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Urban heat islands advance the timing of reproduction in a social insect

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ABSTRACT

For many species, the timing of life cycle events is advancing under contemporary global climate change. However, much less is known regarding phenological shifts as a result of other sources of anthropogenic change, such as urban warming. In both cases, progress has been hampered by a focus on phenological traits such as the timing of emergence, rather than the phenology of more directly related fitness traits such as the timing of reproduction. Here we explore how urban heat island effects shape the timing of reproduction in an acorn-dwelling ant. We used a common garden experiment with acorn ants collected from three cities in the eastern United States along a latitudinal gradient and reared long-term in the laboratory under five temperature treatments. This allowed us to quantify the effects of temperature on reproductive phenology across three scales—a biogeographic temperature cline, three urban vs. rural temperature comparisons, and five laboratory rearing temperatures. At our northernmost and southernmost cities (spanning 6° of latitude), we found both urbanization and warmer laboratory rearing temperature significantly advanced reproductive phenology; ants from the lowest latitude city also had earlier reproductive phenology compared with the higher latitude cities. In the field, the differences in urban versus rural acorn ant reproductive phenology translate to approximately one month earlier reproduction in the urban populations. For insects with synchronous mating events, such as ants, shifts in the already short window of time to reproduce could limit mating across environments, potentially leading to reproductive isolation between urban and rural populations.

1. Introduction

The timing of life cycle events—phenology—is often strongly dependent on temperature, and as the climate continues to warm through anthropogenically-mediated disturbances, changes in phenology are inevitable (Scranton and Amarasekare, 2017). Already, plants and animals have shifted the timing of emergence and seasonal activity patterns earlier in the year by substantial margins—phenological events are earlier by weeks to months under recent climate change over intervals as short as a decade (Parmesan and Yohe, 2003; Thackeray et al., 2010). Although the exploration of phenological shifts under recent climate change is increasingly common (Brown et al., 2015; Cohen et al., 2018), how phenology is altered by other sources of anthropogenic warming, specifically urbanization, has received less attention.

Through the urban heat island effects generated by the urbanization process, we should expect the timing of life cycle events to occur earlier in cities compared with nearby undeveloped areas (Diamond et al., 2014; Neil and Wu, 2006; White et al., 2002). The acceleration of phenological events in cities has support from the literature, though notably, the overwhelming focus is on plant systems, e.g. earlier bud burst and earlier onset of flowering (Jochner and Menzel, 2015). How temperature rise in cities impacts the phenology of animals is largely unknown. As exceptions, urban birds have earlier arrival and migration dates and start singing earlier in the season (Møller et al., 2015) urban mosquitoes have earlier first appearance and peak larval abundance (Townroe and Callaghan, 2014), urban dragonflies end their flight period earlier (Villalobos-Jiménez and Hassall, 2017), and some butterfly species have earlier first appearance and peak abundance in urban areas (Altermatt, 2012; Diamond et al., 2014).

Indeed, the butterfly case study of Diamond et al. (2014) highlights several important features of exploring phenological shifts in cities. First, there was a large amount of variation across the butterfly assemblage in whether individual species exhibited advanced, delayed, or no shift in phenology in response to urban warming. This pattern mirrors, and perhaps even magnifies the general findings for the impacts of climate change on phenology: although the dominant trend is towards earlier phenology, there is still much variation among species in the magnitude and direction of the phenological response (Forrest, 2016; Kharouba et al., 2018). Thus building a numerically large and

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taxonomically diverse dataset of phenological responses to anthropogenic warming, including urbanization, must remain a research priority. Second, there was a strong signal of background climate in how cities impacted phenology. In general, urban warming advanced phenology at high latitude, cooler sites. However, for nearly half of the 20-species butterfly assemblage, when urban warming occurred at warmer, lower latitude sites, emergence phenology was delayed (potentially through thermal stress effects) rather than advanced (Diamond et al., 2014; Scranton and Amarasekare, 2017). The fact that urban warming may have non-linear temperature effects on phenology owing to background climate further emphasizes the need for greater population-level coverage in phenological responses to urbanization across space.

Here, we address these research gaps, by exploring how the reproductive phenology of tiny ants that live entirely within acorns is altered by urban warming across three separate urban-rural gradients situated along a latitudinal cline in background temperature. We focus on reproductive phenology (in our specific system, the timing of the production of sexual reproductive individuals within the colony), as this is arguably the best link between phenological change and fitness (Dunn et al., 2011; Hedhly et al., 2009; Visser et al., 2006). Although investigating reproductive phenology is not uncommon in studies of urban phenology in plants, it is less common in animal systems (but see Charmantier and Gienapp, 2014), and is particularly rare in studies of social species of insects as we studied here (Kaspari et al., 2001). We collected entire colonies of acorn ants from urban and rural habitats across three cities in the eastern United States, including (in order from lowest latitude to highest) Knoxville, Tennessee, Cincinnati, Ohio and Cleveland, Ohio, a span of 6° latitude. We then reared acorn ant colonies under one of five different temperature treatments from 21° to 29 °C and quantified the thermal sensitivity of reproductive phenology for acorn ants from each city and each habitat type. Thus our study explores how reproductive phenology is altered by broad-scale temperature variation among regions, localized temperature variation across urban-rural gradients, and temperature variation imposed within the laboratory environment. Based on a simple model of linear temperature dependence of phenology, we expected acorn ant reproductive phenology would be shifted earlier at lower latitude sites, in urban habitats relative to rural habitats, and within warmer rearing temperatures in the laboratory environment.

2. Material and methods

2.1. Acorn ant natural history and field collections

Acorns ants (*Temnothorax curvispinosus*) inhabit pre-formed cavities, typically tree nuts (e.g., oak and hickory) from previous years' masts that they excavate to form a nest. Acorn ants are social organisms with colonies comprising reproductive and non-reproductive individuals. These non-reproductive individuals, or workers, are present in the colony year-round. Apart from the colony queens, reproductive individuals, either male drones or female foundresses, are only produced at particular times of the year, typically between June and August (Kannowski, 1959; Talbot, 1957). These reproductive individuals, or alates (Fig. 1c), are winged and engage in dispersal (male-biased, Bourke and Heinze, 1994) and mating to begin new colonies. Founding colonies are therefore small, often only an incipient queen and occasionally a limited number of workers from her natal colony (Bourke and Heinze, 1994; Peeters and Molet, 2010); by contrast, at maturity, colonies can reach over 200 workers with multiple queens (often sisters, Bourke and Heinze, 1994; Herbers and Johnson, 2007). Typically, new colonies take several months to a year or more to produce alates (Herbers, 1990). Mating success can frequently be low due to both the narrow window of overlap of the timing of alate production and flight and the limited longevity of alates (from only a few days to several weeks, depending on location; Dunn et al., 2007; Talbot, 1957). Like many phenological traits, mating flights are strongly temperature dependent (Dunn et al., 2007), and could be altered by habitat-driven changes in temperature including the generation of urban heat islands. Because acorn ants are generalist scavenger feeders (Herbers and Johnson, 2007), this system allows us to isolate the effects of temperature on reproductive phenology from cascading phenological shifts of their food resources.

Acorn ants are widely distributed throughout the eastern United States, inhabiting forest patches in urban environments and intact forest stands in rural environments (Diamond et al., 2017). Evolved urban-rural divergence in thermal tolerance has been previously documented in this species (Diamond et al., 2018, 2017). In this study, we collected whole colonies (queens, workers, and brood, or immature ants) of acorn ants from urban and rural habitats across three different cities in the eastern United States to test the repeatability of urban-driven shifts in the timing of reproductive phenology. Because the cities span 6° of latitude: Knoxville, TN (36°N), Cincinnati, OH (39°N), and Cleveland, OH (42°N) (Fig. 1a), we were further able to explore how the latitudinal cline in background temperature impacted reproductive phenology. We used percent developed impervious surface area (ISA) to classify urban versus rural habitats. Rural habitats were those with 0% developed ISA and urban habitats ranged from 40% to 60% developed ISA (Diamond et al., 2018). To ensure we captured peak alate production within the timeframe of our laboratory common garden experiment (see below), we sampled the southernmost (warmest) site, Knoxville, early in the year, at the onset of alate production (27 April – 7 June 2016), with Cleveland (coolest) and Cincinnati (second coolest) both sampled June and July 2016. The latitudinal differences in nest site temperature span 3.5 °C in peak growing season temperature (21.6 °C in Knoxville, 21.2 °C in Cincinnati and 18.1 °C in Cleveland within rural, undeveloped sites), and the nest site differences between urban and rural habitats within each city exceed 1 °C (3.43 °C in Knoxville, 1.14 °C in Cincinnati and 4.11 °C in Cleveland; Fig. 1b; (Diamond et al., 2018).

2.2. Common garden experiment

After we collected ant colonies from the field, we allowed them to acclimate to laboratory conditions at 25 °C for ~ 48 h. During this acclimation period, we conducted an initial demographic census to determine if colony sizes were similar between source populations (see Appendix A, Table A.1). We then transferred each colony to a 120 mL plastic cup with resource tubes of sugar water (25% sucrose solution), tap water, and dead mealworms supplied ad libitum. Colonies (including representatives from each city and source population) were assigned to a temperature treatment of either 21, 23, 25, 27, or 29 °C with a standard photoperiod of 14:10 L:D. These temperature treatments refer to the baseline temperature. To simulate a diurnal fluctuation in temperature, we increased the baseline temperature by 5 °C for 2 h (13:00–15:00) and decreased the nocturnal temperature by 5 °C for the 10 h simulating a nighttime photoperiod (20:00–6:00) (Diamond et al., 2018). Temperature treatments are considered to be non-stressful temperatures for this species (Diamond et al., 2013; Penick et al., 2017) and are within the normal range of environmental temperatures for both urban and rural habitats for peak ant activity (June-July) across our study sites (Diamond et al., 2018).

We maintained colonies under the laboratory temperature treatments for 10 weeks. This design allowed for relatively long-term acclimation of field-caught queens to their respective temperature treatments (though queens typically live five or more years in this species; Keller, 1998); the generation of new cohorts of non-reproductive worker ants with no prior field experience (workers typically live only 30 days on average, Modlmeier et al., 2013); and the generation of a new cohort of reproductive ants (*i.e.* alates) with no prior field experience. As the timing of alate production was our focal variable of interest, we counted the number of alates (with replacement) every two weeks over the course of the experiment, leading to 6 census points for

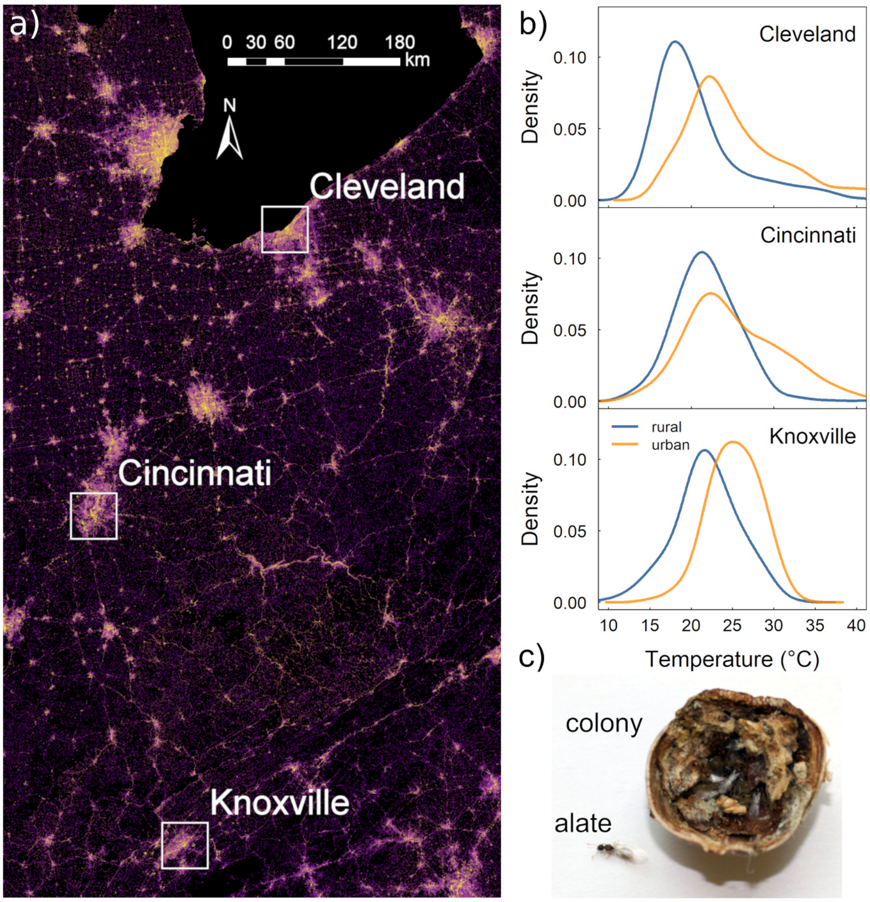

Fig. 1. (a) Map showing the three focal cities. Shading indicates the extent of urbanization and is based on percent developed impervious surface area (Imhoff et al., 2010) with purple to yellow shades indicating greater urbanization. (b) Kernel density distributions (smoothed histograms) for acorn ant nest site temperatures during the growing season in rural and urban habitats from each of the three cities (Diamond et al., 2018). (c) A female alate is shown next to her natal colony.

each colony (initial census from the field plus five additional census points until day 70 in the respective temperature treatment). We had a total of 67 colonies from Knoxville produce alates during the experiment (33 urban, 34 rural); 30 colonies from Cincinnati (21 urban, 9 rural); and 55 colonies from Cleveland (31 urban, 24 rural).

From these data, we computed the ordinal date of maximum alate production. We focused on the date of maximum alate production for two reasons. First, the date of maximum alate production is the most biologically relevant as a proxy for mating phenology (we were unable to track individual nuptial flights in our experimental design), as chance encounters among a limited number of individuals early or late in alate production are likely to be less meaningful than the timing of when most alates are present (Noordijk et al., 2008). Second, the date of maximum alate production is preferable to model from a statistical standpoint. Biases in estimation of the first and final phenophases are well known (van Strien et al., 2008); for example, one abnormally early individual could lead to an erroneous estimate of the date of first appearance. Indeed, with our two-week measurement interval for number of alates produced, such biases are likely to be present in our experimental design. For completeness, we did model the first and final dates of alate production (see below), but we focus our interpretations on the maximum date of alate production for the reasons cited above.

2.3. Statistical analyses

We used generalized linear models with a Gaussian distribution and log link function to explore how the ordinal date of maximum alate production was altered by temperature across different scales including: 1) the laboratory rearing temperature, 2) source population, i.e. warm urban versus cool rural, and 3) the latitudinal gradient in background climate, i.e. from warm lower latitudes to cool higher latitudes. We first fit a global model with the ordinal date of maximum alate production as the response variable, and rearing temperature (as a continuous variable), source population (urban or rural) (as a two-level factor), city (as a three-level factor), and all interactions up to and including their three-way interaction. We then fit a series of subsequent models (all of the same general form, but with different data subsets and predictors, see Appendix A, Table A.2) to further evaluate and explore the effects of different sources of temperature change on acorn ant reproductive phenology. We adopted a similar approach to model the first and final dates of alate production (Appendix A, A.1 Supplementary material and methods). All analyses were performed in R version 3.4.4 (R Development Core Team, 2018).

3. Results

We found substantial effects of temperature on the timing of reproduction (date of maximum alate production) in acorn ant colonies across regional to local scales. Our global model revealed significant main effects of city (χ^2 = 38.4, P < 0.0001), source population (χ^2 = 8.086, P = 0.00446), and laboratory rearing temperature (χ^2 = 15.6, P < 0.0001), though none of the interactions between these terms were statistically significant. In each case, warmer environments (lower latitude sites, urban habitats and warmer laboratory temperature treatments) advanced the ordinal date of maximum alate production relative to cooler environments (Fig. 2, Table 1). Among cities, Knoxville (the lowest latitude city) had by far the earliest timing of alate production (estimated mean ordinal day = 183, SE = 2.16, marginalized over the effects of rearing temperature and source population) compared with Cincinnati (mean ordinal day = 203, SE = 3.57, the middle-latitude city) and Cleveland (mean ordinal day = 200, SE = 2.38, the highest latitude city). Although the differences between Knoxville and Cleveland and between Knoxville and Cincinnati were significantly different (respectively: z = 5.22, P < 0.0001; z = 4.83,

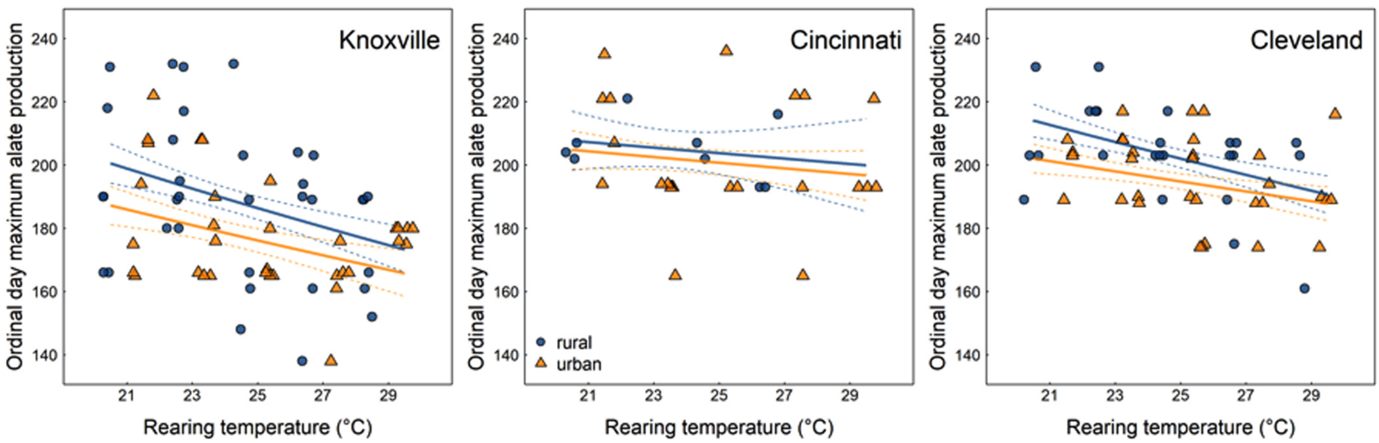

Fig. 2. Ordinal date of maximum alate production as a function of rearing temperature for each city and population (urban versus rural). Predicted values ± 1 SE in solid lines and dashed lines, respectively, for each city and urban or rural population. Points are jittered within each combination of population and temperature treatment.

Table 1
Model estimates (with standard errors) and likelihood ratio tests for the significance of source population, rearing temperature, and their interaction on the ordinal date of maximum alate production. For source population the difference is expressed as urban – rural. Significant *P*-values at the 0.05 level are indicated in bold font.

City	Term	Estimate	SE	χ^2	*P*
Cleveland	Rearing temperature	− 0.0129	0.00532	8.20	**0.00419**
	Source population	− 0.157	0.181	4.71	**0.0299**
	Temperature × Source	0.00473	0.00727	0.426	0.514
Cincinnati	Rearing temperature	− 0.00424	0.0113	0.607	0.436
	Source population	− 0.00923	0.318	0.184	0.668
	Temperature × Source	− 0.000226	0.0131	0.000300	0.986
Knoxville	Rearing temperature	− 0.0162	0.00682	9.23	**0.00238**
	Source population	− 0.126	0.244	4.69	**0.0304**
	Temperature × Source	0.00271	0.00987	0.0750	0.784

$P < 0.0001$), Cincinnati and Cleveland had statistically indistinguishable alate phenology ($z = 0.704$, $P = 0.761$). On the basis that the cities generally differed substantially in their mean timing of maximum alate production, we performed all subsequent analyses of the effects of laboratory rearing temperature and habitat on alate production for each city considered separately.

In our separate models of alate production for each city, Knoxville and Cleveland (at the warm and cool endpoints of our latitudinal gradient), we detected significant main effects of rearing temperature and source population (Fig. 2, Table 1). Warmer laboratory rearing temperatures led to earlier dates of maximum alate production (computed as the mean difference from the warmest to coolest rearing temperature, 29–21 °C) by 19.2 and 24.4 days for Knoxville urban and rural populations, respectively; and by 12.7 and 20.9 days for Cleveland urban and rural populations, respectively. Similarly, colonies from warmer, urban source populations had earlier dates of maximum alate production by 8.07–13.3 days in Knoxville (when reared under the 29 and 21 °C laboratory temperature treatments, respectively) and by 3.79–12.0 days in Cleveland compared with colonies from rural environments in each city. However, these comparisons assume urban and rural populations are experiencing the same temperatures. A more ecologically-relevant comparison would be to use populations reared under the laboratory temperature conditions closest to the environmental temperatures naturally experienced by each source population. For example, we should compare the rural population alate phenology when reared under 21 °C, as these cooler laboratory temperatures are more comparable to rural environmental temperatures, with the urban population alate phenology when reared under 29 °C, as these warmer laboratory temperatures are more comparable to urban environmental temperatures (Fig. 1b). By this comparison, the difference in timing of the production of alates is 32.5 days across urban and rural habitats in Knoxville and 24.7 days in Cleveland.

Our analyses of first and final dates of alate production support our findings from the analyses of the date of maximum alate production, though again we emphasize caution in their strict interpretation owing to issues surrounding bias in their estimation (Appendix A, A.1 Supplementary material and methods). We found that earlier dates of maximum alate production were correlated with earlier first dates of alate production and with earlier final dates of alate production; we also found that individual models of first and final dates of alate production trended in the same direction as for maximum alate production (Appendix A, Tables A.3, A.4), suggesting that the entire window of alate production shifts earlier in cities.

Although urbanization and laboratory rearing temperature both significantly impacted reproductive phenology (date of maximum alate production) in Knoxville and Cleveland, we found no indication that the magnitude of phenological advancement under warmer laboratory rearing temperatures differed among the urban and rural populations for either Knoxville or Cleveland (i.e., no differences in slope, Fig. 2). Specifically, we did not detect a significant interaction between the rearing temperature and source population terms (Table 1). By contrast, for the middle latitude city in our study, Cincinnati, we detected no significant effects of rearing temperature, source population, or their interaction on the ordinal date of maximum alate production (Fig. 2, Table 1), suggesting these Cincinnati populations were remarkably robust to temperature variation.

4. Discussion

How urban warming impacts the reproductive phenology of animals is increasingly relevant with the acceleration of urban development across many parts of the world (Jochner and Menzel, 2015; Seto et al., 2012). Here, we used a five-temperature common garden experiment to quantify the effects of urban temperature rise on the timing of reproduction in a social insect across three urban-rural gradients. We found that for a majority of the urban-rural gradients we examined, urban populations exhibited earlier reproductive phenology compared with rural populations, and we found that reproductive phenology was advanced under warmer laboratory rearing temperatures, though urban and rural populations had similar rates of advancement across the rearing temperatures. Our study demonstrates how urbanization can, to some degree, predictably influence reproductive phenology, and suggests that cities, as replicated warming environments, can be further exploited to examine the temperature-dependence of fitness-related phenological traits.

The consistent effects of temperature at different scales—across laboratory rearing temperature, across urban-rural gradients, and across a latitudinal cline in temperature—support a linear relationship between temperature rise and advanced reproductive phenology in our acorn ant study system. That reproductive phenology continues to advance with temperature, even in the warmest habitats we studied, i.e. acorn ants from urban habitats at our lowest latitude city, Knoxville, Tennessee (36°N latitude) is perhaps unsurprising as this species is highly thermophilic compared with other ant species (Penick et al., 2017), and ants as a taxon are highly thermophilic compared with other groups (Cerdá and Retana, 2000; Diamond et al., 2012). By contrast, some species of butterflies, a much less thermophilic taxon, exhibit phenological delays when urban warming occurs against the backdrop of already warm climatic conditions, likely as a thermal stress response (Diamond et al., 2014). Indeed, whether the advancement of acorn ant reproductive phenology is adaptive remains to be seen, though this is a plausible if not likely outcome as acorn ants from warmer urban environments produce higher numbers of alates (as an estimate of fitness) under warmer rearing temperatures in the laboratory compared to rural populations (Diamond et al., 2018). More generally, phenological advancement can allow organisms to gain earlier access to limiting resources (Herbers, 1989); for acorn ants, this could mean earlier access to empty nest sites (acorns) for newly mated foundresses (MacLean et al., 2017).

As cities represent replicated warming experiments, a major question is the extent to which organismal responses to urban warming are consistent versus idiosyncratic across different urban-rural gradients (Diamond et al., 2018; Johnson and Munshi-South, 2017). For two out of our three cities, we found remarkably consistent responses of acorn ant reproductive phenology to urbanization, wherein acorn ants from urban environments exhibited advanced phenology compared with acorn ants from nearby rural environments. Importantly, these two cities, Cleveland and Knoxville occur at the high and low latitude extremes of our latitudinal cline in urbanization, indicating a lack of latitudinal bias in the effects of urbanization on phenology in our study. Further, we were able to recover a latitudinal signal in acorn ant reproductive phenology, such that our lowest latitude site had the earliest phenology compared with either of the two higher latitude sites (even though these two higher latitude sites were not statistically different from one another). Together, these results suggest that while our sampling design and approach was robust, since we were able to demonstrate the predicted latitudinal cline in reproductive phenology, we found mixed support for the effects of urbanization on reproductive phenology. It is worth mentioning here that for a number of other acorn ant traits including thermal tolerance, Cincinnati (which shows no signal of either urbanization or laboratory rearing temperature on reproductive phenology) is an outlier with respect to the other two urbanization gradients, Cleveland and Knoxville. Acorn ants in Knoxville and Cleveland exhibit evolved increases in heat tolerance and losses in cold tolerance in urban populations, and exhibit plastic responses to laboratory rearing temperature such that heat tolerance was improved and cold tolerance was diminished with increasing rearing temperature. By contrast, acorn ants in Cincinnati exhibit no differentiation in heat tolerance across the urban-rural gradient, and even exhibit countergradient patterns in cold tolerance, with urban ants surprisingly being more tolerant of cold (Diamond et al., 2018). Together, these results suggest the evolutionary process is unfolding in a unique, unpredictable manner in Cincinnati. Further, there were overall relatively few colonies from Cincinnati that produced alates, which hindered our ability to estimate reproductive phenology; as a consequence, our results could reflect limited statistical power, though the inconsistent results of Cincinnati are supported by the suites of traits discussed above.

The mechanisms underlying the phenotypic shifts in phenology we observed between urban and rural populations (at least in Knoxville and Cleveland) cannot be fully disentangled with our experimental design. Although field-caught colonies were reared long-term (10 weeks) in the laboratory environment, it is possible that maternal effects of queens who had been conditioned in their respective urban or rural environments could contribute to the differences in the timing of alate production that we observed. Such maternal effects are likely to be small and important in early development (Massamba-N'Siala et al., 2014), and appear not to play a role in acorn ant thermal tolerance (unpublished results), however, we still cannot exclude the possibility of their existence in the timing of alate production. Results from other systems provide evidence that phenology can evolve rapidly in response to recent climate change (Bradshaw and Holzapfel, 2001; Dubois and Cheptou, 2017), and within the acorn ant system, many other traits appear to be rapidly evolving in cities, most notably heat and cold tolerance (Diamond et al., 2018, 2017). But it would be premature to assert that reproductive phenology is likewise evolving in this system. To do so would require breeding colonies in the lab under common garden for a complete generation and comparing the phenology of urban and rural populations.

Regardless of the mechanism underlying the phenological shifts we observed across the urban-rural gradients, our findings have important implications for reproductive barriers between urban and rural populations. Depending on the city of origin, we estimated that under the temperature conditions in their natal environments, urban acorn ants achieve their maximum alate production at either just under or just over a month before rural acorn ants achieve their maximum alate production. Such phenological differentiation in the timing of reproduction across environments could substantially limit the probability of cross-environment mating and drive reproductive isolation between urban and rural populations. For example, prominent examples of reproductive isolation and divergence in tephritid flies (*Rhagoletis pomonella* and *Eurosta solidaginis*) are driven, in large part, by diapause and emergence timing (Craig et al., 1993; Dambroski and Feder, 2007; Filchak et al., 2000). The potential for cities to generate reproductive isolation and potentially even speciation, either through differences in phenology or other traits could therefore be relatively high (Thompson et al., 2018).

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Author declaration

SED, RAM, and LDC designed the experiment. LDC, SAS, and AP collected data. SED, RAM, and LDC analyzed data. SED and LDC wrote the first draft and all authors contributed to revisions of the manuscript.

Declarations of interest

None.

Ethics

All applicable institution and national guidelines for the care and use of animals were followed.

Data availability

The data generated and analyzed during the current study are available at Mendeley Data: doi:10.17632/tgw3gyd955.1

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2019.01.004

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