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Sociometry of *Solenopsis geminata* (Hymenoptera: Formicidae) reveals variation in colony-level phenotypes in fire ants

Waring TRIBLE, David DeWayne SHOEMAKER & Dietrich GOTZEK



Abstract

In social insects, natural selection operates at the level of the colony, rather than the individual, but our understanding of how colony-level phenotypes arise and vary between species is lacking. Here, we test how colony-level phenotypes vary within the fire ants by measuring the composition of colonies of the tropical fire ant, *Solenopsis geminata*, over a wide range of sizes at multiple times throughout the year. Similar to the well-studied fire ant species *S. invicta*, we find that *S. geminata* colony composition varies strongly with colony size, such that as colonies grow they produce increasingly large workers as well as queens and males. However, major production increases more rapidly with colony size in *S. geminata* than in *S. invicta*, which may explain our observation that *S. geminata* also possesses a smaller maximum colony size. Unlike *S. geminata* or *S. invicta* in the USA, we find no evidence for seasonal variation in colony composition in *S. geminata* in Costa Rica. *Solenopsis geminata* colonies from Costa Rica also exhibit continuous variation in queen number, from one to two to hundreds. Overall, this research describes how colony-level features vary within the fire ants, providing basic data that can be used to study the mechanisms underlying the development and evolution of colony-level phenotypes in social insects.

Key words: Fire ant, sociometry, sociogenesis, colony-level phenotype, colony size, evo-devo.

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Waring Tribble (contact author), Laboratory of Social Evolution and Behavior, The Rockefeller University, 1230 York Avenue, Box 71, New York, New York, 10065, USA. E-mail: wtribble@rockefeller.edu

David DeWayne Shoemaker, Entomology and Plant Pathology Department, University of Tennessee, 371 Plant Biotechnology Building, Knoxville, TN, 37966, USA.

Dietrich Gotzek, Department of Entomology, Smithsonian Institution, Washington, DC, 20013, USA.

Introduction

The primary unit of natural selection in social insects is the colony rather than the individual organism (HÖLDOBLER & WILSON 2009). Social insect species can therefore adapt to their environments via modifications to colony-level properties, such as colony size and colony composition (HÖLDOBLER & WILSON 2009). For example, striking morphological variation occurs among individuals in ant colonies, including morphologically divergent female castes, such as workers and queens, and distinctive males. These forms are not found at all times in all colonies but are dynamically regulated, both in relation to colony development and season (TSCHINKEL 2011). Similar to describing the morphology of a solitary organism, the description of social insect colonies, or sociometry, requires carefully measuring colony composition, such as number and type of workers, brood, and sexuals (TSCHINKEL 1991). To understand how colony composition varies with colony development (termed sociogenesis) and with season, these data must be collected over the full range of colony sizes at multiple time points throughout the year (WILSON 1985, TSCHINKEL 1991). Sociometry and sociogenesis give a broad overview of a species' biology, providing an essential starting point for describing the mechanisms by which social insects

evolve differences in colony-level phenotypes such as caste production, colony size, and queen number (WILSON 1985, TSCHINKEL 1991, TSCHINKEL 2011, BURCHILL & MOREAU 2016). These studies also present a new frontier for evolutionary developmental biology, allowing researchers to describe how developmental rules can be modified to produce adaptive phenotypes not only at the level of the individual but also at the level of the group (YANG 2007).

In this study, we describe the sociometry and sociogenesis of the tropical fire ant, *Solenopsis geminata* (FABRICIUS, 1804), in its native range in Costa Rica (GOTZEK & al. 2015), allowing for comparisons with similar data for an *S. geminata* population in Florida and the model ant species *S. invicta* BUREN, 1972 (MCINNES 1994, TSCHINKEL 2006). This description of *S. geminata* in Costa Rica helps to provide a clearer picture of fire ant biology in general, allowing us to describe common properties of this group and infer how fire ants have evolved differences in colony-level traits. In particular, *S. geminata* is a tropical species with dramatic worker polymorphism (TSCHINKEL 2013). *Solenopsis invicta*, on the other hand, is sub-tropical and exhibits more subtle worker polymorphism (TSCHINKEL 2013). *Solenopsis geminata*, like *S. invicta*, is also socially polymorphic,

with both single-queen (monogyne) and multiple-queen (polygyne) colonies (BANKS & al. 1973, ADAMS & BANKS 1976), but is not known to possess the *Gp-9b* allele or social chromosome that is associated with polygyny in *S. invicta* (ROSS & al. 2003, KRIEGER & ROSS 2005, WANG & al. 2013; D. Gotzek, unpubl.). Finally, like *S. invicta*, *S. geminata* is an invasive species worldwide (WETTERER 2011, GOTZEK & al. 2015), and can have both positive and negative effects on agriculture through its influence on ants and other insects (PHILPOTT 2006, TRIBLE & CARROLL 2014). Understanding the sociometry and sociogenesis of *S. geminata* will therefore provide a valuable resource for future studies in ant biology, ecology, and evolution.

Methods

Our methods follow those employed by W.R. Tschinkel as closely as possible (TSCHINKEL 1993, 2011). We excavated seven to nine *Solenopsis geminata* colonies in the Monteverde cloud forest habitat (elevation 1150 m) at the University of Georgia Costa Rica campus in San Luis, Costa Rica, in November 2011, May 2012, June 2012, and February 2013. The rainy season in Monteverde is approximately May through November, so we included collection dates during the rainy and dry seasons as well as the two transitions between seasons. Colonies were identified by observing nest mounds in disturbed areas such as roadsides or grassy fields. At each time point, colonies were chosen to represent a large range of colony sizes, from the smallest to the largest colonies possible. Each colony was fully excavated into a large plastic bin by digging directly below the nest mound until no further ants were seen emerging from the soil. This method has been estimated to capture approximately 80–90% of ants in the colony (TSCHINKEL 1991). However, it is important to note that foraging workers outside of the nest are not recovered using this method (TSCHINKEL 2011). The soil (mixed with ants) was then transferred at least seven times between two bins until thoroughly mixed and then weighed. After weighing, three 500-g samples of soil and ants were collected from different parts of the bin. These samples were taken back into the lab, and all ants (workers, sexuals, and brood) were sorted live from the soil using an aspirator and preserved in 70% ethanol. Worker number and colony composition was estimated by extrapolating data from these three 500-g samples to the total mass of the colony.

To estimate worker size variation, we arranged heads from the smallest to largest workers identified in this study into a size class scale and measured maximum head width using the ocular scale on an SZ61 Olympus stereoscope at a field of view of approximately 2.2 mm. The full range of head widths was broken into five 0.325 mm increments, Class 1 through Class 5, and individual worker adults were manually sorted into categories using this scale. Overall, each sample was sorted into 12 categories of ants: worker adults (Class 1 through Class 5), worker larvae, worker pupae, alate queens, dealate queens, males, sexual larvae, and sexual pupae. All ants in each category were counted, dried in an oven, and weighed. These measurements were averaged across the three samples for each colony, and then extrapolated using the mass of the colony to estimate the total number and mass of ants in each category in each colony. Mass for individual workers, males, and brood was estimated by weighing all ants in each category for each sample and dividing the total mass by the number of individuals. This allowed us to obtain average, but not individual, masses for

workers, males, and brood. Alate and dealate queens were weighed individually. Three colonies were excluded from all analyses involving worker or sexual brood because the brood molded during storage.

A combination of Fisher's exact tests and linear regressions were performed using Prism 7 with an alpha of $p = 0.05$. We tested whether colonies with more than 20,000 workers ($n = 16$) were more likely to possess Class 5 workers and sexuals than colonies with fewer than 20,000 workers ($n = 16$) using one-way 2×2 Fisher's exact tests. We tested for seasonal variation in the presence of sexuals using two-way 2×4 Fisher's exact tests.

Linear regressions were used to test whether numbers and mass of workers and brood varied with colony size (TSCHINKEL 1988). Count data were log-transformed for all regression analyses, while mass data were not. Residuals were normally distributed in all analyses except one, the regression of minor workers and colony size. A quadratic regression was found to have normally distributed residuals, but these results were virtually indistinguishable from the linear regression results, so for simplicity only the linear regression results are reported here. We tested for seasonal variation in numbers and mass of workers and brood using the residuals of the linear regression analyses. Residuals were classified as above or below the linear regression line and tested for variation between time points using 2×4 Fisher's exact tests.

We verified social organization of 16 colonies using microsatellite loci for which workers had been collected into 95% ethanol. We genotyped 12 workers per colony using 14 variable microsatellite loci, using previously reported primers and cycling profiles (ROSS & al. 2010, ASCUNCE & al. 2009, KRIEGER & ROSS 2005, ASCUNCE & al. 2011). Worker genotypes that were not consistent with singly mated single queen colony social organization (i.e., monogyne) were considered polygyne.

Results

Our final dataset included 32 colonies from four time points throughout the year (Fig. 1), and is available in Table S1 as digital supplementary material to this article, at the journal's web pages. The largest colony had approximately 140,000 workers, and the next three largest colonies had approximately 80,000 workers. *Solenopsis geminata* in Costa Rica therefore forms substantially smaller colonies compared with *S. invicta* in its introduced range in the USA, where colonies frequently exceed 200,000 workers (TSCHINKEL 2006). These results are consistent with MCINNIS (1994), who found that *S. geminata* colonies are smaller than *S. invicta* colonies in Florida, and TSCHINKEL (1987), who found that mated *S. geminata* queens possess fewer sperm than mated *S. invicta* queens. Workers are continuously polymorphic over a large size range, from under 0.1 mg to 2.6 mg dry mass and 0.58 mm to 2.4 mm in head width (Fig. 2). Following TSCHINKEL (1988), we provisionally define the smallest set of workers, Class 1, as minor workers, and larger workers, Class 2 - 5, as major workers (this is a subjective distinction; see Discussion). Winged queens are substantially larger than workers, with an average dry mass of 6.25 mg, but have a head width of approximately 1.6 mm, similar to that of Class 4 workers (Fig. 2). The smallest winged queen we observed had a mass of 2.24 mg, only slightly lighter than the largest major. Males had an average dry mass of 1.46 mg and a head width of approximately 0.9 mm (Fig. 2).

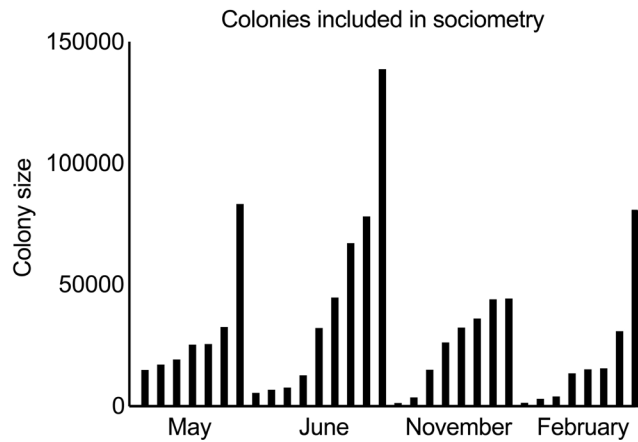


Fig. 1: The 32 colonies employed in sociometric analysis. Within each season, we attempted to select colonies that represent the full range of *Solenopsis geminata* colony sizes.

Variation with colony size: As colonies increase in worker number they produce increasingly large workers, as well as producing males and queens (Fig. 3a, b). The smallest colonies, with under 2000 workers, produced only minor workers (Class 1), and all other colonies produced major workers (Class 2 and higher). Class 5 workers and sexuals were present in significantly more colonies with above 20,000 workers than below 20,000 workers ($p = 0.001$ and $p = 0.027$, respectively, one-tailed Fisher's exact test). Colonies varied widely in biomass, from 0.2 g to 48.7 g dry mass, and larger colonies dedicated a larger proportion of colony biomass to major workers and sexual production (Fig. 3c).

The slope of the log-log regression of total worker number and minor worker number is significantly less than 1, whereas the slope of major workers and colony size is significantly greater than 1 ($p < 0.05$, Fig. 4a). Thus, as colonies increase in size, the proportion of minor workers decreases and the proportion of major workers increases (these findings are also observed with respect to colony biomass). The slope of the log-log regression of total worker number and major worker number is 1.7 in *S. geminata*, but is 1.3 in *S. invicta* (TSCHINKEL 1988, 2006). These slopes indicate that a ten-fold increase in worker number is accompanied by a 50-fold increase in major number in *S. geminata*, but only a 20-fold increase in *S. invicta*. Average worker mass increased with colony size (slope = 0.060 ± 0.022), as did the mass of workers within each size class, on average (Fig. 4b). However, these linear regressions were only significantly greater than zero with all workers, Class 1 workers, and Class 4 workers.

Worker brood number also increased significantly as colonies increased in size (Fig. S1). The 95% confidence interval for the slope of this log-log regression encompasses values both above and below 1, so it is not possible to determine whether the worker: worker brood ratios increase or decrease with colony size. However, the point estimate for the slope is 0.8, indicating that worker: worker brood ratios may increase as colonies increase in size, as is observed in *S. invicta* (TSCHINKEL 1993).

Seasonality: Sexual production occurred in May, July, and November. Using a Fisher's exact test, we observed no significant variation in sexual production between the collection dates ($p = 0.744$). Colonies produced worker brood year-round, and no significant differences were observed in the number or mass of brood ($p = 0.239$ and $p =$

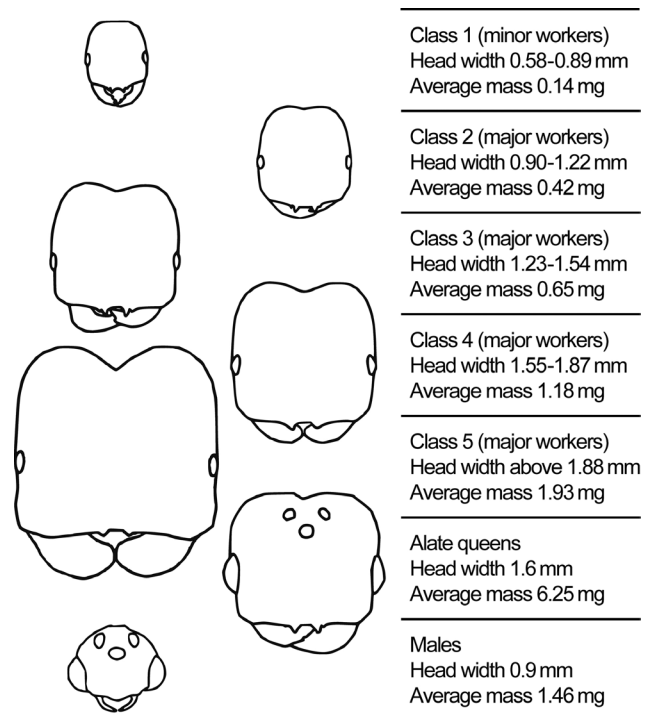


Fig. 2: Adult morphological variation in *Solenopsis geminata*. Workers were split into five size classes. Single representatives were measured for queen and male head widths. Outlines were generated using images from AntWeb.org.

0.619) (Fig. 5). There was also no significant difference in the number of major workers or average worker mass ($p = 0.096$ and 0.193) (Fig. 5). Therefore, no strong patterns of seasonality were observed in our sociometric data, indicating that *Solenopsis geminata* colonies in Costa Rica likely grow and reproduce at an equal rate year-round. However, *S. geminata* in Florida do exhibit seasonal variation in sexual production, indicating that lack of seasonality in our data may result from the difference in climate between Florida and Costa Rica (McINNES 1994). *Solenopsis invicta* also exhibits strong seasonal variation in sexual production, worker brood production, and worker size (TSCHINKEL 1993, 2006). While we did not observe any seasonal variation in *S. geminata* in Costa Rica, it is also possible that some subtler seasonal effects (such as effects restricted to specific colony sizes) may exist.

Queen number: We performed microsatellite analyses for a set of 16 colonies in our study population to test whether colonies were monogyne or polygyne, by genotyping 12 workers per colony at 14 loci. Microsatellites confirmed existence of three monogyne and 13 polygyne colonies in *S. geminata* in Costa Rica (Tab. S1). Dealate queens were recovered from 13 of the 32 colonies included in this study. During colony excavations, any dealate queens that we observed were hand collected, and we identified colonies with two to 10 queens in this manner (Fig. 6). In two of these colonies, two and three queens were found in a single chamber with eggs, and we find it likely that these were the only queens present in these colonies. Queens were also identified in colonies as part of the sociometric samples, leading to estimates ranging from 11 to 293 queens per colony (Fig. 6). Thus, our data are consistent with continuous variation in queen number in *S. geminata* in Costa Rica, from one to hundreds. It is

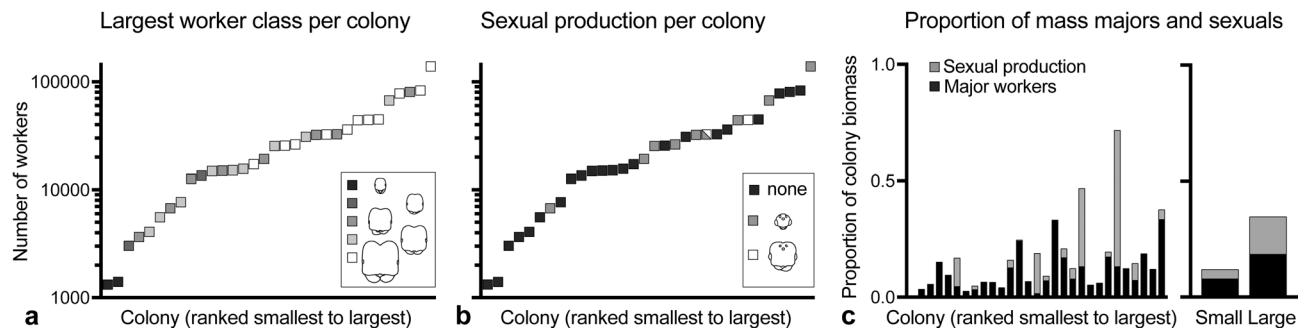


Fig. 3: Variation in caste production with colony size. (a) Largest worker class in each colony. On average, larger classes of workers are found in larger colonies ($p = 0.001$, Fisher's exact test). For example, the largest workers in the smallest two colonies we collected were Class 1, whereas the largest workers in the largest two colonies we collected were Class 5. (b) Production of sexuals (males and alate queens) in each colony. Sexuals were primarily produced in colonies of over 20,000 workers ($p = 0.027$, Fisher's exact test). Alate queens were found in only two colonies, both of which also possessed Class 5 workers. (c) Proportion of colony biomass dedicated to major workers and sexual production (sexual brood, alate queens, and males); the remainder of colony biomass is dedicated to minor workers and worker brood. Left: all colonies. Right: average of colonies under 20,000 workers (Small) and over 20,000 workers (Large).

important to note that in many colonies dealate queens were observed in only one of the three sociometric samples, so the variance in these estimates of queen number may be large (see Tab. S2).

Variation in queen number was not significantly associated with colony size ($p = 0.99$, linear regression). Our microsatellite analyses recovered just three monogyne colonies, so we were not able to statistically test whether monogyne colonies are smaller on average than polygyne colonies, as has been observed in *Solenopsis invicta*. We did not recover a sufficient number of queens to test whether microgyne and macrogyne queens exist in this population, though both have been observed in a monogyne *S. geminata* population in Florida (McINNES & TSCHINKEL 1995).

Discussion

We present the first description of the sociometry and sociogenesis of the tropical fire ant, *Solenopsis geminata*. This is the first study to genetically confirm that both monogyny and polygyny exist in this species in Costa Rica, and it describes many other attributes of colony composition. At a broad level, the patterns we observe in *S. geminata* are consistent with patterns of colony growth seen throughout the eusocial Hymenoptera: As colonies increase in size, they produce increasingly large workers, then queens and males. However, we observe specific differences in colony-level properties of *S. geminata* in Costa Rica relative to *S. geminata* in Florida and to the closely related species *S. invicta*.

Solenopsis geminata colonies on average are smaller than *S. invicta* colonies (TSCHINKEL 1987, 1993, 2006), but *S. geminata* colonies from Costa Rica were larger on average than *S. geminata* colonies collected by McINNES (1994) in Florida. Both *S. geminata* and *S. invicta* in Florida exhibit clear seasonal variation in sexual production that we did not observe (TSCHINKEL 1993, McINNES 1994, TSCHINKEL 2006). Similarly, McINNES (1994) reported a lack of surface activity of *S. geminata* colonies during the winter months, while we observed colony surface activity year-round. All *S. geminata* colonies collected by McINNES (1994) were monogyne, whereas we found both monogyne and polygyne colonies in Costa Rica (Fig. 6; Tab. S1). Variation in sexual production with colony size is observed throughout

the social insects, including *S. invicta* and our data from *S. geminata*. Surprisingly, McINNES (1994) did not observe any variation in sexual production as a function of colony size in *S. geminata* in Florida. These data illustrate that colony-level properties in fire ants can vary widely, paving the way for future researchers to identify the combination of genetic and environmental factors that produce this variation.

Our study adds to a growing list of traits that vary within the nominal *Solenopsis geminata* species. *Solenopsis geminata* colonies from Costa Rica and Florida (McINNES 1994) differ in size, seasonality, and social polymorphism. *Solenopsis geminata* colonies from Florida and Costa Rica also differ genetically and in worker allometries (TSCHINKEL 2013, GOTZEK & al. 2015). These differences may be associated with two *S. geminata* color morphs, red and black, which also differ in cuticular hydrocarbon profiles (GOTZEK & al. 2015, HU & al. 2017). Future research is required to determine whether the nominal *S. geminata* may actually represent a species complex consisting of multiple species or whether *S. geminata* simply possesses a large degree of intraspecific variation (TRAGER 1991, SHOEMAKER & al. 2006).

Female size in fire ants is clearly a function of colony size: as colonies grow, they produce increasingly large workers, then queens (Figs. 3, 4) (TSCHINKEL 1988). It was recently proposed that caste development in ants is a function of female body size, such that smaller individuals develop worker-like morphology and larger individuals develop queen-like morphology (TRIBBLE & KRONAUER 2017). This proposal is consistent with our data from *S. geminata*: Minors, majors, and queens differed in average mass, and the largest major had a similar mass to that of the smallest queen (note that we measured adult mass, which includes developmentally irrelevant factors such as gut contents and developing eggs inside ovaries) (TRIBBLE & KRONAUER 2017). Following TSCHINKEL (1988), we provisionally defined the smallest set of workers, Class 1, as minor workers and larger workers, Class 2 - 5, as major workers. However, our data were not of sufficiently high resolution to delimit castes using a normal score analysis as in TSCHINKEL (1988), so these definitions should be interpreted with caution.

We emphasize that this hypothesis of size-based caste determination does not imply that differences in caste

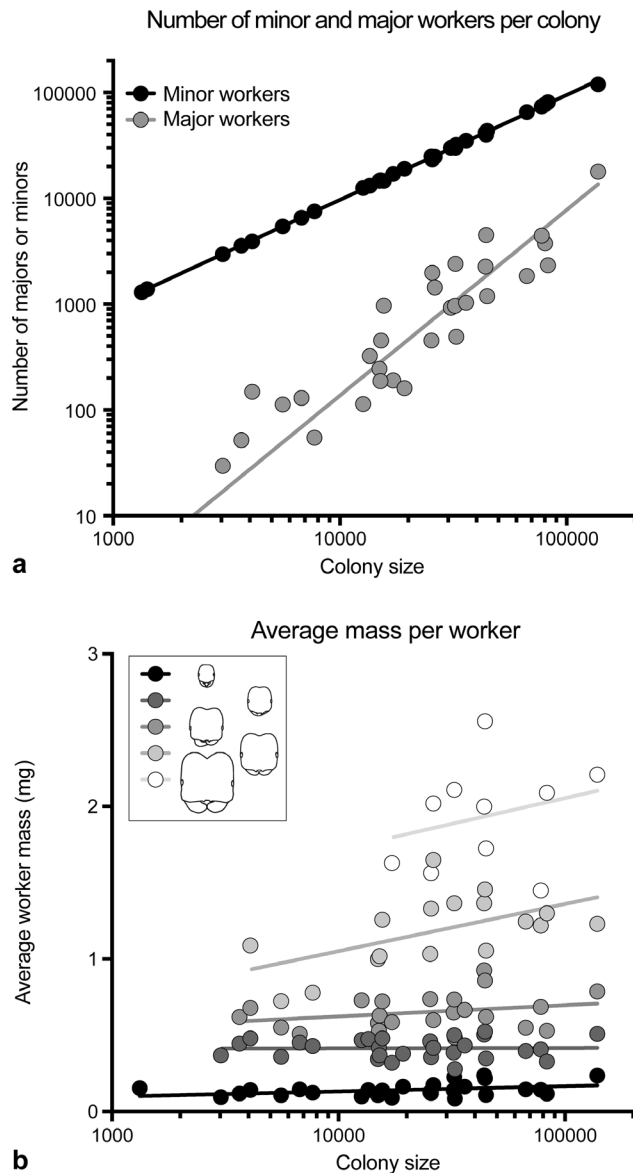


Fig. 4: Changes in worker frequency and mass with colony size. (a) number of minor and major workers in each colony. The slope (best-fit values \pm standard error, SE) for minor workers (Class 1) is 0.984 ± 0.004 and for major workers (Class 2 - 5) 1.742 ± 0.121 , indicating that the proportion of minor workers decreases and the proportion of major workers increases as colonies increase in worker number. (b) Average mass per worker class in each colony. The slopes (best-fit values \pm SE) are: Class 1 = 0.034 ± 0.016 , Class 2 = 0.002 ± 0.030 , Class 3 = 0.074 ± 0.056 , Class 4 = 0.309 ± 0.119 , Class 5 = 0.337 ± 0.422 .

development result primarily from nutritional variation during the larval stage. In holometabolous insects, larval growth typically follows a progression called Dyar's rule, in which the head width is fixed within each larval instar, and increases by a fixed multiple (e.g. $1.4\times$) between instars (DYAR & RHINEBECK 1890). In *Solenopsis invicta*, the head-widths of larvae increase across instars as predicted by Dyar's rule, but larger colonies produce larger first-instar larvae (O'NEAL & MARKIN 1975). These results imply that the factors causing variation in adult female body size in fire

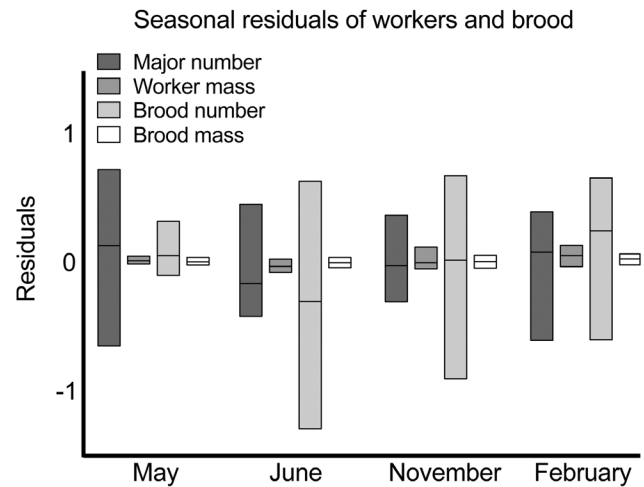


Fig. 5: Residuals, separated by season, of linear regression with colony size and major number, mass per worker, worker brood number, and mass per worker brood. Residuals do not differ significantly between seasons for any category, illustrating no strong seasonal variation in *S. geminata* colony composition ($p > 0.05$, Fisher's exact test). Residuals for major number and brood number correspond to log-transformed count values, and residuals for worker mass and brood mass correspond to milligrams.

ants are present at the time that larvae hatch from eggs and that adult size is therefore regulated primarily by maternal effects (O'NEAL & MARKIN 1975, TRIBLE & KRONAUER 2017).

Here we show that average colony sizes vary between three fire ant populations: *Solenopsis geminata* forms smaller colonies than *S. invicta* in Florida, but the *S. geminata* colonies we surveyed in Costa Rica were larger than those surveyed by MCINNIS (1994) in Florida. In a general sense, it is logical that worker morphology and potentially sexual production might change more rapidly with colony size in populations with smaller colonies in order to reach a mature colony composition before the maximum colony size is reached. Intriguingly, phenotypic differences between the three fire ant populations indicate that this may be the case. In *S. geminata* in Costa Rica, the slope of major production and colony size is much higher than in *S. invicta* (Fig. 4a), which could allow *S. geminata* colonies to attain a mature worker composition at their smaller maximum size. In terms of allometry, worker morphology changes more rapidly as a function of body size in *S. geminata* in Florida than in Costa Rica (i.e., the slope of the allometry is higher), which could allow an additional reduction in colony size while maintaining the complete range of worker morphologies (TSCHINKEL 2013). Clearly, the potential association of caste development and colony size in fire ants warrants further attention.

We also observed continuous variation in queen number in *Solenopsis geminata*, from one to two to hundreds (Fig. 6). In *S. invicta*, polygyny is associated with a supergene, marked by the *Gp-9b* allele, and is inherited in a Mendelian manner (ROSS & KELLER 1998, WANG & al. 2013). In mature *S. invicta* colonies in its invasive range, queen number is dimorphic, with colonies possessing either one or many queens (GOTZEK & ROSS 2007). The continuous variation in queen number we observe in *S. geminata* appears to be inconsistent with the dimorphic variation observed in

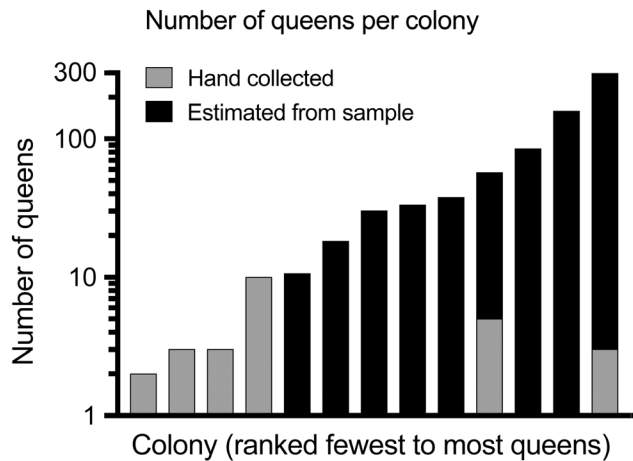


Fig. 6: Number of dealate queens in the 13 colonies in which queens were collected. In the complete dataset, queen numbers ranged widely, from 1 (inferred from microsatellite analysis) to 2 or 3 (inferred from hand collections) to 293 (inferred from sociometric estimates). Note that hand-collected and sociometric estimates of queen number refer to dealates, many of which may not be reproductively active (TSCHINKEL 2006).

S. invicta. However, our data were collected from a range of colony sizes (rather than solely mature colonies), so we cannot conclusively determine whether the distribution of colony queen numbers in *S. geminata* truly differs from *S. invicta*. The mechanisms that control queen number in *S. geminata* are not known, but our data imply that they may have a strong environmental component, or, if genetic, are likely to be regulated by mechanisms that can produce quantitative variation.

Solenopsis geminata is an economically important invasive species in tropical areas worldwide, and is a close relative of the model ant species *S. invicta*. We have demonstrated a substantial amount of variation in colony-level traits exists within the fire ants, including seasonality, caste production, colony size, and queen number. This study illustrates how sociometric data can generate testable predictions regarding the development and evolution of colony-level phenotypes, such as caste development, colony size, and queen number. Sociometry and sociogenesis provide a rich source of information that will help us to understand fundamental aspects of social insect biology, ecology, and evolution, and we hope to encourage the current generation of social insect researchers to continue to collect this type of basic biological data (TSCHINKEL 1991, BURCHILL & MOREAU 2016).

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