

Geographic Variation of Caste Structure among Ant Populations

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Summary

Morphologically distinct worker castes of eusocial insects specialize in different tasks. The relative proportions of these castes and their body sizes represent the demography of a colony that is predicted to vary adaptively with environments. Despite strong theoretical foundations, there has been little empirical evidence for the evolution of colony demography in nature. We show that geographically distinct populations of the ant *Pheidole morrisi* differ in worker caste ratios and worker body sizes in a manner consistent with microevolutionary divergence. We further show that the developmental mechanism for caste determination accounts for the unique pattern of covariation observed in these two traits. Behavioral data reveal that the frequency of different tasks performed by workers changes in a caste-specific manner when caste ratios are altered and demonstrate the importance of the major caste in colony defense. The population-level variation documented here for *P. morrisi* colonies supports the predictions of adaptive demography theory and illustrates that developmental mechanisms can play a significant role in shaping the evolution of phenotype at the colony level.

Results and Discussion

The defining characteristic of eusocial insects is a colonial lifestyle in which individuals belong to castes that specialize in different tasks. Because a colony operates as a developmental and functional superorganismic unit [1, 2], the distribution of castes within a colony should reflect the colony's needs in a given environment and represent an adaptive demography [3–5]. When competitors and resources in the environment change, the adaptive demography of a colony is predicted to change in such a way as to optimize colony performance in the new environment [6, 7]. However, despite strong theoretical foundations, there have been limited empirical data that address this prediction [8, 9]. The few documented cases of demographic shifts have been plastic responses to short-term environmental changes [10–14], but theory also predicts that colonies in different geographic populations will exhibit different phenotypes as the result of evolutionary divergence [3, 15]. In the present study, we examine whether worker demography differs between natural populations of the ant *Pheidole*

morrisi to test this evolutionary prediction. Three study populations, located in Florida (FL), North Carolina (NC), and New York (NY) respectively, were chosen because they occur in very different biotic and climatic environments.

Evidence for the Divergence of Colony Phenotypes—Measures of Field Colonies

The three populations differed significantly in the percentage of majors in a colony and in worker body sizes. The FL population had a significantly greater mean percentage of majors ($15.0 \pm 0.9\%$ standard error [SE]) than both northern populations (NC = $10.3 \pm 0.5\%$ SE; NY = $10.6 \pm 0.8\%$ SE), which exhibited no statistical difference between them (Figure 1A; FL versus NC/NY: $F [1,70] = 27.077$, $p < 0.0001$; NC versus NY: $F [1,69] = 0.073$, $p = 0.787$, one-way ANOVA). The FL population also had significantly smaller mean body sizes for both worker castes, whereas NY and NC populations, again, did not differ in this demographic characteristic (Figure 1B; FL versus NC/NY: $t[56] = -4.466$, $p < 0.0001$; NC versus NY: $t[56] = 1.199$, $p = 0.235$, linear mixed effects [LME] ANOVA). The season of collection had no significant effect on body size ($t[56] = -0.458$, $p = 0.649$).

Evidence for the Divergence of Colony Phenotypes—Common-Garden Experiments

Cross-generational breeding experiments cannot be done in most ant species, and therefore common-garden experiments are used for assessing whether phenotypic differences between geographic populations are due to genetic or environmental differences. Common-garden experiments produced the same patterns of worker caste proportions and body sizes observed in the field; FL colonies produced a significantly higher percentage of majors than colonies from NC and NY (Figure 1C; FL versus NC/NY: $F [1,34] = 15.794$, $p = 0.0004$; NC versus NY: $F [1,33] = 0.080$, $p = 0.779$, one-way ANOVA), and the mean body size of FL minor and major pupae was smaller than that of pupae from NC and NY (Figure 1D; FL versus NC/NY: $t[33] = -5.717$, $p < 0.0001$; NC versus NY: $t[33] = -1.914$, $p = 0.064$, LME ANOVA). In another member of the genus, *Pheidole dentata*, individual colonies were found to possess characteristic minor/major caste ratios that after disruption would be homeostatically restored in a matter of weeks [16]. Likewise, different geographic populations of *Pheidole morrisi* exhibit characteristic differences in caste ratios that persist in a common rearing environment even after disruption (see Experimental Procedures). This pattern is consistent with the hypothesis that microevolutionary divergence in caste structure has occurred between the populations.

A Developmental Mechanism for Demographic Evolution

The peculiar association of a higher percentage of majors with a lower mean body size for both worker castes

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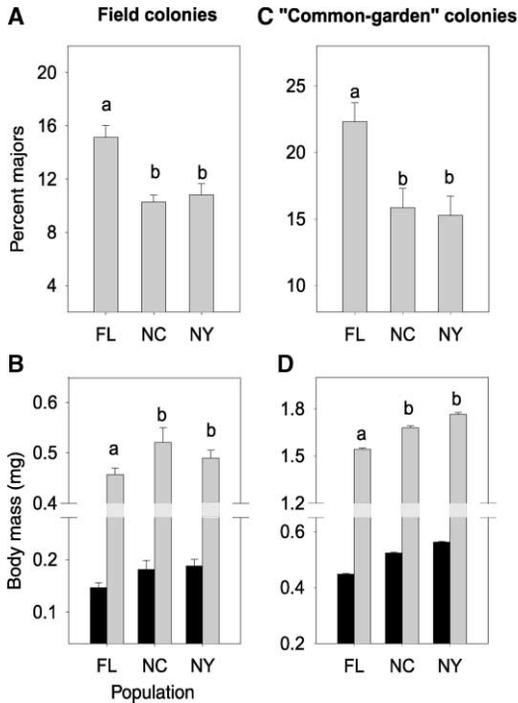


Figure 1. Worker Caste Proportions and Body Sizes of Field and Laboratory Colonies from Three Populations

Comparison of worker demography in three populations collected in the field (A and B), and from laboratory common-garden experiments (C and D). Scale bars represent means \pm SE.

(A) The percent majors per colony did not differ between NC and NY ($F [1,69] = 0.073, p = 0.787$) but were significantly lower than for the FL population ($F [1,70] = 27.077, p < 0.0001$, one-way ANOVA). (B) The mean dry fat-free body mass of workers (black: minor workers; gray: major workers) was not significantly different in the NY and NC populations ($t[56] = 1.199, p = 0.235$). However, the mean body masses of workers in FL colonies were significantly lower (approximately 10%) than in NY and NC ($t[56] = -4.466, p < 0.0001$, LME ANOVA, Helmert contrasts).

(C) Laboratory common-garden colonies generated the same pattern found in the field, with FL colonies producing more majors (FL versus NC/NY: $F [1,34] = 15.794, p = 0.0004$) (NC versus NY: $F [1,33] = 0.080, p = 0.779$, one-way ANOVA).

(D) The mean body masses of worker pupae in lab colonies also followed the same pattern as those from the field, with FL minors and majors each having smaller mean body size (FL versus NC/NY: $t[33] = -5.717, p < 0.0001$) (NC versus NY: $t[33] = -1.914, p = 0.064$, LME ANOVA, Helmert contrasts). In each panel, means with the same lowercase letter were not significantly different.

can be explained by the developmental mechanism that controls caste determination in *Pheidole* [17–19]. Early in larval development, any worker larva has the potential to become either a minor or a major worker. In the middle of the last larval instar, larvae that are above a well-defined threshold size for becoming major workers postpone pupation and grow to a much larger body size, whereas larvae below this threshold size finish their development as minor workers. Above-threshold larvae also reprogram the growth of their head imaginal disks so that the heads of majors are disproportionately large relative to their body size (Figure 2A).

This caste determination mechanism predicts that the proportion of majors will be correlated with the mean

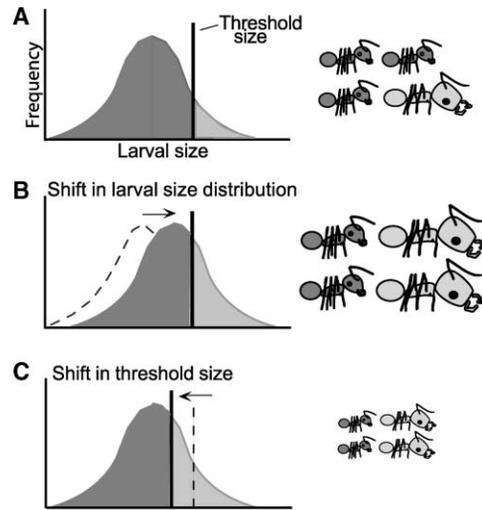


Figure 2. How Changes in the Developmental Mechanism of Worker Caste Determination in *Pheidole* Are Predicted to Affect the Proportion of Minors and Majors Produced as Well as Mean Body Sizes of These Castes

(A) Most larvae develop into minors (dark). However, larvae above a threshold size take an alternate developmental trajectory and become majors (light).

(B) If the mean size of larvae can be increased relative to a fixed threshold size, a larger proportion of majors will result, and both majors and minors will be of greater mean size than in scenario (A). (C) A greater proportion of majors is also produced if the larval size distribution is the same as in (A) but the threshold size for major determination is lower. In this case, the mean sizes of both minors and majors should be lower than in (A). The data presented here are consistent with (A) representing NY and NC populations and (C) representing the FL population.

body size of both worker castes and that the mean body size of minor and major worker castes will be positively correlated with each other. Thus, an increase in the percentage of majors a colony produces can be accomplished in two ways: by an upward shift of larval size distribution relative to a given threshold size (Figure 2B) or by a downward shift of the threshold size relative to a given larval size distribution (Figure 2C). Each case predicts a different correlated change in worker body size, and each is likely to be accomplished through a different mechanism; if the larval size distribution shifts upward relative to a given threshold size, then the increase in the proportion of larvae developing into majors will be accompanied by an *increase* in the mean size of both minors and majors (Figure 2B versus 2A). Such an upward shift can be achieved by a change in the nursing behavior of workers toward larvae. If, by contrast, the threshold size shifts downward relative to a given larval size distribution, then the increase in the proportion of larvae developing into majors will be accompanied by a *decrease* in the mean size of both minors and majors (Figure 2C versus 2A). Such a downward shift in threshold size would involve a change the developmental properties intrinsic to the larvae.

The observed pattern of demographic change found with latitude in *P. morrisi* (Figure 2C) only matches the predictions of the threshold shift model. Colonies from FL produce more majors than colonies from NC and NY

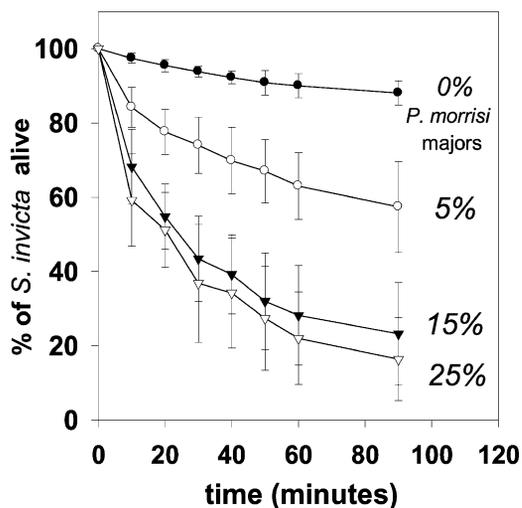


Figure 3. The Effect of Increasing the Percentage of Majors in Groups of 100 *P. morrisi* Matched against Groups of 50 Individuals of a Common Florida Competitor, *S. invicta*

Increasing the proportion of majors significantly enhances the defensive abilities of *P. morrisi*. For trials of this size, no significant increase in defensive ability was found above 15% majors. Each treatment includes ten replicates. Points represent means \pm SE.

(Figure 1A), and both worker castes are smaller in FL than in NC and NY (Figure 1B) [S1]. Accordingly, change in the threshold size intrinsic to larvae, rather than a change in nurse worker behavior, is the likely mechanism underlying the observed differences in caste structure. Indeed, shifts in threshold size are thought to be a common feature in the evolution of polyphenic traits in insects [20] and have been documented in other insect taxa [21].

Caste Proportions and Defense

The observed differences in worker demography raise the question of whether such differences are adaptive, or simply neutral, in regards to colony function. The increased proportion of major “soldiers” in FL colonies could be an adaptation that enhances colony defense in an environment with high interspecific competition. This hypothesis seems plausible given that a number of species that compete for the same resources as *P. morrisi*, especially ants of the genus *Solenopsis*, are common in the FL site but absent or rare in the NC and NY sites [22, 23]. It is also consistent with a general trend of increasing ant predation and competition that exists with decreasing latitude [24].

To test the feasibility of the defense hypothesis, we conducted laboratory contests of *P. morrisi* against a dominant FL competitor, the fire ant *Solenopsis invicta* (see Experimental Procedures). The defensive ability (measured as the number of fire ants killed in a given time period) was positively correlated with the number of *P. morrisi* soldiers in our experimental groups (Figure 3). The gain in defensive ability with increasing percentage of majors was substantial when majors ranged between 0% and 15% (10 min observation, Welch’s t test, one-sided, all $p < 0.005$), but groups containing more than 15% majors did not appear to gain significant addi-

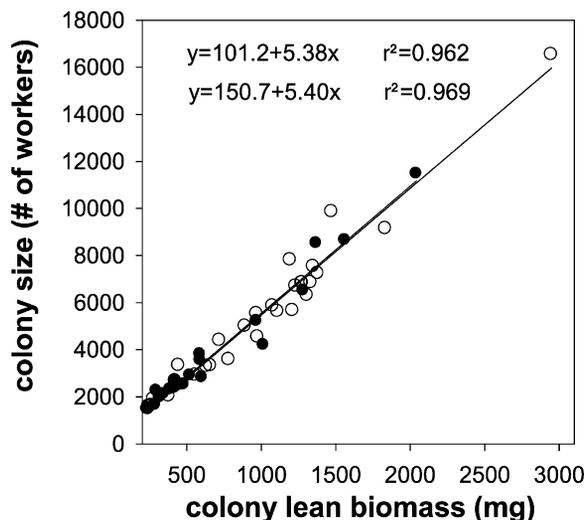


Figure 4. Total Lean Biomass versus Total Number of Workers, Minors and Majors, for NY, NC, and FL Colonies

White dots indicate NY and NC colonies, and black dots indicate FL colonies (NY = 9, NC = 20, and FL = 20 colonies). Despite the difference in the proportion of majors and mean workers sizes between NY/NC and FL colonies, they follow the same allometric relationship between total lean biomass and colony size in worker number (test for different linear regressions, for site, $t[48] = 0.158$, $p = 0.875$; for site \times total biomass $t[47] = 0.057$, $p = 0.955$). Although FL colonies invest approximately 10% more of their lean biomass into the major caste than NY and NC colonies, the 10% lower mean body size of minor and majors in this population compensates for this so that the total number of workers produced is equivalent to NC and NY colonies.

tional defensive ability. This finding demonstrates that *Pheidole* majors can have a significant defensive function and suggests that the additional 5% majors present in FL colonies (beyond the 10% observed for NC and NY colonies) may indeed confer a defensive advantage in the highly competitive FL environment.

Developmental Constraint and Colonial Phenotype

Because the proportion of worker castes and body size are developmentally linked in *P. morrisi*, any fitness gains that a colony achieves by altering one aspect of demography has to be balanced against fitness losses that result from the correlated change in the value of the other demographic parameter. The defensive advantage of a greater percentage of majors in FL, for example, must be traded off against the potential negative consequences of smaller mean body size of the individuals in this caste [25]. Indeed, the lean body mass of *P. morrisi* majors was positively correlated with their defensive ability (number of *S. invicta* killed per standard trial, Pearson’s correlation, $r[98] = 0.256$; $p = 0.01$). However, the loss incurred by this trade-off appears to be minimal; groups of FL ants with 15% majors more than compensate for their smaller body size because they are estimated to kill as many as 20%–30% more *S. invicta* than groups of *P. morrisi* with a caste and body size composition typical of NC/NY (see Experimental Procedures). It therefore appears that the defensive advantage of hav-

evolution of colony phenotype through constraints that entail functional trade-offs.

Experimental Procedures

Demographic Measures in Field Colonies

Study populations of *Pheidole morrisi* were located in Tallahassee, Florida (30° 45' N, 84° 30' W); Hoffman, North Carolina (35° 03' N, 79° 55' W); and Farmingville, New York (40° 84' N, 73° 04' W). Stefan Cover of the Museum of Comparative Zoology at Harvard University confirmed species identifications; voucher specimens are deposited at the Museum of Comparative Zoology.

Whole colonies were extracted from each site in June and July of 2000. In October of 2000, whole colony extractions were repeated for NC and FL, and mound samples were taken at the NY site, for a total of 15 NY, 29 NC, and 29 FL sampled colonies. The demographic sampling method was modeled after that of Tschinkel (1993) [25]. For each colony, total extracted soil, which included all ants of the colony, was uniformly mixed. Four to six approximately 500 g samples of this mix were taken. With the total mass of the mix known, the number and proportions of the ants in a given soil sample provided an estimate of the total colony size and caste ratio of the colony. The estimates based on the four to six samples were averaged, and the resulting mean value for caste ratio and colony size were considered the measure for that colony. Because only mound samples extending 1–2 ft into the soil were available in NY during the fall of 2000, samples from this site and season were not included for estimates of colony caste ratios from the field. In the few instances when it was practicable to count the absolute number of workers, it was done and considered to be the measure for that colony. Inclusion of such colonies in the statistical analyses of the data set did not significantly affect the results and so were included in the final analysis.

Body masses were obtained from frozen samples collected from colonies ($n = 10$ colonies per site, per season; total $n = 60$). The frozen sample sizes of ants averaged 461 ± 18 SE for minors and 117 ± 8 SE for majors per colony. Samples were sorted by caste and dried in an oven for 48 hr. Dried samples were then serially extracted in hexane so that all fat was removed. The dry, fat-free mass of all the ants in a sample was taken and divided by the total number to give a single mean value for the body mass of the caste in that colony.

Common-Garden Experiments

Colony fragments, including queens, were collected from FL, NC, and NY in spring 2002. These colonies were housed in wax-coated artificial nests made from dental plaster, the glass was covered with red cellophane, and the colonies were kept in Fluon-coated plastic bins of standard size: 38 cm \times 15 cm \times 6 cm. The colonies were kept at 26.5°C and 55% humidity and a 12L:12D photoperiod and were fed a 1:1 honey-water mixture and mealworms ad libitum from the time of collection until the time of measurement.

Immediately after collection, colony size and caste ratio were standardized to one queen with approximately 1100 workers containing 10% majors and a small number of larvae for 12 NY, 12 NC, and 11 FL colonies. Lab colonies were then acclimatized for 4–5 weeks so that the seed larvae were allowed to achieve eclosion and so that all brood from this time onward were laboratory-laid and -reared larvae. All majors were removed at this time, and the colonies were allowed to develop for an additional 2 weeks, at which time all pupae present were removed and the proportions of minor and major pupae produced by the colonies were recorded. The initial removal of majors was necessary for eliminating their inhibitory influence on the production of additional major workers [18, 28]. Thirty-six minor pupae and up to 36 major pupae were randomly chosen from each colony for a total of 426 minors, 297 majors (NY); 393 minors, 326 majors (NC); and 469 minors, 361 majors (FL). Pupal wet masses were measured individually on a Cahn 25 automatic electrobalance. Counting all pupae present in the colonies at this time provided the percent-major value for workers in the colony. Note that, in contrast to the field data, the common-garden data collected indicate pupal production rather than the standing proportions of adult majors. Pupal data with all majors removed from the

nests were taken so that the production could be determined separately from any other regulatory influences, such as major pheromones, that might affect caste ratios.

Caste Proportion Defense Experiments

One hundred *P. morrisi* in the described proportions of minor and major workers were added to nests at least 12–24 hr previous to the addition of *S. invicta* and allowed to settle in the nest. Immediately before addition of *S. invicta*, all food items and foil nest covers were removed. Fifty *S. invicta* of medium size class (mean \pm SD = 0.25 ± 0.07 mg) were then added. Casualties on both sides were recorded at the time intervals shown in Figure 3. Ants with severely restricted motion, a severed abdomen or head, or numerous missing appendages were scored as “dead.”

Ants for these experiments were maintained at 25°C, 50%–70% humidity, and a 16L:8D photoperiod and fed a 1:1 honey-water mixture and mealworms ad libitum. Colonies from the three sites were raised in the laboratory for at least 2 months before the study. Workers from the same three FL, three NC, and four NY *P. morrisi* colonies were used for the 0%, 5%, 15%, and 25% major experimental trials for a total of ten replicates per percent-major trial. *Solenopsis invicta* was collected from three colonies in Durham, North Carolina. For all trials nests were 60 mm petri dishes filled with moistened dental plaster and covered in aluminum foil to block light. These nests were housed in an arena 17.5 \times 12.5 \times 6 cm in size.

Major Body Size Defense Experiments

A single *P. morrisi* major worker was added to a 60 mm diameter petri dish containing five medium-size class *S. invicta* (as defined above). The number of dead *S. invicta* was recorded 15 min after addition to the arena. *S. invicta* were counted as “dead” as described above. Trials in which *P. morrisi* and *S. invicta* did not physically engage during the 15 min period were excluded from the analysis. The lean (fat-free) body size of the 100 individual *P. morrisi* majors used in the trials ($n = 40$ FL, 20 NC, and 40 NY) was also determined as described above.

A regression equation of this data was used with the mean values of major body size from FL and NC/NY for estimating the average size-dependent difference in fighting ability of an individual major from the different populations. This value was multiplied by the average difference in the percentage of majors between FL and NC/NY (5%) to give the net difference in defensive ability. The value range is calculated from the upper and lower 95% confidence interval values for the slope and intercept of the regression equation.

Observations of Worker Caste Behavioral Repertoires

Five FL colonies composed of one queen, 400 workers, and approximately 75 larvae were established in nest and laboratory rearing conditions as described in “Caste Proportion Defense Experiments.” In the first set of trials each colony contained 300 minor workers and 100 major workers (25% major workers). These colonies were kept covered with red cellophane, and over a period of 5 consecutive days 12 observation sessions were conducted on each colony. 60 majors were then removed from the same colonies and replaced with minor workers so that colonies were composed of 10% majors. After one day of acclimatization, another 12 observation sessions were conducted over a 5 day period.

These observations sessions were conducted at 40 \times magnification under a dissecting microscope on a custom mount that allowed the whole internal nest area to be observed by movement of the microscope without disturbing the colony. The entire internal area of the nest was systematically scanned in a predetermined pattern. The behaviors of all minors and majors that could be viewed under the scope were scored with a tally counter. Seven categories of behavior were recognized: (1) rest/being groomed, (2) self-grooming, (3) allo-grooming (the grooming of another individual worker of either caste), (4) tending brood, (5) trophallaxis (regurgitation) to another worker, (6) tending of the queen (including mouth feeding or touching with antennae), and (7) tending the dead or a food item. The twelve scans of the entire colony were conducted at random times during the light cycle of the insectary. These scans were then summed within a colony to give one set of count values for the behaviors in that colony at that caste ratio. These counts were

used for generating likelihood β distributions that estimated the proportion of the total behavioral acts of the colony that were accounted for by that behavioral category. These five resulting β distributions, one for each colony, were then plotted for each behavioral category. A log-likelihood ratio test compared the overall behavioral profiles of workers in the 10% and 25% conditions.

Notes on Statistical Analyses

The distribution of values for the percentage of majors in colonies in both the common-garden and field colonies met normality assumptions, so no transformations were carried out on the data prior to analysis. Individual colonies were considered to be random effects, and all others factors, including colony size, season, site, and caste, were considered to be fixed effects in a linear mixed effects model ANOVA of body sizes in field colonies. In certain size ranges, colony size is known to influence mean worker size [25]. The mean size of colonies from which samples were drawn for the analysis of worker body size did not significantly differ between the three sites ($F_{(2,46)} = 1.716$, $p = 0.191$, one-way ANOVA). Because all sampled colonies had 1500 or more individuals, no correlation was found between colony size and worker caste ratio, as was sometimes found in smaller "incipient" colonies of dimorphic ants [7]; therefore, colony size was not included in subsequent analyses. All statistical analyses were performed in S-Plus 2000.

Supplemental Data

A supplemental figure is available with this article online at <http://www.current-biology.com/cgi/content/full/14/6/514/DC1>.

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