

Extreme polygyny in the previously unstudied subtropical ant *Temnothorax tuscaloosae* with implications for the biogeographic study of the evolution of polygyny

B. Guénard¹  · J. Z. Shik^{2,3} · D. Booher⁴ · D. Lubertazzi⁵ · G. Alpert⁵

Received: 1 February 2016 / Revised: 4 July 2016 / Accepted: 9 July 2016
© International Union for the Study of Social Insects (IUSSI) 2016

Abstract Evolutionary ecologists often seek to explain life history variation at geographical scales. Progress in this regard has been slower in social insects than in unitary organisms, due in part to limited information about species distributions and the complexity of studying colony-level traits. *Temnothorax* ants have proven useful for investigating associations between ecology and life history traits in ants, because they have small colonies (<200 workers) with substantial variation in traits like queen number within and across populations. Yet most of these studies have focused on <10 *Temnothorax* species from northern latitudes, although the genus contains >400 species that range from temperate to tropical biomes. Here, we generate the first global map of *Temnothorax* diversity and demonstrate that previous ecological hypotheses for colony life history

variation in the genus are based on data and studies confined to a small part of the overall genus-level distribution. For example, studies of northern-latitude species have suggested that overwintering success is a prominent selective force favoring polygyny but little is known about polygyny in *Temnothorax* species from other regions. We report the highest observed queen numbers known in the genus in the subtropical species *T. tuscaloosae* and confirm that all queens in these polygynous colonies are mated and actively laying eggs. Behavioral laboratory experiments further indicate that this species has a fluid nest structure and lacks intraspecific aggression at the whole-forest scale. These results extend the biogeographical extent of life history studies for this genus and point to evolutionary discoveries awaiting comparative research of the global *Temnothorax* fauna.

B. Guénard and J. Z. Shik with equal contributions to the study.

Electronic supplementary material The online version of this article (doi:10.1007/s00040-016-0498-7) contains supplementary material, which is available to authorized users.

✉ B. Guénard
bguenard@hku.hk

- ¹ School of Biological Sciences, University of Hong Kong, Pok Fu Lam, Hong Kong
- ² Smithsonian Tropical Research Institute, Balboa, Republic of Panama
- ³ Department of Biology, Centre for Social Evolution, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark
- ⁴ Department of Ecology and Evolutionary Biology, University of California Los Angeles, 621 Charles E. Young Drive S, Los Angeles, CA 90095-1606, USA
- ⁵ Museum of Comparative Zoology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

Keywords Biogeography · Macroecology · Life history · Polygyny · Formicidae · *Temnothorax*

Introduction

Biogeography has for long provided context to understand how ecological mechanisms shape life history variation (e.g. Lack 1947; James 1970; Ricklefs 1987; Brown et al. 1993; Santos and Cannatella 2011). This is especially true for social insects (e.g. ants, bees and termites; Brown and Wilson 1956; Kaspari and Vargo 1995; Kaspari 2005). However, despite important recent advances in our understanding of the mechanisms governing large-scale patterns of social insect diversity and community structure (e.g. Sanders et al. 2003; Kaspari et al. 2004; Lessard et al. 2012; Guénard et al. 2012a), these insights have been slow to inform our understanding of social evolution. This is partly

due to limited data on species distributions (Guénard et al. 2012a) and reflects an added layer of complexity in social insects. Many life history traits belong not to individual insects but to entire colonies (Hölldobler and Wilson 2008). Over 15,000 species of ants exhibit orders-of-magnitude variation in both colony size (worker mass and number) at reproduction (Kaspari and Vargo 1995; Bourke 1999; Heinze 2006; Hou et al. 2010) and the mass and number of queens performing this reproductive work (Shik 2008; Shik et al. 2012).

Queen number, in particular, is a central feature of ant colonies, because it governs nestmate relatedness and shapes the balance between cooperation and conflict (Hölldobler and Wilson 1977; Herbers 1993; Herbers 1993; Hughes et al. 2008). However, measurements of critical life history traits like queen number have been published for surprisingly few species (i.e. <400 of the >15,000 species), let alone across populations and environments (Tschinkel 2011). While the functional benefits of genetic diversity in ant societies have been supported by recent theoretical advances (e.g., social heterosis, Nonacs and Kapheim 2010) and empirical results (e.g., social immunity, Ugelvig et al. 2010), relatively few ant lineages have been used to study the ecological mechanisms favoring colonies with multiple egg-laying queens. The ant genus *Temnothorax* (Hymenoptera: Formicidae) has been useful in studying how ecology shapes colony life history, as their small colonies (<200 workers) are amenable to experimental manipulations and exhibit variation in queen number within and between species (Herbers and Johnson 2007; Herbers 1986a, b; Foitzik et al. 2004; Heinze and Rueppell 2014; Herbers 1993; Bourke and Heinze 1994). Yet even within this relatively well-studied genus, research has been limited to <6 % (ca. 25 of 431) of *Temnothorax* species and subspecies (Heinze 2006, Antcat.org 2016). Moreover, many studies have focused on four North American species restricted to higher latitudes (Talbot 1957; Alloway et al. 1982; Herbers and Stuart 1996; Herbers and Johnson 2007) and higher altitudes (Rüppell et al. 1998; Rüppell et al. 2001a, b; but see Heinze and Rueppell 2014).

While *Temnothorax* species actually range much further south, ecological explanations for *Temnothorax* polygyny, such as duration and intensity of winter (Herbers 1986a), parasite pressure (Bourke and Heinze 1994), and patchy resource availability (Foitzik et al. 2004) stem from observations of a small part of the geographic and taxonomic range. The present study provides both the first global map of *Temnothorax* species diversity and the first description of the current biogeographical extent of hypothesis testing in *Temnothorax*. We do this using a new ant distribution database (Guénard et al. 2012a). We then performed the first study of polygyny in *Temnothorax tuscaloosae* (Wilson 1951), a subtropical species distributed across the

southeastern United States. Surveying colony structure from several populations, we tested whether colonies of *T. tuscaloosae* tend to have different numbers of queens than species at northern latitudes. We used dissections to determine if queens in polygynous colonies were mated and laying eggs, and performed a lab experiment to test whether queen number in *T. tuscaloosae* was associated with fluid colony structure, with workers and queens freely moving among spatially discrete nests. By providing a subtropical comparison for a wealth of existing northern-latitude studies of *Temnothorax*, this study highlights the value of biogeographical information for understanding life history evolution in social insects.

Materials and methods

Temnothorax biogeography

We used the Global Ant Biodiversity Informatics database (GABI) to map patterns of *Temnothorax* species richness, with the visualization of individual species available on antmaps.org. This database is a comprehensive set of ant-species occurrence data that consolidates specimen and literature records from many sources. We focused on the Nearctic, Neotropical, and Palearctic regions since these areas are the focus of the most detailed studies of *Temnothorax* ecology and biogeography. Only validly described species have been included in this study (morphospecies were excluded).

Queen number in *T. tuscaloosae*

Temnothorax tuscaloosae is restricted to the southeastern United States (Supplementary Material Appendix 1 Table S1) and has remained unstudied since its initial description from two colonies collected near Tuscaloosa, Alabama over 60 years ago (Wilson 1951). Additional localities were added from Alabama (MacGown and Forster 2005), Mississippi (MacGown and Brown 2006) and North Carolina (Carter 1962; Guénard et al. 2012b). *Temnothorax tuscaloosae* appears common in North Carolina where it is known from seven counties (Supplementary Material Appendix 1 Table S1). We report here the first records of *T. tuscaloosae* from Tennessee, South Carolina, Georgia, and Virginia, with the latter representing the northernmost record for the species (Supplementary Material Appendix 1; Table S1).

To assess colony-level worker and queen numbers in *T. tuscaloosae*, we collected entire colonies nesting inside acorns and hickory nuts found within the leaf litter (as per Alloway et al. 1982; Foitzik and Heinze 1998). Details on collection information are presented in Table 1. Within

Table 1 Collection information on the three species *T. curvispinosus*, *T. longispinosus* and *T. tuscaloosae* used in this study

Species name	# Nests	Decimal Lat./Long.	State	Collection date	Refs.
<i>T. curvispinosus</i>	11	37.5639°N; -81.0164° W	WV	Oct. 2011	1
	29	34.6135° N; -85.0889°W	GA	Aug. 2014	1
	19	33.95°N, -83.333°W	GA	Aug.–Sept. 2014	1
	62	35.7626°N; -78.6844°W	NC	Oct.–Nov. 2011	1
<i>T. curvispinosus</i>	258	N/A	IL	May–June	2
Literature records	467	39.17°N, -86.53°W	IN	Mar.–Apr. 2004	3
	525	42.45°N, -84.02°W	MI	June–Aug. 1954/55	4
	23	N/A	MI	June	2
	9	33.99°N, -88.92°W	MS	Oct. 2003	5
	63	40.36°N, -74.67°W	NJ	Oct. 1987	6
	38	41.75°N, -80.96°W	OH	Mar.–Dec. 1940/42	7
	492	40.05°N, -82.98°W	OH	Mar.–June 1977/78	8
	57	41.76°N, -80.96°W	OH	June	9
	645	40.24°N, -82.95°W	OH	Fall 2003	10
	98	38.06°N, -80.49°W	WV	Spring 1983/88	11
<i>T. longispinosus</i>	17	42.48°N, -74.17°W	NY	Oct 1983	12
Literature records	97	41.75°N, -80.96°W	OH	Mar.–Dec 1940/42	7
<i>T. tuscaloosae</i>	2	36.6846°N; -78.3853°W	VA	Nov. 2007	1
	41	35.3695°N; -80.0973°W	NC	Oct. 2011	1
	179	35.7629°N; -78.6858°W	NC	Oct.–Nov 2011	1
	13	34.6135°N; -85.0889°W	GA	Aug. 2014	1

Shaded rows represent records extracted from the literature

References (Refs.) used for each set of records are as follows: 1—This study, 2—Stuart (1992), 3—Linksvayer (2008), 4—Talbot, (1957), 5—MacGown (2006), 6—Evans and Pierce (1995), 7—Headley (1943), 8—Alloway et al. (1982), 9—Stuart (1985), 10—Johnson and Herbers (2006), 11—Herbers and Stuart (1996), 12—Herbers and Tucker (1986)

72 h of returning colonies to the lab in plastic vials, we counted all workers and queens and noted the presence of brood. All means in the text are provided \pm SD.

To interpret demographic trends in *T. tuscaloosae*, we compared its demography with two facultative polygynous *Temnothorax* species: *T. curvispinosus* and *T. longispinosus*. These two species have ranges that overlap *T. tuscaloosae* but also occur at higher latitudes. Data for *T. curvispinosus* were obtained from colonies collected across a range that extends from New England (Ellison et al. 2012) to northern Florida (Deyrup 2003). Supplementing the literature review with our own collections, our analysis included 2763 colonies from 15 sites from Mississippi (34 N) to Michigan (42 N) and elevations from 33 m to 1139 m. Collection information details for *T. curvispinosus* and *T. longispinosus* are presented in Table 1.

Testing for functional polygyny

We tested for functional polygyny in *T. tuscaloosae* by assessing queen reproductive status in 19 colonies. Queens were examined within 2 weeks of collection. A total of 240 queens were dissected (as per Buschinger and Alloway 1978)

from colonies spanning the range of worker and queen number encountered during field collections. After pinning each queen under water in a wax-filled dish under a dissecting microscope, we used fine forceps to remove the subgenital plate. This typically resulted in the removal of the hindgut, poison gland, Dufour's gland, and reproductive system. We placed these organs on a microscope slide and added a drop of water and coverslip before examination under a compound microscope at 100 \times and 200 \times magnification. We assessed mating status by noting the presence of sperm in the spermatheca and egg-laying status by counting the number of developed ovarioles and noting whether they contained mature or developing eggs. We also noted the presence of corpora lutea—small yellow granules at the base of the ovarioles. These are degraded nurse cells from recently laid eggs and have previously been used to assess egg-laying activity (Peeters 1993). We further dissected 12 workers from one colony to assess worker reproductive status.

Testing for polydomous nest structure

We tested whether *T. tuscaloosae* workers discriminate among nestmates and non-nestmates to explore whether

polygyny may be associated with polydomous nest structure. We did this with fusion experiments of paired nests in the following distance treatments ($n = 14$): (1) *Control* (ants from the same hickory nut nest), (2) *Plot* (nests from the same 0.42 m² plot), (3) *Tree* (nests located in separate plots 1 and 5 m from the base of a single tree), and (4) *Forest* (nests from under four hickory trees (*Carya* sp.) separated by 50–200 m). Polydomy would be supported if workers from treatments ostensibly representing the same colony (control, plot, and tree) freely mix, while aggression is expressed at long distances where workers are unlikely to come from the same colony (e.g. at the forest scale). All nests for the nest fusion experiment were harvested at the Lake Raleigh forest.

Each paired nest had two queens and 12 workers, a ratio that reflected the mean ratio of queens to workers from field-collected colonies (see “[Results](#)”). Each colony was placed in a 1.5-ml microcentrifuge vial, and connected by a 5-cm-long clear Tygon tubing (0.5 cm inner diameter) to a vial containing another colony. Daily counts of workers and queens within each tube were conducted during five consecutive days to note the position of individuals between nests. These counts did not discriminate the colony affinity of individuals. A conservative queen relocation standard was used for this test, as we considered colonies fused when ≥ 3 queens were observed in the same vial (as per Kikuchi et al. 2007; Tsuji 2010). We used the GENMOD procedure in SAS with an autoregressive (AR(1)) covariance structure to perform a repeated measures logistic regression testing for differences in nest fusion over 5 days among the four nest distance treatments.

Results

Temnothorax biogeography

The best studied *Temnothorax* species come from habitats at the northern edge of the global *Temnothorax* distribution. In the New World, *Temnothorax* diversity peaks at intermediate latitudes (30°N), with 46 species in the southwestern United States region (Arizona, California, New Mexico and Texas) and 47 species in the Caribbean region (Bahamas, Cuba and Hispaniola) (Fig. 1). In contrast, only nine species are known from the Northeastern United States (Fig. 1). In the Old World, *Temnothorax* diversity peaks between 30 and 40° of latitude (N), principally within the western half of the Mediterranean basin where 109 species have been reported from Morocco, Spain and Algeria (59, 53 and 51 species, respectively) (Fig. 1). The eastern part of the Mediterranean basin also supports a high diversity of

Temnothorax species with 63 species reported from the Greece–Turkey region (48 and 47 species reported, respectively, from Greece and Turkey).

Functional polygyny in *T. tuscaloosae*

Temnothorax tuscaloosae is among the most polygynous of any known *Temnothorax* species. Queen dissections indicate that these colonies are functionally polygynous. Queen number was remarkable in a number of ways. First, the average *T. tuscaloosae* colony had 10.4 ± 7.7 queens (range 1–38 queens)—ten times more than species from the higher latitude *T. curvispinosus* (1.0 ± 0.75) and *T. longispinosus* (1.2 ± 2.3). Second, while larger *T. tuscaloosae* colonies tended to have more queens ($R^2 = 0.48$; $p = 0.0001$; Supplementary Material Appendix 2 Fig. S1), high queen counts relative to other *Temnothorax* species were not explained solely by colony size. Worker numbers were similar in colonies of *T. tuscaloosae* (54.5 ± 41.8), *T. curvispinosus* (68 ± 56.4), and *T. longispinosus* (44.9 ± 35.0) (Table 2). Third, using the classification of Herbers (1984), 77.3 % of *T. tuscaloosae* colonies were polygynous (≥ 5 queens per nest), and only 1.3 % were monogynous. In contrast, most of the *T. curvispinosus* colonies collected were monogynous (83 %), 9 % oligogynous (2–4 queens) and none were polygynous.

Functional polygyny was clearly evident in *T. tuscaloosae*. Most queens (236 of 240) had six ovarioles ranging in length from short stubs to more than one queen body length, were mated (239 of 240 had spermathecae filled with sperm), and were actively laying eggs (235 of 240 had corpora lutea and ovarioles with developing oocytes). Evidence of active egg laying among all queens was observed even when >20 queens occurred in a single nest (Supplementary Appendix 1, Table S2). Worker dissections indicated that some (4 of 12) had two short ovarioles. One worker had a mature egg that was likely unfertilized given that workers lack spermathecae.

Lack of intraspecific aggression and fluid nest structure in *T. tuscaloosae*

Functional polygyny in *T. tuscaloosae* was associated with extremely fluid colony structure. Nest fusion in the lab increased over time (Day: $\chi^2_{1, 56} = 9.81$; $P = 0.002$), and most nests fused after 24 h regardless of the distance treatment (Day \times Distance: $\chi^2_{3, 56} = 3.71$; $P = 0.295$) (Fig. 2). Distance did not influence the probability of fusion during the experiment ($\chi^2_{3, 56} = 5.41$; $P = 0.144$), with 92.9 % of the colonies from the same forest treatment fusing after 5 days (Fig. 2). No aggressive behavior between individuals was observed.

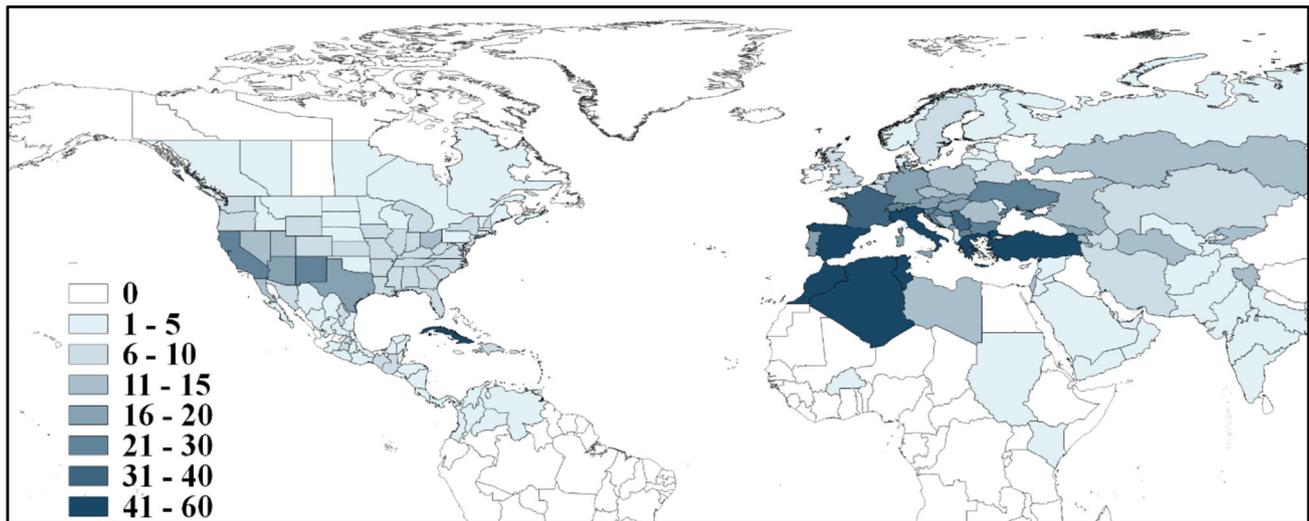


Fig. 1 Map of the diversity of *Temnothorax* species in the western Holarctic, northern Neotropical and northern Ethiopian realms. Boundaries reflect political divisions, i.e., country and, in some cases, state/provincial divisions. *White color* represents an absence of *Temnothorax* species and *light to dark blue* represents an increasing

number of species along a continuous gradient. Data were obtained from the Global Ant Biodiversity Informatics database (GABI). Species-level distribution maps are available at <http://antmaps.org> (Color figure online)

Table 2 Sociometry across *Temnothorax* species

Species	No. of nests	Mean no. of workers	Mean No. of queens	Worker:queen
<i>T. curvispinosus</i> ^{a,b,c}	102	68	1.02	63.3
	70	70	0.97	74.9
<i>T. longispinosus</i> ^{a,d}	114	43.7	1.19	38.8
<i>T. pergandei</i> ²	5	37.8	0.8	33.5
	2	85	1	85
<i>T. shaumii</i>	1	55	1	55
<i>T. tuscaloosae</i>	235	54.5	10.4	6.5

Italicized numbers are from previously published studies, non-italicized numbers collected in the present study

References: ^aHeadley (1943), ^bStuart et al. (1993), ^cMacGown (2006), ^dHerbers and Tucker (1986)

Discussion

We report the first comprehensive demographic study of a subtropical species of *Temnothorax* and interpret our findings using the first comprehensive global species distribution map for the genus. This map reveals subtropical centers of diversity, from 20 to 35 N in the New World, and 30 to 40 N in the Old World. The results reveal regions that can be most profitably studied to understand the costs and benefits of polygynous colony structure. We also show that the most polygynous colonies (up to 38 queens) known for the genus occur in the subtropical species *T. tuscaloosae*. These colonies are further shown to be functionally polygynous (all queens mated and egg-laying) and have highly fluid colony structures given that they freely accept foreign workers and queens into their nests. High queen numbers in

this subtropical species suggest that polygyny in *Temnothorax* can be generated by additional ecological factors besides cold winters, while also highlighting the benefits of maximizing the evolutionary and ecological extent of hypothesis testing when exploring ecological factors shaping life histories.

Globally, 431 *Temnothorax* species and subspecies have been described (as per Ward et al. 2015; Antcat.org 2016) with 140 species known from the New World (Antcat.org 2016; Antmaps.org 2015). Species occur throughout North America and range southward to Colombia, reaching peak diversity in the Caribbean region and the southwestern United States (Fig. 1). There are also numerous undescribed species known from Hispaniola (Lubertazzi and Alpert 2014, Lubertazzi, personal observation) and the southern United States (Booher, unpublished data). The western

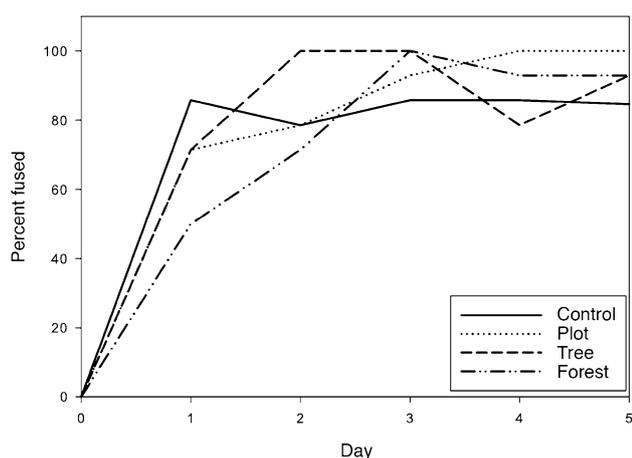


Fig. 2 Queens from different nests readily fused in *T. tuscaloosae* within 5 days. Nests were paired according to the following distance treatments: Control (same colony), Plot (from the same 0.42 m² quadrat), Tree (from the separate quadrats placed under the same tree), Forest (from trees separated by >50 m). Nests were considered fused when ≥ 3 queens were found in the same nest

Palaearctic region exhibits a similar trend. The highest diversity occurs in regions around the Mediterranean basin and decreases at higher latitudes (Fig. 1; Heinze and Buschinger 1988). The western Mediterranean region currently exhibits the overall peak of known diversity in *Temnothorax*, but we also note that several species have recently been described from the eastern part of the Mediterranean basin (Csősz et al. 2014, 2015; Salata and Borowiec 2015; Seifert and Csősz 2015). Similarly, future taxonomic studies from other parts of the world are likely to increase the total number of species, especially for studies focused on warmer regions, e.g., recent descriptions of new Afrotropical species (Prebus 2015). The wide distribution of the genus across diverse habitats suggests that they are likely to exhibit a range of life history strategies. More comprehensive knowledge of the diversity of *Temnothorax* across its range coupled with a better understanding of the ecological conditions and life history details of more species from throughout this distribution will provide more opportunity to develop hypotheses regarding the evolution of colony traits in ants and within social systems more generally.

Temnothorax tuscaloosae lacks aggression between nests, with rapid nest mixing at distances of up to 200 m, far greater than a colony's foraging radius, and at distances where nests are less likely to contain close relatives. This lack of intraspecific aggression at the whole-forest scale suggests a scenario of unicoloniality rather than polydomy, although population genetic studies will be needed to fully resolve the underlying colony fusion dynamics. Results for *T. tuscaloosae* contrast with distance-dependent aggression in *Temnothorax nylanderii*, a closely related higher-latitude

species that shows evidence of the 'dear enemy' phenomenon (Heinze et al. 1996). *Temnothorax tuscaloosae* may thus provide an interesting system for exploring the sensory mechanisms governing nestmate recognition and evolutionary within-colony conflicts of interest shaped by nestmate relatedness. Behavioral observations at even larger biogeographic scales suggest that climate shapes colony aggression, with the ant *T. rugatulus* mobilizing fewer but more aggressive workers to threats in northern habitats (Bengston and Dornhaus 2014). It will be useful to test whether such behavioral patterns extend even further south in *Temnothorax* species with tropical distributions.

Bourke and Heinze (1994) outlined how cold winters, social parasitism, and habitat patchiness might favor the evolution of polygyny in the lineage containing *Temnothorax*. This study shows the extreme polygyny exhibited by the subtropical *T. tuscaloosae*, and suggests that cold tolerance might not be as dominant as a selective pressure. While this is likely important at higher latitudes, other factors besides overwintering performance might govern polygyny in this subtropical species. It should be noted though that northern populations of *T. tuscaloosae* (e.g., Raleigh region; NOAA.gov 2016) experience winter conditions characterized by yearly episodic freezing temperatures over short periods, but with higher minimum temperatures when compared to regions where most previous studies have been conducted (e.g. Herbers 1986a; Herbers and Johnson 2007). While the measurements of queen mortality of *T. tuscaloosae* during winter were not realized in this study, it can be assumed that winter conditions might have a limited impact on queen mortality and, thus, on the selection of polygyny, particularly for southern populations. Moreover, these ecological dynamics may be underappreciated since polygyny appears prevalent in other subtropical species that were described from nest series containing several dealate queens, e.g., *T. palustris* in Florida, Deyrup and Cover 2004; *T. nigricans* in Cuba (presented as *Macromischa melanocephala*), Wheeler 1931. It will be important to perform additional manipulative field experiments to explore the specific advantages provided by extreme functional polygyny and fluid nest structure in subtropical species like *T. tuscaloosae*.

A promising factor may be social parasitism, a costly scenario where queen ants from closely related species consume a host colony's resources and contribute reproductive ants, but not workers. Polygynous colonies are predicted to benefit by having extra queens to offset these costs or resist the attacks of social parasites (Bourke and Heinze 1994). At larger spatial scales, a link between social parasitism and polygyny would be supported if populations have higher queen numbers when they overlap with distributions of social parasites. We note that *T. tuscaloosae* lacks known social parasites, and none was observed in any of the

235 nests collected at different locations. While frequencies of social parasitism observed in other North American *Temnothorax* populations have been reported to range from 2 to 12 % (Herbers 1986a), the extreme polygyny in *T. tuscaloosae* appears to have evolved independently of parasite pressure. More generally, the biogeography of *Temnothorax* indicates that areas with the highest potential host diversity (Fig. 1) have the fewest species of social parasites (Supplementary 1, Fig. S2). Further demographic studies will help in determining the large-scale phylogenetic correspondence between queen number and the occurrence of social parasites.

Resource patchiness with limited nest sites is also thought to favor polygyny in *Temnothorax* populations (Herbers 1986b; Foitzik et al. 2004). However, nest site limitation appeared low in our focal *T. tuscaloosae* population given that there were many seemingly suitable but empty acorn and hickory nests. And yet, nest site limitation may promote polygyny in this ant. First, *T. tuscaloosae* colonies may track patchy resources (e.g., food or nest sites) as they exhibited patchy distributions, with nest densities averaging 4.9 nests per 0.42 m², but ranging from 0 to 12 nests in neighboring 1-m² plots. Second, the fluid colony structure exhibited by *T. tuscaloosae* may enable colonies to dynamically track ephemeral resources (Herbers 1986b; Debout et al. 2007) such as hickory nuts with fast decomposition rates (Foitzik and Heinze 1998, Heinze 2006). Polygynous colonies likely have backup queens, which in some colonies, are consumed by predators as they relocate among rapidly decomposing nest sites.

To conclude, our study provides a new subtropical perspective when thinking about variation in queen number in this model ant genus. Such perspectives are made possible by combining basic demographic and natural history studies with modern databasing efforts like the Global Ant Biodiversity Informatics database and its associated visualizations (e.g., Antmaps.org 2015, Janicki et al. 2016). While based on a single-species study, our data highlight the importance of an inclusive approach when studying the ecological characteristics of species present within regions which traditionally have received less ecological studies but represent centers of diversity. Queen number is one of the better studied traits in a subset of geographically biased *Temnothorax* species, with variation in this trait, overall, having contributed much to our ongoing understanding of social evolution. A biogeographical perspective will be key toward exploring how ecology influences and shapes colony phenotypes—and predicting how colonies may respond in a changing climate.

Acknowledgments We dedicate this paper to E. O. Wilson who described *T. tuscaloosae* in 1951 in his first taxonomic paper on ants. We would like to thank Tim Linksvayer and Joe MacGown for

providing us records, respectively, from Tennessee and Mississippi. Collections in State Parks of North Carolina were supported by a permit from the NC Division of Parks and Recreation. Collections in Georgia National Forests were supported by a permit from the US Forest Service. Collections conducted in Virginia, West Virginia and Lake Raleigh (NC) were performed on private lands with permission from their respective owners. Ed Olson and John Blake for their assistance in locating field sites at Savannah River Site, a National Environmental Research Park. Logistical support was provided by the Department of Energy-Savannah River Operations Office through the U.S. Forest Service Savannah River under Interagency Agreement DE-AI09-00SR22188. Mike Joyce and the U.S. Forest service for permitting research insect collections.

References

- Alloway TM, Buschinger A, Talbot M, Stuart R, Thomas C (1982) Polygyny and polydomy in three North American species of the genus *Leptothorax* Mayr (Hymenoptera: Formicidae). *Psyche* 89:249–274
- Antcat.org (2016) <http://www.antcat.org/>. Accessed 7 Jan 2016
- Antmaps.org (2015) <http://www.antmaps.org/>. Accessed 30 Dec 2015
- Bengston SE, Dornhaus A (2014) Be meek or be bold? A colony-level behavioural syndrome in ants. *Proc R Soc B* 281:20140518
- Bourke AFG (1999) Colony size, social complexity and reproductive conflict in social insects. *J Evol Biol* 12:245–257
- Bourke AF, Heinze J (1994) The ecology of communal breeding: the case of multiple-queen leptothoracine ants. *Phil Trans R Soc B* 345:359–372
- Brown WL Jr, Wilson EO (1956) Character displacement. *Syst Zool* 5:49–64
- Brown JH, Marquet PA, Taper ML (1993) Evolution of body size: consequences of an energetic definition of fitness. *Am Nat* 142:573–584
- Buschinger A, Alloway TM (1978) Caste polymorphism in *Harpaxogenus canadensis* M.R. Smith (Hym., Formicidae). *Insectes Soc* 25:339–350
- Carter WG (1962) Ant distribution in North Carolina. *J Elisha Mitchell Sci Soc* 78:150–204
- Csősz S, Seifert B, Müller B, Trindl A, Schulz A, Heinze J (2014) Cryptic diversity in the Mediterranean *Temnothorax lichtensteini* species complex (Hymenoptera: Formicidae). *Org Divers Evol* 14:75–88
- Csősz S, Heinze J, Mikó I (2015) Taxonomic synopsis of the Ponto-Mediterranean ants of *Temnothorax nylanderii* species-group. *PLoS One* 10:e0140000. doi:10.1371/journal.pone.0140000
- Debout G, Schatz B, Elias M, McKey D (2007) Polydomy in ants: what we know, what we think we know, and what remains to be done. *Biol J Linnean Soc* 90:319–348
- Deyrup M (2003) An updated list of Florida ants (Hymenoptera: Formicidae). *Fla Entomol* 86:43–48
- Deyrup M, Cover S (2004) A new species of the ant genus *Leptothorax* from Florida, with a key to the *Leptothorax* of the southeast (Hymenoptera: Formicidae). *Fla Entomol* 87:51–59
- Ellison AM, Gotelli NJ, Farnsworth EJ, Alpert GD (2012) A field guide to the ants of New England. Yale University Press, New Haven
- Evans JD, Pierce NE (1995) Effects of diet quality and queen number on growth in *Leptothoracine* ant colonies (Hymenoptera: Formicidae). *J N Y Entomol Soc* 103:91–99
- Foitzik S, Heinze J (1998) Nest site limitation and colony takeover in the ant *Leptothorax nylanderii*. *Behav Ecol* 9:367–375

- Foitzik S, Backus VL, Trindl A, Herbers JM (2004) Ecology of *Leptothorax* ants: impact of food, nest sites, and social parasites. *Behav Ecol Sociobiol* 55:484–493
- Guénard B, Weiser MD, Dunn RR (2012a) Global models of ant diversity suggest regions where new discoveries are most likely are under disproportionate deforestation threat. *Proc Natl Acad Sci USA* 109:7368–7373
- Guénard B, McCaffrey KA, Lucky A, Dunn RR (2012b) Ants of North Carolina: an updated list (Hymenoptera: Formicidae). *Zootaxa* 3552:1–36
- Headley AE (1943) Population studies of two species of ants, *Leptothorax longispinosus* Roger and *Leptothorax curvispinosus* Mayr. *Ann Entomol Soc Am* 36:743–753
- Heinze J (2006) Life in a nutshell—social evolution in formicoxenine ants. In: Kipyatkov VE (ed) *Life cycles in social insects: behaviour, ecology and evolution*. Petersburg University Press, St. Petersburg, pp 49–61
- Heinze J, Buschinger A (1988) Polygyny and functional monogyny in *Leptothorax* ants (Hymenoptera: Formicidae). *Psyche* 95:309–326
- Heinze J, Rueppell O (2014) The frequency of multi-queen colonies increases with altitude in a Nearctic ant. *Ecol Entomol* 39:527–529
- Heinze J, Foitzik S, Hippert A, Hölldobler B (1996) Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderii*. *Ethology* 102:510–522
- Herbers JM (1984) Queen-worker conflict and eusocial evolution in a polygynous ant species. *Evolution* 38:631–643
- Herbers JM (1986a) Effects of ecological parameters on queen number in *Leptothorax longispinosus* (Hymenoptera; Formicidae). *J Kans Entomol Soc* 59:675–686
- Herbers JM (1986b) Nest site limitation and facultative polygyny in the ant *Leptothorax longispinosus*. *Behav Ecol Sociobiol* 19:115–122
- Herbers JM (1993) Ecological determinants of queen number in ants. In: Keller L (ed) *Queen number and sociality in insects*. Oxford University Press, New York, pp 262–293
- Herbers JM, Johnson CA (2007) Social structure and winter survival in acorn ants. *Oikos* 116:829–835
- Herbers JM, Stuart RJ (1996) Patterns of reproduction in Southern versus Northern populations of *Leptothorax* ants (Hymenoptera: Formicidae). *Ann Entomol Soc Am* 89:354–360
- Herbers JM, Tucker CW (1986) Population fluidity in *Leptothorax longispinosus* (Hymenoptera; Formicidae). *Psyche* 93:217–229
- Hölldobler B, Wilson EO (1977) The number of queens: an important trait in ant evolution. *Naturwissenschaften* 64:8–15
- Hölldobler B, Wilson EO (2008) *The Superorganism: the beauty, elegance, and strangeness of insect societies*. W. W. Norton and Company, New York
- Hou C, Kaspari M, Vender Zanden HB, Gillooly JF (2010) Energetic basis of colonial living in social insects. *Proc Natl Acad Sci USA* 107:3634–3638
- Hughes WHO, Oldroyd BP, Beekman M, Ratnieks FLW (2008) Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science* 320:1213–1216
- James FC (1970) Geographic size variation in birds and its relationship to climate. *Ecology* 51:365–390
- Janicki JH, Narula N, Ziegler M, Guénard B, Economo EP (2016) Visualizing and interacting with large-volume biodiversity data using client-server web mapping applications: the design and implementation of antmaps.org. *Ecol Inform* 32:185–193
- Johnson CA, Herbers JM (2006) Impact of parasite sympatry on the geographic mosaic of coevolution. *Ecology* 87:382–394
- Kaspari M (2005) Global energy gradients and the regulation of body size: worker mass and worker number in ant colonies. *Proc Natl Acad Sci USA* 102:5079–5083
- Kaspari M, Vargo EL (1995) Colony size as a buffer against seasonality: Bergmann's rule in social insects. *Am Nat* 145:610–632
- Kaspari M, Ward PS, Yuan M (2004) Energy gradients and the geographic distribution of local ant diversity. *Oecologia* 140:407–414
- Kikuchi T, Tsuji K, Ohnishi H, Le Breton J (2007) Caste-biased acceptance of non-nestmates in a polygynous ponerine ant. *Anim Behav* 73:559–565
- Lack DL (1947) The significance of clutch size, parts 1 and 2. *Ibis* 89:302–352
- Lessard JP, Borregaard MK, Fordyce JA, Rahbek C, Weiser MD, Dunn RR, Sanders NJ (2012) Strong influence of regional species pools on continent-wide structuring of local communities. *Proc R Soc B* 279:266–274
- Linksvayer TA (2008) Queen-worker-brood coadaptation rather than conflict may drive colony resource allocation in the ant *Temnothorax curvispinosus*. *Behav Ecol Sociobiol* 62:647–657
- Lubertazzi D, Alpert GD (2014) The ants (Hymenoptera: Formicidae) of Jaragua National Park. *J Insects*. Article ID 104157:6 pages. doi:10.1155/2014/104157
- MacGown JA (2006) Hickory nuts used as nesting sites by ants (Hymenoptera: Formicidae). *Marginalia Insecta* 1:1–3
- MacGown JA, Brown RL (2006) Survey of ants (Hymenoptera: Formicidae) of the Tombigbee National Forest in Mississippi. *J Kans Entomol Soc* 79:325–340
- MacGown JA, Forster JA (2005) A preliminary list of the ants (Hymenoptera: Formicidae) of Alabama, USA. *Entomol News* 116:61–74
- NOAA.gov (2016), National Oceanic and Atmospheric Administration, National Centers for Environmental Information. <http://www.ncdc.noaa.gov/>. Accessed 16 June 2016
- Nonacs P, Kapheim KM (2010) Social heterosis and the maintenance of genetic diversity. *J Evol Biol* 20:2253–2265
- Peeters C (1993) Monogyny and polygyny in ponerine ants with or without queens. In: Keller L (ed) *Queen number and sociality in insects*. Oxford University Press, Oxford, pp 234–261
- Prebus M (2015) Palearctic elements in the old world tropics: a taxonomic revision of the ant genus *Temnothorax* Mayr (Hymenoptera, Formicidae) for the Afrotropical biogeographical region. *Zookeys* 483:23–57
- Ricklefs RE (1987) Community diversity: relative roles of local and regional processes. *Science* 235:167–171
- Rüppell O, Heinze J, Hölldobler B (1998) Size-dimorphism in the queens of the North American ant *Leptothorax rugatulus* (Emery). *Insectes Soc* 45:67–77
- Rüppell O, Heinze J, Hölldobler B (2001a) Alternative reproductive tactics in the queen-size-dimorphic ant *Leptothorax rugatulus* (Emery) and their consequences for genetic population structure. *Behav Ecol Sociobiol* 50:189–197
- Rüppell O, Heinze J, Hölldobler B (2001b) Genetic and social structure of the queen size dimorphic ant *Leptothorax* cf. *andrei*. *Ecol Entomol* 26:76–82
- Salata S, Borowiec L (2015) Redescription of *Temnothorax antigoni* (Forel, 1911) and description of its new social parasite *Temnothorax curtisetosus* sp. n. from Turkey (Hymenoptera, Formicidae). *ZooKeys* 523:129–148
- Sanders NJ, Gotelli NJ, Heller NE, Gordon DM (2003) Community disassembly by an invasive species. *Proc Natl Acad Sci USA* 100:2474–2477
- Santos JC, Cannatella DC (2011) Phenotypic integration emerges from aposematism and scale in poison frogs. *Proc Natl Acad Sci USA* 108:6175–6180
- Seifert B, Csösz S (2015) *Temnothorax crasecundus* sp. n.—a cryptic Eurocaucasian ant species (Hymenoptera, Formicidae) discovered by Nest Centroid Clustering. *ZooKeys* 479:37–64

- Shik JZ (2008) Ant colony size and the scaling of reproductive effort. *Funct Ecol* 22:674–681
- Shik JZ, Hou C, Kay A, Kaspari M, Gillooly JF (2012) Toward a general life history model of the superorganism: predicting the survival, growth, and reproduction of ant societies. *Biol Lett* 8:1059–1062
- Stuart RJ (1985) Spontaneous polydomy in laboratory colonies of the ant *Leptothorax curvispinosus* Mayr (Hymenoptera: Formicidae). *Psyche* 92:71–82
- Stuart RJ (1992) Nestmate recognition and the ontogeny of acceptability in the ant, *Leptothorax curvispinosus*. *Behav Ecol Sociobiol* 30:403–408
- Stuart RJ, Gresham-Bissett L, Alloway TM (1993) Queen adoption in the polygynous and polydomous ant, *Leptothorax curvispinosus*. *Behav Ecol* 4:276–281
- Talbot M (1957) Population studies of the slave-making ant *Leptothorax duloticus* and its slave, *Leptothorax curvispinosus*. *Ecology* 38:449–456
- Tschinkel WR (2011) Back to basics: sociometry and sociogenesis of ant societies (Hymenoptera: Formicidae). *Myrmecol News* 14:49–54
- Tsuji K (2010) What brings peace to the world of ants (Hymenoptera: Formicidae)? *Myrmecol News* 13:131–132
- Ugelvig LV, Kronauer DJC, Schrempf A, Heinze J, Cremer S (2010) Rapid anti-pathogen response in ant societies relies on high genetic diversity. *Proc R Soc B* 277:2821–2828
- Ward PS, Brady SG, Fisher BL, Schultz TR (2015) The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). *Syst Entomol* 40:61–81. doi:[10.1111/syen.12090](https://doi.org/10.1111/syen.12090)
- Wheeler WM (1931) New and little-known ants of the genera *Macromischa*, *Creosomyrmex* and *Antillaemyrmex*. *Bull Mus Comp Zool* 72:1–34
- Wilson EO (1951) A new *Leptothorax* from Alabama (Hymenoptera: Formicidae). *Psyche* 57:128–130