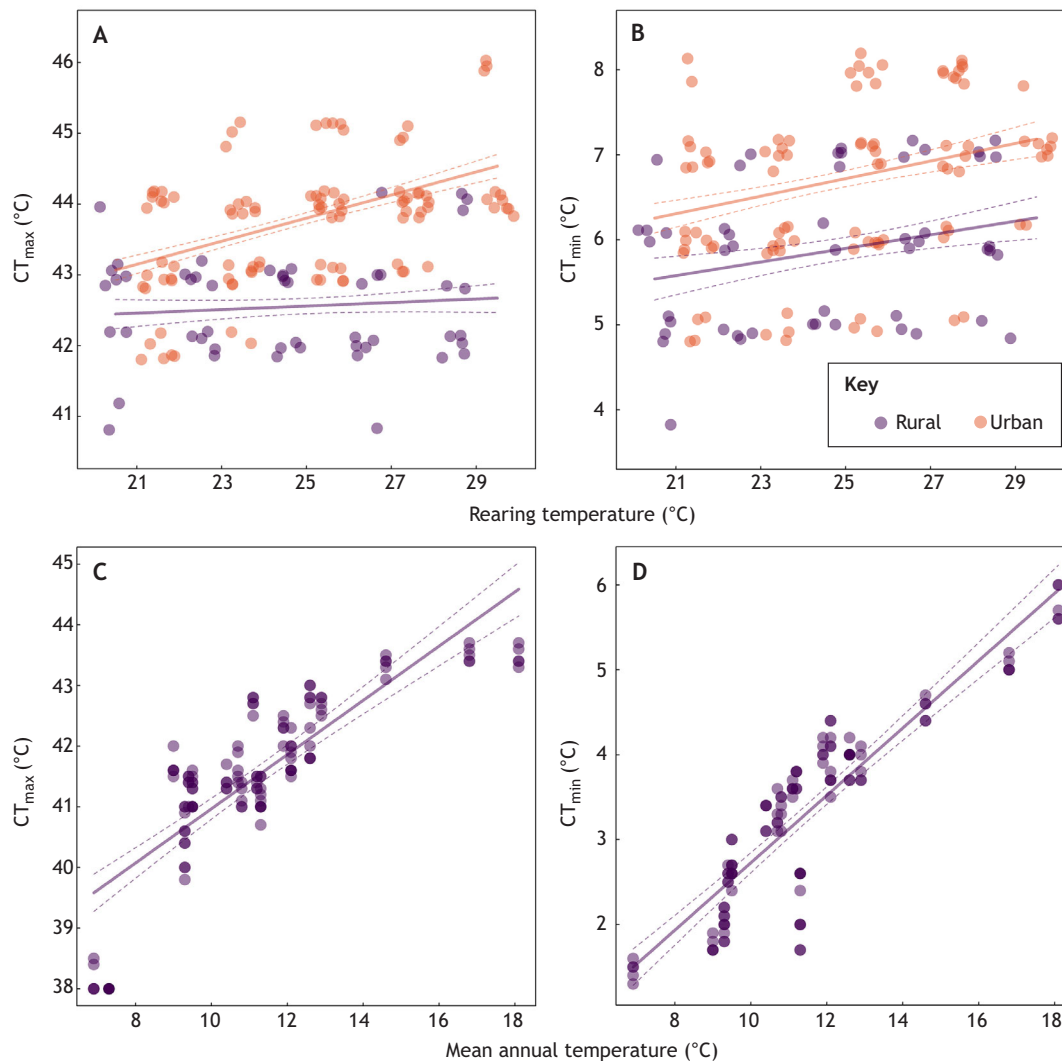
Fig. 1. North American woodland ant (*Lasius americanus*) thermal tolerance responses to warming. Thermal tolerances of ants collected from urban and rural populations and reared under laboratory common garden conditions. (A) Heat tolerance (the critical thermal maximum, CT_{max}); (B) cold tolerance (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 ±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
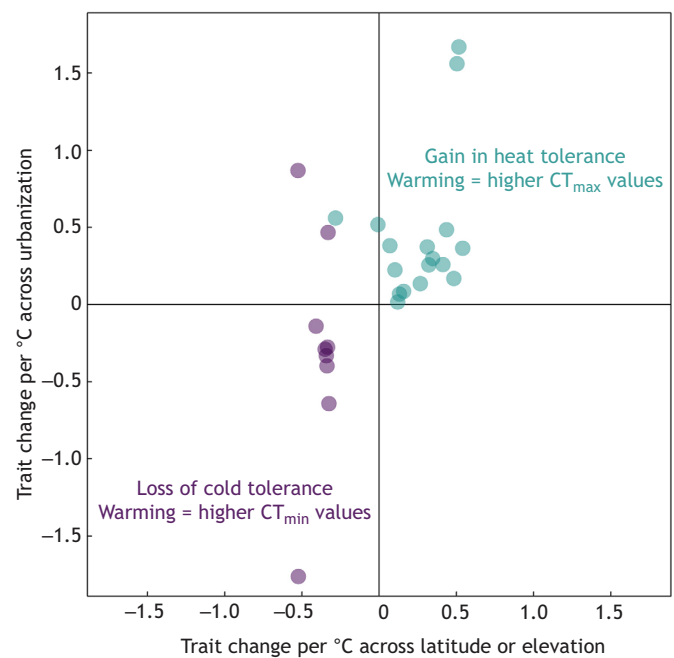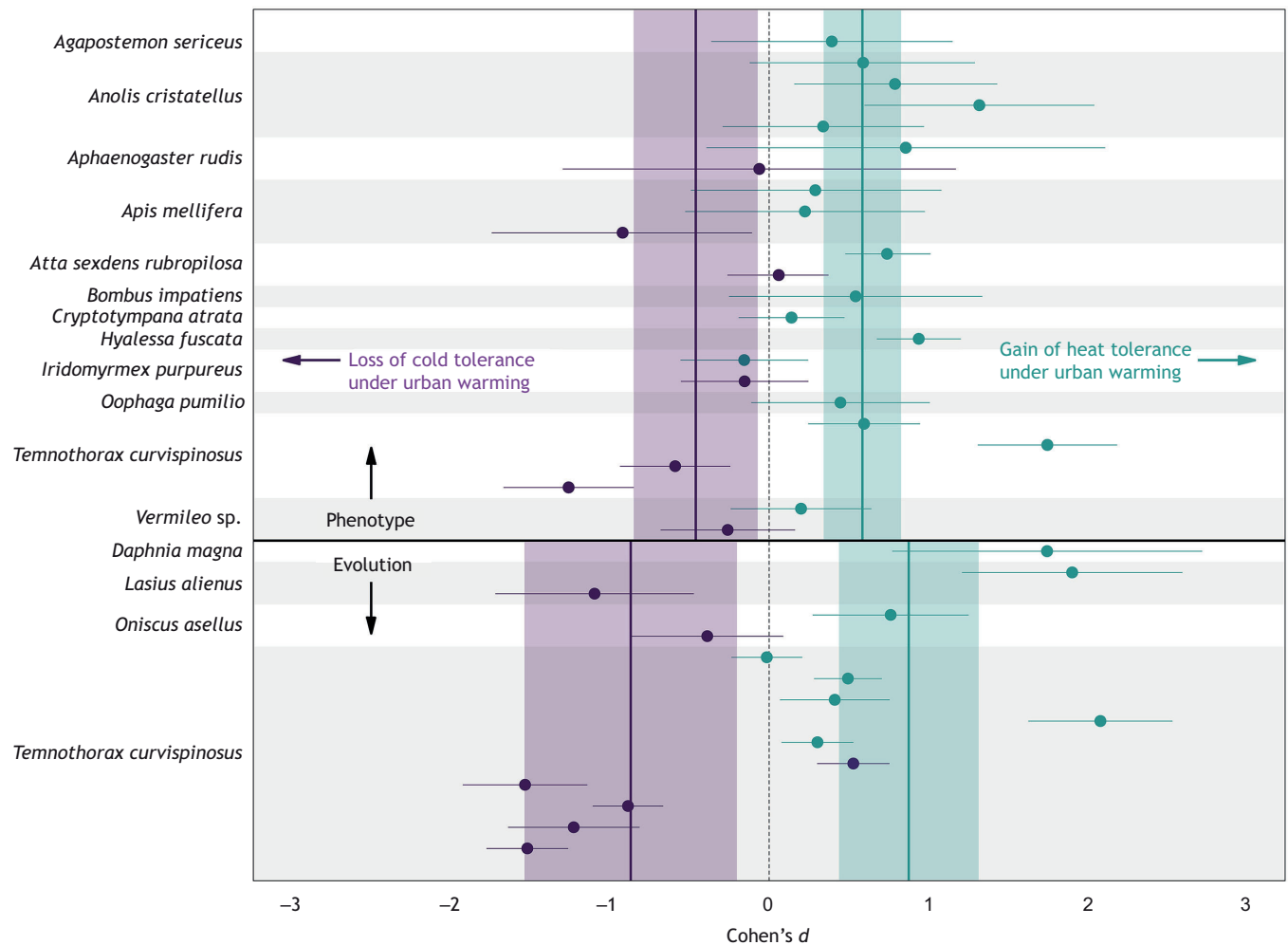

Fig. 2. Magnitude and direction of the trait change in heat and cold tolerance per 1°C change in environmental temperature across urban heat island gradients versus across biogeographical gradients in latitude or elevation. Each point corresponds to one species' shift in thermal tolerance across urbanization and biogeographical gradients. Heat tolerance responses are represented by green symbols, and cold tolerance responses by purple symbols. Note that 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
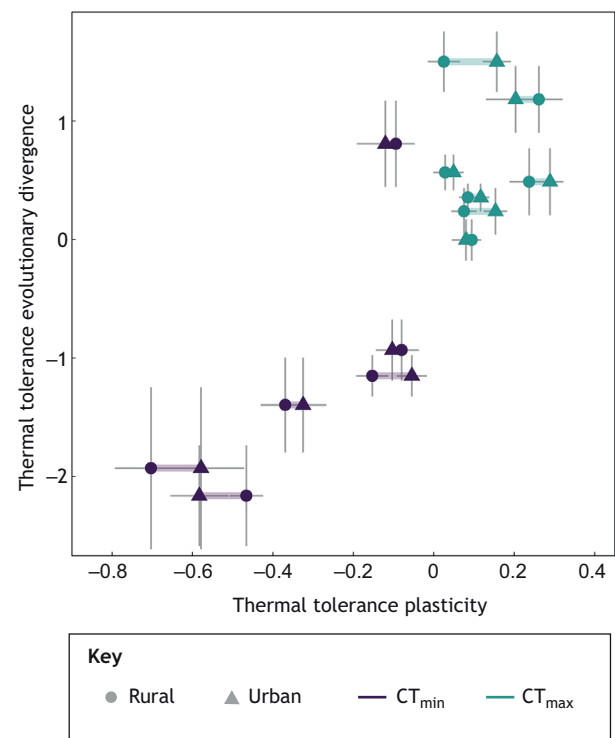
Fig. 4. Magnitude and direction of evolutionary divergence and plasticity of heat and cold tolerance responses to urban heat islands. Rural population responses are indicated by circles. Urban population responses are indicated by triangles. Cold tolerance responses are indicated by purple symbols. Heat 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±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±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±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±0.0223, 95% CI [−0.0243, 0.0630], z=0.870, P=0.384; and cold tolerance: estimate=0.0094±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 tolerances 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
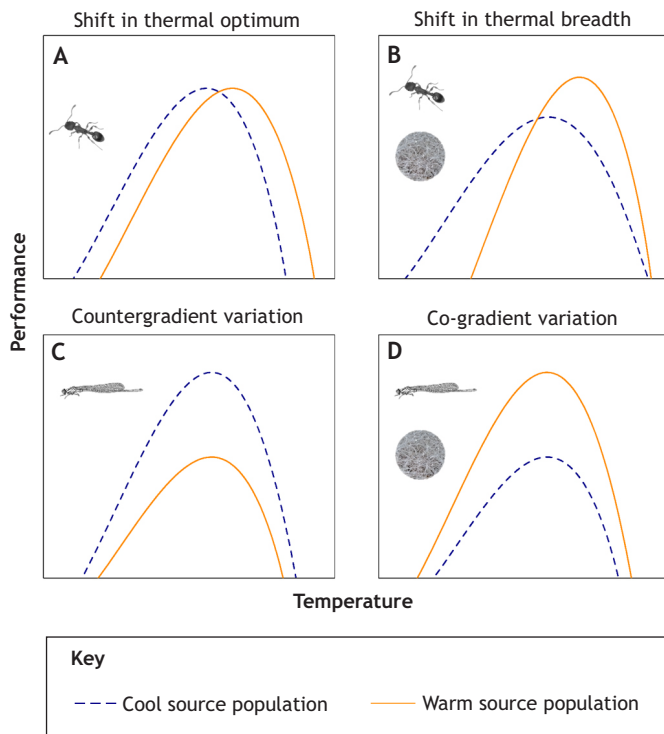
Fig. 5. Hypotheses for the evolution of thermal performance curves (following Angilletta, 2009). (A) Shift in the thermal optimum; (B) shift in thermal breadth; (C) countergradient variation; (D) co-gradient variation. In each panel, the orange line corresponds to a warm source population, and the dashed 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.

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

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

References

Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., Hunt, V. M., Apgar, T. M. and Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proc. Natl. Acad. Sci. USA* **114**, 8951-8956. doi:10.1073/pnas.1606034114

Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford: Oxford University Press.

Bennett, J. M., Calosi, P., Clusella-Trullas, S., Martínez, B., Sunday, J., Algar, A. C., Araújo, M. B., Hawkins, B. A., Keith, S., Kühn, I. et al. (2018). GlobTherm, a global database on thermal tolerances for aquatic and terrestrial organisms. *Sci. Data* **5**, 180022. doi:10.1038/sdata.2018.22

Blois, J. L., Williams, J. W., Fitzpatrick, M. C., Jackson, S. T. Ferrier, S. (2013). Space can substitute for time in predicting climate-change effects on biodiversity. *Proc. Natl. Acad. Sci. USA* **110**, 9374-9379. doi:10.1073/pnas.1220228110

Brady, S. P., Bolnick, D. I., Angert, A. L., Gonzalez, A., Barrett, R. D. H., Crispo, E., Derry, A. M., Eckert, C. G., Fraser, D. J., Fussmann, G. F. et al. (2019). Causes of maladaptation. *Evol. Appl.* **12**, 1229-1242. doi:10.1111/eva.12844

Brans, K. I. and De Meester, L. (2018). City life on fast lanes: urbanization induces an evolutionary shift towards a faster life style in the water flea *Daphnia*. *Funct. Ecol.* **32**, 2225-2240. doi:10.1111/1365-2435.13184

Brans, K. I., Jansen, M., Vanoverbeke, J., Tüzün, N., Stoks, R. and De Meester, L. (2017). The heat is on: genetic adaptation to urbanization mediated by thermal tolerance and body size. *Glob. Change Biol.* **23**, 5218-5227. doi:10.1111/gcb.13784

Brans, K. I., Stoks, R. and De Meester, L. (2018). Urbanization drives genetic differentiation in physiology and structures the evolution of pace-of-life syndromes in the water flea *Daphnia magna*. *Proc. R. Soc. B Biol. Sci.* **285**, 20180169. doi:10.1098/rspb.2018.0169

Buckley, L. B. and Huey, R. B. (2016). How extreme temperatures impact organisms and the evolution of their thermal tolerance. *Integr. Comp. Biol.* **56**, 98-109. doi:10.1093/icb/icw004

Bush, A., Mokany, K., Catullo, R., Hoffmann, A., Kellermann, V., Sgrò, C., McEvey, S. and Ferrier, S. (2016). Incorporating evolutionary adaptation in species distribution modelling reduces projected vulnerability to climate change. *Ecol. Lett.* **19**, 1468-1478. doi:10.1111/ele.12696

Campbell-Staton, S. C., Winchell, K. M., Rochette, N. C., Fredette, J., Maayan, I., Schweizer, R. M. and Catchen, J. (2020). Parallel selection on thermal physiology facilitates repeated adaptation of city lizards to urban heat islands. *Nat. Ecol. Evol.* **4**, 652-658. doi:10.1038/s41559-020-1131-8

Caruso, C. M., Martin, R. A., Sletvold, N., Morrissey, M. B., Wade, M. J., Augustine, K. E., Carlson, S. M., MacColl, A. D. C., Siepielski, A. M. and Kingsolver, J. G. (2017). What are the environmental determinants of phenotypic selection? A meta-analysis of experimental studies. *Am. Nat.* **190**, 363-376. doi:10.1086/692760

Chick, L. D., Waters, J. and Diamond, S. E. (2020). Pedal to the metal: cities power evolutionary divergence by accelerating metabolic rate and running speed. *Evol. Appl.* doi:10.1111/eva.13083

Chown, S. L. and Duffy, G. A. (2015). Thermal physiology and urbanization: perspectives on exit, entry and transformation rules. *Funct. Ecol.* **29**, 902-912. doi:10.1111/1365-2435.12478

Chown, S. L. and Gaston, K. J. (2008). Macrophysiology for a changing world. *Proc. R. Soc. B Biol. Sci.* **275**, 1469-1478. doi:10.1098/rspb.2008.0137

Chown, S. L. and Gaston, K. J. (2016). Macrophysiology – progress and prospects. *Funct. Ecol.* **30**, 330-344. doi:10.1111/1365-2435.12510

Chown, S. L., Slabber, S., McGeoch, M. A., Janion, C. and Leinaas, H. P. (2007). Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proc. R. Soc. B Biol. Sci.* **274**, 2531-2537. doi:10.1098/rspb.2007.0772

Clusella-Trullas, S., Blackburn, T. M. and Chown, S. L. (2011). Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *Am. Nat.* **177**, 738-751. doi:10.1086/660021

Damgaard, C. (2019). A critique of the space-for-time substitution practice in community ecology. *Trends Ecol. Evol.* **34**, 416-421. doi:10.1016/j.tree.2019.01.013

Diamond, S. E. (2017). Evolutionary potential of upper thermal tolerance: biogeographic patterns and expectations under climate change. *Ann. N. Y. Acad. Sci.* **1389**, 5-19. doi:10.1111/nyas.13223

Diamond, S. E. (2018). Contemporary climate-driven range shifts: putting evolution back on the table. *Funct. Ecol.* **32**, 1652-1665. doi:10.1111/1365-2435.13095

Diamond, S. (2020). Thermal tolerance of *Lasius americanus* across urbanization and geographical gradients. *Dryad, Dataset*. doi:10.5061/dryad.zw3r22871

Diamond, S. E. and Martin, R. A. (2020a). Evolutionary consequences of the urban heat island. In *Urban Evolutionary Biology*, pp. 91-110. Oxford, New York: Oxford University Press.

Diamond, S. E. and Martin, R. A. (2020b). Evolution is a double-edged sword, not a silver bullet, to confront climate change. *Ann. N. Y. Acad. Sci.* **1469**, 38-51. doi:10.1111/nyas.14410

Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A. and Martin, R. A. (2018a). Evolution of thermal tolerance and its fitness consequences: parallel and non-parallel responses to urban heat islands across three cities. *Proc. R. Soc. B* **285**, 20180036. doi:10.1098/rspb.2018.0036

Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A. and Zhao, C. (2018b). Evolution of plasticity in the city: urban acorn ants can better tolerate more rapid increases in environmental temperature. *Conserv. Physiol.* **6**, coy030. doi:10.1093/conphys/coy030

Donihue, C. M. and Lambert, M. R. (2015). Adaptive evolution in urban ecosystems. *Ambio* **44**, 194-203. doi:10.1007/s13280-014-0547-2

Ellison, A. M., Gotelli, N. J., Farnsworth, E. J. and Alpert, G. D. (2012). *A Field Guide to the Ants of New England*. Yale University Press.

Elmendorf, S. C., Henry, G. H. R., Hollister, R. D., Fosaa, A. M., Gould, W. A., Hermanutz, L., Hofgaard, A., Jónsdóttir, I. S., Jorgenson, J. C., Lévesque, E. et al. (2015). Experiment, monitoring, and gradient methods used to infer climate change effects on plant communities yield consistent patterns. *Proc. Natl. Acad. Sci. USA* **112**, 448-452. doi:10.1073/pnas.1410088112

Gorton, A. J., Moeller, D. A. and Tiffin, P. (2018). Little plant, big city: a test of adaptation to urban environments in common ragweed (*Ambrosia artemisiifolia*). *Proc. Biol. Sci.* **285**, 20180968. doi:10.1098/rspb.2018.0968

Gunderson, A. R. and Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B Biol. Sci.* **282**, 20150401. doi:10.1098/rspb.2015.0401

Helmuth, B. (2009). From cells to coastlines: how can we use physiology to forecast the impacts of climate change? *J. Exp. Biol.* **212**, 753-760. doi:10.1242/jeb.023861

Hendry, A. P. and Kinnison, M. T. (1999). Perspective: the pace of modern life: measuring rates of contemporary microevolution. *Evolution* **53**, 1637-1653. doi:10.1111/j.1558-5646.1999.tb04550.x

Hoffmann, A. A. and Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature* **470**, 479-485. doi:10.1038/nature09670

Hoffmann, A. A., Chown, S. L. and Clusella-Trullas, S. (2013). Upper thermal limits in terrestrial ectotherms: how constrained are they? *Funct. Ecol.* **27**, 934-949. doi:10.1111/j.1365-2435.2012.02036.x

Hölldobler, B. and Wilson, E. O. (1990). *The Ants*. Berlin, Heidelberg: Springer-Verlag.

Huey, R. B. and Kingsolver, J. G. (1993). Evolution of resistance to high temperature in ectotherms. *Am. Nat.* **142**, S21-S46. doi:10.1086/285521

Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Pérez, H. J. Á. and Garland, T. (2009). Why tropical forest lizards are vulnerable to climate warming. *Proc. R. Soc. Lond. B Biol. Sci.* **276**, 1939-1948. doi:10.1098/rspb.2008.1957

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M. and Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **367**, 1665-1679. doi:10.1098/rstb.2012.0005

Imhoff, M. L., Zhang, P., Wolfe, R. E. and Bounoua, L. (2010). Remote sensing of the urban heat island effect across biomes in the continental USA. *Remote Sens. Environ.* **114**, 504-513. doi:10.1016/j.rse.2009.10.008

Johnson, M. T. J. and Munshi-South, J. (2017). Evolution of life in urban environments. *Science* **358**, eaam8327. doi:10.1126/science.aam8327

Keller, L. (1998). Queen lifespan and colony characteristics in ants and termites. *Insectes Soc.* **45**, 235-246. doi:10.1007/s000400050084

Kingsolver, J. G. and Buckley, L. B. (2017). Quantifying thermal extremes and biological variation to predict evolutionary responses to changing climate. *Phil. Trans. R. Soc. B* **372**, 20160147. doi:10.1098/rstb.2016.0147

Lambert, M. R. and Donihue, C. M. (2020). Urban biodiversity management using evolutionary tools. *Nat. Ecol. Evol.* **4**, 903-910. doi:10.1038/s41559-020-1193-7

Magozzi, S. and Calosi, P. (2015). Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming. *Glob. Change Biol.* **21**, 181-194. doi:10.1111/gcb.12695

Martin, R. A., Chick, L. D., Garvin, M. L. and Diamond, S. E. (2020). In a nutshell, a reciprocal transplant experiment reveals local adaptation and fitness trade-offs in response to urban evolution in an acorn-dwelling ant. *bioRxiv* 2020.08.21.251025. doi:10.1101/2020.08.21.251025

Martin, R. A., Chick, L. D., Yilmaz, A. R. and Diamond, S. E. (2019). Evolution, not transgenerational plasticity, explains the adaptive divergence of acorn ant thermal tolerance across an urban-rural temperature cline. *Evol. Appl.* **12**, 1678-1687. doi:10.1111/eva.12826

McLean, M. A., Angilletta, M. J. and Williams, K. S. (2005). If you can't stand the heat, stay out of the city: thermal reaction norms of chitinolytic fungi in an urban heat island. *J. Therm. Biol.* **30**, 384-391. doi:10.1016/j.jtherbio.2005.03.002

Merilä, J. and Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol. Appl.* **7**, 1-14. doi:10.1111/eva.12137

Mitchell-Olds, T. and Shaw, R. G. (1987). Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* **41**, 1149-1161. doi:10.1111/j.1558-5646.1987.tb02457.x

Muñoz, M. M. and Losos, J. B. (2017). Thermoregulatory behavior simultaneously promotes and forestalls evolution in a tropical lizard. *Am. Nat.* **191**, E15-E26. doi:10.1086/694779

Overgaard, J., Kearney, M. R. and Hoffmann, A. A. (2014). Sensitivity to thermal extremes in Australian *Drosophila* implies similar impacts of climate change on the distribution of widespread and tropical species. *Glob. Change Biol.* **20**, 1738-1750. doi:10.1111/gcb.12521

Penick, C. A., Diamond, S. E., Sanders, N. J. and Dunn, R. R. (2017). Beyond thermal limits: comprehensive metrics of performance identify key axes of thermal adaptation in ants. *Funct. Ecol.* **31**, 1091-1100. doi:10.1111/1365-2435.12818

Radchuk, V., Reed, T., Teplitsky, C., Pol, M. van de, Charmantier, A., Hassall, C., Adamík, P., Adriaensen, F., Ahola, M. P., et al. (2019). Adaptive responses of animals to climate change are most likely insufficient. *Nat. Commun.* **10**, 1-14. doi:10.1038/s41467-019-10924-4

Razgour, O., Forester, B., Taggart, J. B., Bekaert, M., Juste, J., Ibáñez, C., Puechmaille, S. J., Novella-Fernandez, R., Alberdi, A. and Manel, S. (2019). Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. *Proc. Natl. Acad. Sci. USA* **116**, 10418-10423. doi:10.1073/pnas.1820663116

Rivkin, L. R., Santangelo, J. S., Alberti, M., Aronson, M. F. J., Keyzer, C. W. de, Diamond, S. E., Fortin, M.-J., Frazee, L. J., Gorton, A. J., et al. (2019). A roadmap for urban evolutionary ecology. *Evol. Appl.* **12**, 384-398. doi:10.1111/eva.12734

Schär, S., Talavera, G., Espadaler, X., Rana, J. D., Andersen, A. A., Cover, S. P. and Vila, R. (2018). Do Holarctic ant species exist? Trans-Beringian dispersal and homoplasy in the Formicidae. *J. Biogeogr.* **45**, 1917-1928. doi:10.1111/jbi.13380

Seebacher, F., White, C. R. and Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Change* **5**, 61-66. doi:10.1038/nclimate2457

Sgrò, C. M., Terblanche, J. S. and Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annu. Rev. Entomol.* **61**, 433-451. doi:10.1146/annurev-ento-010715-023859

Shephard, A. M., Mitchell, T. S., Henry, S. B., Oberhauser, K. S., Kobiela, M. E. and Snell-Rood, E. C. (2020). Assessing zinc tolerance in two butterfly species: consequences for conservation in polluted environments. *Insect Conserv. Divers.* **13**, 201-210. doi:10.1111/icad.12404

Sinclair, B. J., Ferguson, L. V., Salehipour-shirazi, G. and MacMillan, H. A. (2013). Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. *Integr. Comp. Biol.* **53**, 545-556. doi:10.1093/icb/ict004

Sinervo, B., Méndez-de-la-Cruz, F., Miles, D. B., Heulin, B., Bastiaans, E., Cruz, M. V.-S., Lara-Resendiz, R., Martínez-Méndez, N., Calderón-Espinosa, M. L., Meza-Lázaro, R. N. et al. (2010). Erosion of lizard diversity by climate change and altered thermal niches. *Science* **328**, 894-899. doi:10.1126/science.1184695

Snell-Rood, E. C., Kobiela, M. E., Sikkink, K. L. and Shephard, A. M. (2018). Mechanisms of plastic rescue in novel environments. *Annu. Rev. Ecol. Evol. Syst.* **49**, 331-354. doi:10.1146/annurev-ecolsys-110617-062622

Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* **213**, 912-920. doi:10.1242/jeb.037473

Sørensen, J. G., Kristensen, T. N. and Overgaard, J. (2016). Evolutionary and ecological patterns of thermal acclimation capacity in *Drosophila*: is it important for keeping up with climate change? *Curr. Opin. Insect Sci.* **17**, 98-104. doi:10.1016/j.cois.2016.08.003

Start, D., Bonner, C., Weis, A. E. and Gilbert, B. (2018). Consumer-resource interactions along urbanization gradients drive natural selection. *Evolution* **72**, 1863-1873. doi:10.1111/evo.13544

Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science* **301**, 65-65. doi:10.1126/science.1083073

Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P. M. et al. (2013). Climate change 2013: the physical science basis. *Contrib. Work. Group Fifth Assess. Rep. Intergov. Panel Clim. Change.*

Sunday, J. M., Bates, A. E. and Dulvy, N. K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proc. R. Soc. Lond. B Biol. Sci.* **278**, 1823-1830. doi:10.1098/rspb.2010.1295

Sunday, J. M., Bates, A. E. and Dulvy, N. K. (2012). Thermal tolerance and the global redistribution of animals. *Nat. Clim. Change* **2**, 686-690. doi:10.1038/nclimate1539

Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K., Longino, J. T. and Huey, R. B. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proc. Natl. Acad. Sci. USA* **111**, 5610-5615. doi:10.1073/pnas.1316145111

Szulkin, M., Garroway, C. J., Corsini, M., Kotarba, A. Z. and Dominoni, D. (2020). How to quantify urbanization when testing for urban evolution? In *Urban Evolutionary Biology*. pp. 13–33, Oxford: Oxford University Press.

Thompson, K. A., Renaudin, M. and Johnson, M. T. J. (2016). Urbanization drives the evolution of parallel clines in plant populations. *Proc. R. Soc. B Biol. Sci.* **283**, 20162180. doi:10.1098/rspb.2016.2180

Tüzün, N. and Stoks, R. (2018). Evolution of geographic variation in thermal performance curves in the face of climate change and implications for biotic interactions. *Curr. Opin. Insect Sci.* **29**, 78-84. doi:10.1016/j.cois.2018.07.004

Tüzün, N., Op de Beeck, L., Brans, K. I., Janssens, L. and Stoks, R. (2017). Microgeographic differentiation in thermal performance curves between rural and urban populations of an aquatic insect. *Evol. Appl.* **10**, 1067-1075. doi:10.1111/eva.12512

Verheyen, J., Tüzün, N. and Stoks, R. (2019). Using natural laboratories to study evolution to global warming: contrasting altitudinal, latitudinal, and urbanization gradients. *Curr. Opin. Insect Sci.* **35**, 10-19. doi:10.1016/j.cois.2019.06.001

Wade, M. J. and Kalisz, S. (1990). The causes of natural selection. *Evolution* **44**, 1947-1955. doi:10.1111/j.1558-5646.1990.tb04301.x

West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. Oxford: Oxford University Press.

Wogan, G. O. U. and Wang, I. J. (2018). The value of space-for-time substitution for studying fine-scale microevolutionary processes. *Ecography* **41**, 1456-1468. doi:10.1111/ecog.03235

Wolkovich, E. M., Cook, B. I., Allen, J. M., Crimmins, T. M., Betancourt, J. L., Travers, S. E., Pau, S., Regetz, J., Davies, T. J., Kraft, N. J. B. et al. (2012). Warming experiments underpredict plant phenological responses to climate change. *Nature* **485**, 494-497. doi:10.1038/nature11014

Yilmaz, A. R., Diamond, S. E. and Martin, R. A. (2020). Evidence for the evolution of thermal tolerance, but not desiccation tolerance, in response to hotter, drier city conditions in a cosmopolitan, terrestrial isopod. *Evol. Appl.* doi:10.1111/eva.13052

Youngsteadt, E., Dale, A. G., Terando, A. J., Dunn, R. R. and Frank, S. D. (2015). Do cities simulate climate change? A comparison of herbivore response to urban and global warming. *Glob. Change Biol.* **21**, 97-105. doi:10.1111/gcb.12692

Zhao, L., Lee, X., Smith, R. B. and Oleson, K. (2014). Strong contributions of local background climate to urban heat islands. *Nature* **511**, 216-219. doi:10.1038/nature13462

Supplementary Information

Supplementary Text

Lasius americanus case study: urban evolution versus biogeographic clines in thermal tolerance

<u>Urban evolution component</u>

Colony collections
        For the urban evolution study, we collected queenright colonies of the North American woodland ant, *Lasius americanus* (formerly, *Lasius alienus*, Schär et al., 2018). We collected newly founded colonies inhabiting tree nuts (acorn and hickory nuts) that allowed us to retain the entire colony. Mature colonies inhabit soil, leaf litter and rotting logs (Ellison et al., 2012), which frequently prevents the collection of entire colonies. Colony collection dates spanned 6 June to 7 June 2016. At the time of collection, colonies contained one queen and 3 to 40 workers (mean ± 1 SD = 16.1 ± 9.72). Colonies originated from sites in the greater Knoxville, Tennessee, USA area (36° N latitude). Rural sites were located within a 2km radius of I.C. King park (35.8912, -83.9473) and urban sites were located within a 2km radius of the University of Tennessee Agriculture Education Center (35.8982, -83.9567). Rural sites were those designated as 0% developed impervious surface area (ISA) whereas urban sites were those with 40-60% ISA. The growing season temperature difference in the microclimates that acorn ants inhabit between these specific urban and rural sites is 3.64 °C (Diamond et al., 2018).

Laboratory rearing
        Field-collected colonies were randomly assigned to one of five temperature treatments in laboratory growth chambers. Note that because colonies generally require the presence of the queen for the production of new workers, we were unable to split members of a single colony across the temperature treatments. The temperature treatments each include a ± 5 °C diurnal temperature shift from a baseline daytime temperature of 21, 23, 25, 27, or 29 °C, synced with a 14:10 L:D photoperiod (following Diamond et al., 2018). Colonies were housed separately and were provided with a continuous supply of sugar water and dead mealworms. Colonies were held within the growth chambers for a minimum of 10 weeks to allow a new cohort of workers to be generated. Lab-born workers were then assessed for heat and cold tolerance.

Physiological trait assays
        We used the critical thermal maximum (CT_{max}) and the critical thermal minimum (CT_{min}) as our measures of heat and cold tolerance, respectively. For CT_{max}, worker ants were placed individually into 1.5 mL Eppendorf tubes which were inserted into a dry-block incubator. The initial temperature of the incubator was set at 34 °C and the temperature was raised by 1 °C every minute until the loss of muscular coordination, at which point the CT_{max} (in °C) was recorded. We employed a similar procedure to assess CT_{min}, except that the initial temperature of the incubator was set at 16 °C and the temperature was decreased by 1 °C every minute until the loss of muscular coordination, at which point the CT_{min} (in °C) was recorded. We assessed heat and cold tolerance of a total of 296 individuals from 5 rural colonies and 12 urban colonies. Because the assessment of heat and cold tolerance is destructive, we were unable to measure heat

and cold tolerance on the same individual. As a consequence, 149 of the total thermal tolerance estimates were for heat tolerance and 147 were for cold tolerance. These individual estimates were generally well balanced across the colony units. For each colony, a minimum of 10 workers were assessed for heat tolerance and another 10 workers for cold tolerance except for one colony where 5 workers were assessed for heat tolerance and 4 workers for cold tolerance.

Data analysis

To quantify the evolution of heat and cold tolerance in response to urban heat island effects, we constructed linear mixed effects models. Separate models were constructed for heat and cold tolerance. We developed models that included the main effects of source population (urban versus rural), rearing temperature (treated as a continuous variable), and their interaction. Colony identity was treated as a random intercept in all models.

Biogeographic cline component

Colony collections

For the biogeographic cline study, we used previously published data on the thermal tolerance of *Lasius americanus* (Diamond and Chick, 2018). Although these methods are described in Diamond and Chick (2018), we provide a brief summary here. We collected colony fragments (*i.e.*, workers only) from *L. americanus* colonies inhabiting forests of eastern North America. There were 24 sites from 33.1 to 42.6 °N latitude, representing a span of 6.9 to 18.1 °C in mean annual temperature. All collection sites were in natural areas, far from urban heat island effects. Colony fragment collections occurred during the growing season (May-September) 2014. Colony fragments were housed individually according to their colony of origin.

Physiological trait assays

Colony fragments were held for a minimum of 48 hours at ambient room temperature (~ 25 °C), and thermal tolerance assays were performed within one week of collection from the field. The methods used to assess CT_{max} and CT_{min} were the same dynamic temperature ramping protocol as for the urban evolution study component described above. A total of 246 workers were assessed for thermal tolerance including 131 for heat tolerance and 115 for cold tolerance.

Data analysis

To quantify the effect of latitudinal variation in temperature on heat and cold tolerance, we constructed linear mixed effects models. We performed separate models for heat and cold tolerance. In each model, mean annual temperature (in °C) was included as a continuous predictor. Colony identity was treated as a random intercept.

Thermal tolerance trait change across urbanization and biogeographic gradients

Literature search

We began our analysis of the magnitude and direction of thermal tolerance trait change across urbanization gradients versus biogeographic gradients by identifying studies on either the

evolution of thermal tolerance in cities, or, urban-driven phenotypic shifts in thermal tolerance. Using these studies as a foundation, we then sought out corresponding data on biogeographic clines in thermal tolerance for that particular species. Urban evolution and phenotypic trait change studies focused on thermal tolerance traits were identified from a recent review of the topic (Diamond and Martin, 2020) and a literature search using Google Scholar with combinations of the following terms: "urban", "urbanization", "land-use change", "city", "human settlement", "evolution", "adaptation", "thermal tolerance", "CT_{max}", "CT_{min}", "heat tolerance", "cold tolerance", "chill coma recovery", "urban heat island", "temperature", "phenotype", and "trait change". The last literature search was performed in April 2020. In some cases, thermal tolerance trait data across biogeographic clines in temperature were not available for the species identified in a given urban study. When we could not find exact species matches between urban and biogeographic studies, we found the phylogenetically closest relative for which biogeographic data were available. In some cases, we were able to identify sister species such as between *Anolis cristatellus* and *Anolis pulchellus*, although in others, the comparison group was quite distant such as between *Apis mellifera* and *Bombus* sp. (see Table S2 for urbanization-biogeographic cline species pairs).

Study inclusion criteria

We placed limits on the studies for inclusion in our analysis with respect to the type of thermal tolerance estimate used. Because we aimed to directly interpret the magnitude of trait change per °C change in environmental temperature, we limited our analyses to those studies that reported heat and cold tolerance estimates (specifically CT_{max} and CT_{min}) in units of °C, rather than other measures such as time to recover from heat or cold stress. All studies from our literature search that met these criteria were included in our analysis.

Data analysis

Our general analytical approach was to standardize the magnitude and direction of thermal tolerance trait change by the magnitude of warming across urbanization and biogeographic gradients (either latitude or elevation). For heat tolerance, values were positive if urban or low latitude/elevation heat tolerance exceeded rural or high latitude/elevation heat tolerance, that is, when urban or low latitude/elevation populations exhibited a greater capacity to tolerate heat stress (urban or low latitude/elevation CT_{max} > rural or high latitude/elevation CT_{max}). For cold tolerance, values were negative if rural or high latitude/elevation cold tolerance exceeded urban or low latitude/elevation cold tolerance, that is, when urban or low latitude/elevation populations lost their capacity to tolerate cold stress (urban or low latitude/elevation CT_{min} > rural or high latitude/elevation CT_{min}).

All environmental temperature differences were expressed as changes in mean temperature across urban versus rural habitats or across latitude/elevation gradients. Differences in temperature between warmed urban sites experiencing heat island effects and rural sites were taken from values reported by the authors in each study. Differences in mean annual temperature across latitude or elevation were obtained from WorldClim (Fick and Hijmans, 2017).

When only the endpoints of the biogeographic gradient were available, we computed the difference of high versus low latitude or elevation trait values (always subtracting the cool environment from the warm environment) and divided by the difference in mean annual temperature across the two sites. When more than two biogeographic data points were available, we computed the slope of the relationship between the trait values and the mean annual

temperature of each site. In the case that multiple urbanization gradient trait and environmental data were available, we re-used the same biogeographic trait and environmental data for each corresponding urbanization gradient. We performed a similar data categorization for the urbanization gradient. When data were reported as urban versus rural (or nonurban), we computed the trait and environmental differences of these two sites (always subtracting the rural environment from the warm environment), and when data were reported for multiple sites within the same urbanization gradient, we computed the slope of the relationship between the trait value and environmental temperature.

When possible, we used raw datasets provided by the authors either in supplementary material of the relevant journal article(s) or through publicly available data repositories. In the case that raw data were not available, we extracted either raw data or summary statistics (trait means) from the article figures and/or tables. To extract data from figures, we used the WebPlotDigitizer software (Rohatgi, 2019). In all cases, our experimental units for analysis were based on the data reported by the authors of each study. When possible, we reported the trait values for each combination of study, city and species. However, some authors reported aggregate statistics with no accompanying raw data, for example, trait data pooled across multiple urbanization gradients, in which case, we followed the conventions of the authors and analyzed the urbanization versus biogeographic trait change per °C environmental temperature change at the level of the entire group of cities.

Given the variation in data sources for this analysis, it was not possible to obtain meaningful standard errors in both the trait change and environmental temperature change across urbanization and biogeographic gradients. As a consequence, we report and analyze mean trait change in heat and cold tolerance across urbanization and biogeographic gradients. Specifically, we did not weight estimates by their standard error during analysis. We performed a Chi-squared contingency table test, counting the number of cases where the urbanization and biogeographic responses were in the same direction versus different directions, and testing whether these counts were significantly different from a 50/50 expectation. We analyzed heat and cold tolerance traits together to have sufficient cell counts to satisfy the assumptions of the Chi-squared contingency analysis, although we did find comparable results when we separated the analyses on the basis of tolerance trait type. In addition, we performed a simple linear model of the tolerance trait change (separately for heat and cold tolerance) as a function of urbanization versus biogeographic cline. This model allowed us to assess whether the magnitude of trait change was significantly greater for either the urbanization cline or for the biogeographic cline. All of the data that formed the basis of these analyses can be found in Table S2.

Meta-analysis of phenotypic versus evolved shifts in thermal tolerance

Literature search and study inclusion criteria

We used the same literature search as the one for the analysis of trait change across urbanization versus biogeographic gradients. Because we were interested in how thermal tolerance traits responded to urbanization when only phenotypes could be measured versus genetic changes could be demonstrated, we were able to expand the range of studies we considered to not only include CT_{max} and CT_{min} but other related traits including heat stress resistance time, heat stress recovery time, and chill coma recovery time.

Phenotypic studies involved assessment of thermal tolerance trait from field-caught individuals (or individuals that were reared briefly in the laboratory, but not for a complete generation). By contrast, evolutionary studies involved laboratory common garden experiments in which the study organism was reared for at least one generation in the laboratory prior to assessment of thermal tolerance traits. In these cases, evolutionary divergence can be disentangled from plastic responses to urban heat island effects.

Data analysis

We performed a formal meta-analysis of the magnitude and direction of phenotypic versus evolved shifts in thermal tolerance traits across urbanization gradients. Our effect size for the meta-analysis was computed as the standardized mean difference between urban and rural trait values using Cohen's *d*. When possible, we computed Cohen's *d* from the raw data using the *cohen.d* function from the *effsize* library in R (Torchiano, 2020). In other cases, we computed Cohen's *d* from means and variances extracted from figures (again, using WebPlotDigitizer), tables, the article text (mostly for information on sample sizes), and from linear model test statistics. Here, we used the *fes*, *mes*, and *tes* functions from the *compute.es* library (Del Re, 2013).

Because Cohen's *d* is defined as the standardized mean difference between two groups, in some cases this required continuous data taken along an urbanization gradient to be transformed into a binary variable of urban versus rural. When this occurred, we followed the authors' assessment of site groupings into more urbanized sites versus more natural sites.

Our final meta-analysis dataset included a total of 39 individual effect sizes for the difference in thermal tolerance traits between urban and rural populations. There were 24 estimates for phenotypic data only and 15 estimates for which evolutionary change was demonstrated. There were 25 estimates of heat tolerance and 14 estimates of cold tolerance. Combined, these estimates came from 15 individual studies and from 15 different species, though not each study reported data on a single species. Some studies reported data from multiple species; and for a couple species in the meta-analysis, multiple studies were focused on the same species. We conducted a formal random effects meta-analysis using the *rma* function from the *metafor* library in R (Viechtbauer, 2010). Because we computed all values of Cohen's *d* either from raw or summary data, we were able to include their associated measurement error variances in our meta-analytic models. We included two moderators in our meta-analytic model, including the type of thermal tolerance trait (heat tolerance versus cold tolerance) and the type of variation (whether only phenotypic data were available versus whether evolution, *i.e.* genetic changes among populations, could be demonstrated). All of the data that formed the basis of these analyses can be found in Table S3.

Evolutionary divergence in thermal tolerance versus plasticity

Literature search and study inclusion criteria

Our final analysis explored the magnitude and direction of evolutionary divergence in thermal tolerance traits versus the magnitude and direction of thermal plasticity. To accomplish this, we used a paired design, identifying species for which data on evolutionary divergence of

thermal tolerance traits in response to urbanization and thermal plasticity of tolerance traits were available.

We used the results of the literature search for the analysis of trait change across urbanization versus biogeographic gradients to identify studies with evolutionary change in thermal tolerance traits across urbanization gradients. We then restricted this list of studies to those that not only compute evolved differences between urban and rural populations but also the plastic response to temperature. We computed the magnitude and direction of evolutionary divergence as the difference in trait means between urban and rural populations, always subtracting the rural from the urban trait mean. Note that we left these values as the mean trait difference between urban and rural populations; we did not standardize these evolutionary divergence measures by the magnitude of warming across the urban heat island gradient as we did in the urban versus biogeographic trait change analysis. We adopted this approach so that the bivariate means and standard errors for evolved and plastic shifts in thermal tolerance would be directly interpretable. We followed the conventions we established earlier for our other analyses, in that a gain of heat tolerance in response to urban warming was assigned a positive value, and a loss of cold tolerance in response to urban warming was assigned a negative value.

Data analysis

We computed thermal tolerance plasticity as the slope of the relationship between the trait and laboratory rearing temperature, that is, plasticity is expressed as the rate of trait change per °C of warming. We preserved the direction of plasticity, such that slopes are allowed to be either positive or negative in response to warming. We used formal meta-analytical models (using the *rma* function from the *metafor* library as described above) to test whether evolutionary divergence in heat and cold tolerance traits and thermal plasticity were significantly different from zero. We also used formal meta-analysis with moderators to test whether there was a significant association between evolutionary divergence and thermal plasticity, and whether urban versus rural population thermal plasticity was significantly different across populations. All of the data that formed the basis of these analyses can be found in Table S4.

Supplementary Tables

Table S1. Estimates, standard errors, test statistics and *P*-values from models of temperature effects on heat tolerance (CT_{max}) and cold tolerance (CT_{min}) of *Lasius americanus*. For the urbanization gradient, the results of linear mixed effects models with the predictors of rearing temperature, habitat type and their interaction plus a random intercept of colony identity are provided. For the biogeographic gradient, the results of linear mixed effects models with the predictor of mean annual temperature plus a random intercept of colony identity are provided.

Gradient	Tolerance trait	Model term	Estimate	SE	*F*	*P*
Urbanization	CT_{max}	Intercept	41.9	0.968	473000	< 0.0001
		Temperature treatment	0.025	0.0385	16.1	0.00203
		Habitat type (rural - urban)	-2.21	1.21	82.8	< 0.0001
		Temperature × Habitat	0.138	0.0486	8.08	0.016
	CT_{min}	Intercept	3.9	1.16	7040	< 0.0001
		Temperature treatment	0.08	0.0463	8.15	0.0156
		Habitat type (rural - urban)	0.238	1.47	25.5	0.000373
		Temperature × Habitat	0.0233	0.059	0.157	0.7
Biogeographic	CT_{max}	Intercept	36.5	0.701	72600	< 0.0001
		Mean annual temperature	0.447	0.0606	54.3	< 0.0001
	CT_{min}	Intercept	-1.24	0.484	1060	< 0.0001
		Mean annual temperature	0.397	0.0413	92.1	< 0.0001

Table S2. Thermal tolerance trait change across urbanization and biogeographic gradients.

	Urbanization				Biogeographic gradient					Trait data	
Study citation	Type of study	Species	City or region	Study citation	Type of study	Species	Cline location	Latitude or elevation	Tolerance type	Urbanization trait change per °C	Biogeographic trait change per °C
Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Cleveland, Ohio, USA	Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Eastern USA	latitude	CTmin	0.314	0.346
Diamond et al., 2017	lab common garden	Temnothorax curvispinosus	Cleveland, Ohio, USA	Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Eastern USA	latitude	CTmin	0.253	0.346
Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Cleveland, Ohio, USA	Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Eastern USA	latitude	CTmax	0.080	0.131
Diamond et al., 2017	lab common garden	Temnothorax curvispinosus	Cleveland, Ohio, USA	Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Eastern USA	latitude	CTmax	0.228	0.131
Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Cincinatti, Ohio, USA	Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Eastern USA	latitude	CTmin	-0.501	0.346
Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Cincinatti, Ohio, USA	Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Eastern USA	latitude	CTmax	-0.002	0.131
Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Knoxville, Tennessee, USA	Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Eastern USA	latitude	CTmin	0.594	0.346
Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Knoxville, Tennessee, USA	Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Eastern USA	latitude	CTmax	0.065	0.131
Martin et al., 2019	lab common garden	Temnothorax curvispinosus	Cleveland, Ohio, USA	Diamond et al., 2018	lab common garden	Temnothorax curvispinosus	Eastern USA	latitude	CTmax	0.375	0.131
Martin et al., 2019	common garden	Temnothorax curvispinosus	Cleveland, Ohio, USA	Diamond et al., 2018	common garden	Temnothorax curvispinosus	Eastern USA	latitude	CTmin	0.398	0.346

Yilmaz et al., in press	lab common garden	Oniscus asellus	Cleveland, Ohio, USA	Castañeda et al., 2004	field caught	Porcellio laevis	Chile	latitude	CTmin	1.723	0.522
Yilmaz et al., in press	lab common garden	Oniscus asellus	Cleveland, Ohio, USA	Castañeda et al., 2004	field caught	Porcellio laevis	Chile	latitude	CTmax	0.545	-0.241
Brans et al., 2017	lab common garden	Daphnia magna	Flanders, Belgium	Geerts et al., 2015	common garden	Daphnia magna	Western Europe	latitude	CTmax	0.525	0.026
This paper	lab common garden	Lasius alienus	Knoxville, Tennessee, USA	Diamond and Chick, 2018	field caught	Lasius alienus	Eastern USA	latitude	CTmax	0.263	0.447
This paper	lab common garden	Lasius alienus	Knoxville, Tennessee, USA	Diamond and Chick, 2018	field caught	Lasius alienus	Eastern USA	latitude	CTmin	0.172	0.397
Warren et al., 2018	field caught	Aphaenogaster rudis/picea	Buffalo, New York, USA	Diamond and Chick, 2018	field caught	Aphaenogaster rudis/picea	Eastern USA	latitude	CTmax	0.353	0.525
Warren et al., 2018	field caught	Aphaenogaster rudis/picea	Buffalo, New York, USA	Diamond and Chick, 2018	field caught	Aphaenogaster rudis/picea	Eastern USA	latitude	CTmin	-0.882	0.512
Sánchez-Echeverría et al., 2019	field caught	Apis mellifera	Pachuca, Hidalgo, Mexico	Oyen et al., 2016	field caught	Bombus sp.	Western USA	elevation	CTmin	0.377	0.319
Sánchez-Echeverría et al., 2019	field caught	Apis mellifera	Pachuca, Hidalgo, Mexico	Oyen et al., 2016	field caught	Bombus sp.	Western USA	elevation	CTmax	0.151	0.494
Burdine and McCluney, 2019	field caught	Apis mellifera	Toledo, Ohio, USA	Oyen et al., 2016	field caught	Bombus sp.	Western USA	elevation	CTmax	1.683	0.494
Burdine and McCluney, 2019	field caught	Bombus impatiens	Toledo, Ohio, USA	Oyen et al., 2016	field caught	Bombus sp.	Western USA	elevation	CTmax	1.546	0.494
Burdine and	field caught	Agapostemon sericeus	Toledo, Ohio, USA	Oyen et al., 2016	field caught	Bombus sp.	Western USA	elevation	CTmax	0.492	0.494

McCluney, 2019 Campbell-Staton et al., 2020	field caught	Anolis cristatellus	Aguadilla, Puerto Rico	Hertz, 1979	field caught	Anolis pulchellus	Puerto Rico	elevation	CTmax	0.295	0.333
Campbell-Staton et al., 2020	field caught	Anolis cristatellus	Arecibo, Puerto Rico	Hertz, 1979	field caught	Anolis pulchellus	Puerto Rico	elevation	CTmax	0.395	0.333
Campbell-Staton et al., 2020	field caught	Anolis cristatellus	Mayagüez, Puerto Rico	Hertz, 1979	field caught	Anolis pulchellus	Puerto Rico	elevation	CTmax	0.274	0.333
Campbell-Staton et al., 2020	field caught	Anolis cristatellus	San Juan, Puerto Rico	Hertz, 1979	field caught	Anolis pulchellus	Puerto Rico	elevation	CTmax	0.114	0.333

Table S3. Data used in the meta-analysis of phenotypic versus evolved shifts in thermal tolerance. Rows are in same order as Fig. 3, from top to bottom.

Study citation	Species	City or region	Trait data source	R function to obtain effect size	Tolerance type	Tolerance moderator	Generation	Phenotype versus evolution	Cohen's d	Lower 95% CI	Upper 95% CI
Burdine and McCluney, 2019	Agapostemon sericeus	Toledo, Ohio, USA	raw	cohen.d	CTmax	max	Field caught	phenotype	0.39	-0.36	1.15
Campbell-Staton et al., 2020	Anolis cristatellus	Aguadilla, Puerto Rico	figure (mean, se, n)	mes	CTmax	max	Field caught	phenotype	0.59	-0.12	1.29
Campbell-Staton et al., 2020	Anolis cristatellus	Arecibo, Puerto Rico	figure (mean, se, n)	mes	CTmax	max	Field caught	phenotype	0.79	0.16	1.43
Campbell-Staton et al., 2020	Anolis cristatellus	Mayagüez, Puerto Rico	figure (mean, se, n)	mes	CTmax	max	Field caught	phenotype	1.32	0.60	2.04
Campbell-Staton et al., 2020	Anolis cristatellus	San Juan, Puerto Rico	figure (mean, se, n)	mes	CTmax	max	Field caught	phenotype	0.34	-0.29	0.97
Warren et al., 2018	Aphaenogaster rudis	Buffalo, New York, USA	raw	cohen.d	CTmax	max	Field caught	phenotype	0.86	-0.39	2.11
Warren et al., 2018	Aphaenogaster rudis	Buffalo, New York, USA	raw	cohen.d	CTmin	min	Field caught	phenotype	-0.06	-1.29	1.17
Sánchez-Echeverría et al., 2019	Apis mellifera	Pachuca, Hidalgo, Mexico	text, test statistic (t, n)	tes	CTmax	max	Field caught	phenotype	0.29	-0.49	1.08
Burdine and McCluney, 2019	Apis mellifera	Toledo, Ohio, USA	raw	cohen.d	CTmax	max	Field caught	phenotype	0.22	-0.53	0.98
Sánchez-Echeverría et al., 2019	Apis mellifera	Pachuca, Hidalgo, Mexico	text, test statistic (t, n)	tes	CTmin	min	Field caught	phenotype	-0.92	-1.74	-0.11

Study	Species	Location	Data type	Metric	Trait	max/min			Value	Lower	Upper
Angilletta et al., 2007	Atta sexdens rubropilosa	São Paulo, Brazil	text, summary statistic (mean, se, n)	mes	Heat stress resistance time	max	Field caught	phenotype	0.74	0.48	1.01
Angilletta et al., 2007	Atta sexdens rubropilosa	São Paulo, Brazil	text, summary statistic (mean, se, n)	mes	Chill coma recovery time	min	Field caught	phenotype	0.06	-0.26	0.37
Burdine and McCluney, 2019	Bombus impatiens	Toledo, Ohio, USA	raw	cohen.d	CTmax	max	Field caught	phenotype	0.54	-0.25	1.34
Nguyen et al., 2020	Cryptotympana atrata	Seoul, South Korea	raw	cohen.d	CTmax	max	Field caught	phenotype	0.14	-0.19	0.47
Nguyen et al., 2020	Hyalessa fuscata	Seoul, South Korea	raw	cohen.d	CTmax	max	Field caught	phenotype	0.94	0.68	1.20
Andrew et al., 2019	Iridomyrmex purpureus	New South Wales, Australia	raw	cohen.d	CTmax	max	Field caught	phenotype	-0.16	-0.55	0.24
Andrew et al., 2019	Iridomyrmex purpureus	New South Wales, Australia	raw	cohen.d	CTmin	min	Field caught	phenotype	-0.15	-0.55	0.24
Rivera-Ordonez et al., 2019	Oophaga pumilio	La Selva, Costa Rica	raw	cohen.d	CTmax	max	Field caught	phenotype	0.45	-0.11	1.01
Diamond et al., 2017	Temnothorax curvispinosus	Cleveland, Ohio, USA	raw	cohen.d	CTmax	max	Field caught	phenotype	0.60	0.25	0.94
Martin et al., 2019	Temnothorax curvispinosus	Cleveland, Ohio, USA	raw	cohen.d	CTmax	max	Field caught	phenotype	1.75	1.31	2.18
Diamond et al., 2017	Temnothorax curvispinosus	Cleveland, Ohio, USA	raw	cohen.d	CTmin	min	Field caught	phenotype	-0.59	-0.93	-0.25
Martin et al., 2019	Temnothorax curvispinosus	Cleveland, Ohio, USA	raw	cohen.d	CTmin	min	Field caught	phenotype	-1.26	-1.67	-0.85
Bar-Ziv and Scharf, 2018	Vermileo sp.	Tel Aviv, Israel	text, test statistic (F, n)	fes	Heat stress recovery time	max	Field caught	phenotype	0.20	-0.24	0.64
Bar-Ziv and Scharf, 2018	Vermileo sp.	Tel Aviv, Israel	text, test statistic (F, n)	fes	Chill coma recovery time	min	Field caught	phenotype	-0.26	-0.68	0.16

Brans et al., 2017	Daphnia magna	Flanders, Belgium	figure (raw data points)	cohen.d	CTmax	max	F2+	evolution	1.75	0.78	2.72
This study	Lasius alienus	Knoxville, Tennessee, USA	raw	cohen.d	CTmax	max	F1	evolution	1.90	1.21	2.59
This study	Lasius alienus	Knoxville, Tennessee, USA	raw	cohen.d	CTmin	min	F1	evolution	-1.10	-1.72	-0.48
Yilmaz et al., in press	Oniscus asellus	Cleveland, Ohio, USA	raw	cohen.d	CTmax	max	F1	evolution	0.76	0.28	1.25
Yilmaz et al., in press	Oniscus asellus	Cleveland, Ohio, USA	raw	cohen.d	CTmin	min	F1	evolution	-0.39	-0.86	0.09
Diamond et al., 2018	Temnothorax curvispinosus	Cincinnati, Ohio, USA	raw	cohen.d	CTmax	max	F1	evolution	-0.01	-0.23	0.21
Diamond et al., 2018	Temnothorax curvispinosus	Cleveland, Ohio, USA	raw	cohen.d	CTmax	max	F1	evolution	0.49	0.28	0.71
Diamond et al., 2017	Temnothorax curvispinosus	Cleveland, Ohio, USA	raw	cohen.d	CTmax	max	F1	evolution	0.41	0.07	0.76
Martin et al., 2019	Temnothorax curvispinosus	Cleveland, Ohio, USA	raw	cohen.d	CTmax	max	F2+	evolution	2.08	1.63	2.53
Diamond et al., 2018	Temnothorax curvispinosus	Knoxville, Tennessee, USA	raw	cohen.d	CTmax	max	F1	evolution	0.30	0.08	0.53
Diamond et al., 2018	Temnothorax curvispinosus	Cincinnati, Ohio, USA	raw	cohen.d	CTmin	min	F1	evolution	0.53	0.30	0.75
Diamond et al., 2017	Temnothorax curvispinosus	Cleveland, Ohio, USA	raw	cohen.d	CTmin	min	F1	evolution	-1.53	-1.92	-1.14
Diamond et al., 2018	Temnothorax curvispinosus	Cleveland, Ohio, USA	raw	cohen.d	CTmin	min	F1	evolution	-0.89	-1.11	-0.67
Martin et al., 2019	Temnothorax curvispinosus	Cleveland, Ohio, USA	raw	cohen.d	CTmin	min	F2+	evolution	-1.23	-1.64	-0.82
Diamond et al., 2018	Temnothorax curvispinosus	Knoxville, Tennessee, USA	raw	cohen.d	CTmin	min	F1	evolution	-1.52	-1.77	-1.26

Table S4. Evolutionary divergence in thermal tolerance versus thermal plasticity. Evolutionary divergence values represent the change in mean thermal tolerance trait values between urban versus rural populations. Thermal plasticity values represent the change in critical temperature per °C increase in laboratory rearing temperature.

Study citation	Species	Tolerance type	Evolutionary divergence (mean)	Evolutionary divergence (se)	Rural thermal plasticity (slope)	Rural thermal plasticity (se)	Urban thermal plasticity (slope)	Urban thermal plasticity (se)
Brans et al., 2017	Daphnia magna	CTmax	1.182	0.273	0.261	0.057	0.204	0.072
Diamond et al., 2017	Temnothorax curvispinosus	CTmax	0.487	0.276	0.237	0.047	0.289	0.031
Diamond et al., 2017	Temnothorax curvispinosus	CTmin	-1.152	0.166	-0.153	0.038	-0.055	0.035
Diamond et al., 2018	Temnothorax curvispinosus	CTmax	0.238	0.191	0.075	0.030	0.153	0.027
Diamond et al., 2018	Temnothorax curvispinosus	CTmax	-0.004	0.168	0.094	0.022	0.080	0.033
Diamond et al., 2018	Temnothorax curvispinosus	CTmax	0.354	0.110	0.085	0.020	0.117	0.019
Diamond et al., 2018	Temnothorax curvispinosus	CTmin	-2.162	0.416	-0.466	0.040	-0.583	0.070
Diamond et al., 2018	Temnothorax curvispinosus	CTmin	0.807	0.356	-0.094	0.045	-0.121	0.069
Diamond et al., 2018	Temnothorax curvispinosus	CTmin	-1.397	0.394	-0.369	0.059	-0.325	0.056
This study	Lasius americanus	CTmax	1.500	0.247	0.025	0.038	0.157	0.033
This study	Lasius americanus	CTmin	-0.933	0.251	-0.080	0.041	-0.103	0.039
Yilmaz et al., in press	Oniscus asellus	CTmax	0.566	0.142	0.028	0.027	0.049	0.023
Yilmaz et al., in press	Oniscus asellus	CTmin	-1.930	0.676	-0.703	0.088	-0.579	0.105

References

Andrew, N. R., Miller, C., Hall, G., Hemmings, Z. and Oliver, I. (2019). Aridity and land use negatively influence a dominant species' upper critical thermal limits. *PeerJ* **6**, e6252.

Angilletta, M. J., Jr, Wilson, R. S., Niehaus, A. C., Sears, M. W., Navas, C. A. and Ribeiro, P. L. (2007). Urban physiology: city ants possess high heat tolerance. *PLoS ONE* **2**, e258.

Bar-Ziv, M. A. and Scharf, I. (2018). Thermal acclimation is not induced by habitat-of-origin, maintenance temperature, or acute exposure to low or high temperatures in a pit-building wormlion (Vermileo sp.). *J Therm Biol* **74**, 181–186.

Brans, K. I., Jansen, M., Vanoverbeke, J., Tüzün, N., Stoks, R. and De Meester, L. (2017). The heat is on: Genetic adaptation to urbanization mediated by thermal tolerance and body size. *Glob Change Biol* 5218–5227.

Burdine, J. D. and McCluney, K. E. (2019). Differential sensitivity of bees to urbanization-driven changes in body temperature and water content. *Sci Rep* **9**, 1–10.

Campbell-Staton, S. C., Winchell, K. M., Rochette, N. C., Fredette, J., Maayan, I., Schweizer, R. M. and Catchen, J. (2020). Parallel selection on thermal physiology facilitates repeated adaptation of city lizards to urban heat islands. *Nat Ecol Evol* **4**, 652–658.

Castañeda, L. E., Lardies, M. A. and Bozinovic, F. (2004). Adaptive latitudinal shifts in the thermal physiology of a terrestrial isopod. *Evol Ecol Res* **6**, 579–593.

Del Re, A. (2013). *compute.es: Compute Effect Sizes*. https://cran.r-project.org/package=compute.es

Diamond, S. E. and Chick, L. (2018). The Janus of macrophysiology: stronger effects of evolutionary history, but weaker effects of climate on upper thermal limits are reversed for lower thermal limits in ants. *Curr Zool* **64**, 223–230.

Diamond, S. E. and Martin, R. A. (2020). Evolutionary consequences of the urban heat island. In *Urban Evolutionary Biology*, pp. 91–110. Oxford, New York: Oxford University Press.

Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A. and Martin, R. A. (2017). Rapid evolution of ant thermal tolerance across an urban-rural temperature cline. *Biol J Linn Soc* **121**, 248–257.

Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A. and Martin, R. A. (2018). Evolution of thermal tolerance and its fitness consequences: parallel and non-parallel responses to urban heat islands across three cities. *Proc R Soc B* **285**, 20180036.

Ellison, A. M., Gotelli, N. J., Farnsworth, E. J. and Alpert, G. D. (2012). *A Field Guide to the Ants of New England*. Yale University Press.

Fick, S. E. and Hijmans, R. J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int J Climatol* **37**, 4302–4315.

Geerts, A. N., De Meester, L. and Stoks, R. (2015). Heat tolerance and its evolutionary potential along a latitudinal gradient in *Daphnia magna*. *Evol Ecol Res* **16**, 517–528.

Hertz, P. E. (1979). Sensitivity to high temperatures in three west Indian grass anoles (Sauria, Iguanidae), with a review of heat sensitivity in the genus *Anolis*. *Comp Biochem Physiol A* **63**, 217–222.

Martin, R. A., Chick, L. D., Yilmaz, A. R. and Diamond, S. E. (2019). Evolution, not transgenerational plasticity, explains the divergence of acorn ant thermal tolerance across an urban-rural temperature cline. *Evol Appl* **12**, 1678–1687.

Nguyen, H. Q., Serret, H., Bae, Y., Ji, S., Chae, S., Kim, Y. I., Ha, J. and Jang, Y. (2020). Not all cicadas increase thermal tolerance in response to a temperature gradient in metropolitan Seoul. *Sci Rep* **10**, 1–12.

Oyen, K. J., Giri, S. and Dillon, M. E. (2016). Altitudinal variation in bumble bee (Bombus) critical thermal limits. *J Therm Biol* **59**, 52–57.

Rivera-Ordonez, J. M., Nowakowski, A. J., Manansala, A., Thompson, M. E. and Todd, B. D. (2019). Thermal niche variation among individuals of the poison frog, *Oophaga pumilio*, in forest and converted habitats. *Biotropica* **51**, 747–756.

Rohatgi, A. (2019). *WebPlotDigitizer*. https://automeris.io/WebPlotDigitizer

Sánchez-Echeverría, K., Castellanos, I., Mendoza-Cuenca, L., Zuria, I. and Sánchez-Rojas, G. (2019). Reduced thermal variability in cities and its impact on honey bee thermal tolerance. *PeerJ* **7**, e7060.

Schär, S., Talavera, G., Espadaler, X., Rana, J. D., Andersen, A. A., Cover, S. P. and Vila, R. (2018). Do Holarctic ant species exist? Trans-Beringian dispersal and homoplasy in the Formicidae. *J Biogeogr* **45**, 1917–1928.

Torchiano, M. (2020). *effsize: Efficient Effect Size Computation*. https://CRAN.R-project.org/package=effsize

Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package. *J Stat Softw* **36**, 1–48.

Warren, R. J., Bayba, S. and Krupp, K. T. (2018). Interacting effects of urbanization and coastal gradients on ant thermal responses. *J Urban Ecol* **4**, juy026.

Yilmaz, A. R., Diamond, S. E. and Martin, R. A. (in press). Evidence for the evolution of thermal tolerance, but not desiccation tolerance, in response to hotter, drier city conditions in a cosmopolitan, terrestrial isopod. *Evol Appl*.