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

Reference	Species	Location	Data	Effect	Trait	max/min	Source	Level			
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

Reference	Species	Location	Type	Method	Metric		Generation		Value	Lower	Upper
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

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