

Caste Determination in a Polymorphic Social Insect: Nutritional, Social, and Genetic Factors

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Social insect colonies can be composed of individuals radically different in form and function. Groups of individuals within a colony that are morphologically or behaviorally distinct are called castes, and colony efficiency and productivity can be increased by division of labor among female castes (Oster and Wilson 1978). A defining feature of eusociality is the development of discrete reproductive (e.g., queen) and nonreproductive (workers) castes, although in many groups the workers can be further subdivided into morphological or behavioral castes (Oster and Wilson 1978).

Whether castes are determined in the egg (maternal or genetic differences) or by the environment of the developing larva has long been a subject of debate. Dietary effects on caste determination in honeybees have been known for more than a century, and many other environmental factors affecting caste determination have been described (Michener 1974; Hölldobler and Wilson 1990). Recently, however, genetic systems of caste determination have been described in a variety of species across separate origins of eusociality (e.g., Kerr 1950; Robinson and Page 1988; Helms Cahan et al. 2002; Hayashi et al. 2007), and maternal effects have been shown in at least two ant species (De Menten et al. 2005; Schwander et al. 2008). Morphological diversity among female castes can be extreme in insect societies. Heritable variation in size has been reported numerous times in ants, among workers in five genera (Fraser et al. 2000; Hughes et al. 2003; Rheindt et al. 2005; Schwander et al. 2005; Jaffe et al. 2007) and between queens and workers in seven genera (Helms Cahan et al. 2002; Helms Cahan and Vinson 2003; Percy et al. 2004; Fournier et al. 2005; Linksvayer 2006; Ohkawara et al. 2006; Hughes and Boomsma 2008), not to mention between queens and workers in honeybees (Tilley and Oldroyd 1997; Moritz et al. 2005). Despite the recent upsurge of research on genetic effects on caste determination, there is still a general lack of understanding of how both genetic and environmental factors simultaneously contribute to

ABSTRACT: We examined how dietary, social, and genetic factors affect individual size and caste in the Florida harvester ant *Pogonomyrmex badius*, which has three discrete female castes. The diet that a larva consumed, as indicated by $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C : N, varied with caste. Both N content and estimated trophic position of dietary input was higher for major than for minor workers and was highest for gynes (reproductive females). The size and resources of a colony affected the size of only minor workers, not that of gynes and major workers. Approximately 19% of patriline showed a bias in which female caste they produced. There were significant genetic effects on female size, and the average sizes of a major worker and a gyne produced by a patriline were correlated, but neither was correlated with minor worker size. Thus, genetic factors influence both caste and size within caste. We conclude that environmental, social, and genetic variation interact to create morphological and physiological variation among females in *P. badius*. However, the relative importance of each type of factor affecting caste determination is caste specific.

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caste determination and how these interactions affect differentiation both between queens and workers and among worker castes. In this study we examine how genetic and environmental factors combine to produce caste and size variation in an ant colony.

Systems where caste is determined exclusively by genetic differences have been considered maladaptive at the colony level because they impose a genetic load (Kerr 1950; Anderson et al. 2006b; Schwander et al. 2006); the ratio of castes is fixed by the genetics of caste determination, and thus, colonies are not able to adjust ratios to environmental demands. On the other hand, alleles that increase the probability of reproduction are advantageous to individuals, albeit at the cost of other alleles within the colony. Thus, genetic effects on queen-worker caste determination are likely to involve trade-offs between colony and individual levels of selection (Oster and Wilson 1978; Bourke and Franks 1995; Keller 1999). The results of this trade-off can be seen in a variety of taxa with documented genetic caste determination and suggest that the genetics of caste determination are a major driver in the evolution of novel modes of social organization (Anderson et al. 2006a; Keller 2007).

The majority of studies that describe a genetic component to caste determination indicate a strong genotype \times environment interaction such that genetic differences affect only the propensity to develop into one caste or another (Chapman et al. 2007; Hughes and Boomsma 2007). Genetic effects on behavioral differences among workers have been well studied in honeybees, and mounting evidence indicates that these effects increase a colony's ability to respond to environmental variation (Oldroyd and Fewell 2007). A recent study on honeybees (Mattila and Seeley 2007) demonstrated that more genetically diverse colonies had increased foraging, nest building, and food storage, which in turn yielded increased reproduction. Furthermore, colonies that have more genetic diversity can have better homeostatic control (Page et al. 1995; Jones et al. 2004, 2007) and higher pathogen resistance (Tarpy 2003; Seeley and Tarpy 2007). The positive effects of increased genetic diversity are, however, not universal, and several studies have failed to detect an effect, either positive or negative (Rosset et al. 2005).

In honeybees, qualitative differences in larval nutrition at critical times during development (e.g., percent royal jelly fed) trigger epigenetic effects (DNA methylation; Kucharski et al. 2008) and endocrine changes in individuals that differentiate the developmental trajectories of queens and workers (Michener 1974; Hartfelder 2000). Curiously, despite a historic knowledge of the importance of royal jelly in queen development in honeybees, the role of qualitative diet differences in caste determination is not well studied or understood in other social insects (but see Gregg

1942; Passera 1974; Hölldobler and Wilson 1990). However, methods such as stable isotope analysis have been successfully applied to discern trophic differences within and among species of ants (Davidson et al. 2003; Tillberg et al. 2007) and may provide a means of discerning cryptic differences in diet among social insect castes.

We investigated environmental and genetic influences on the three female castes of *Pogonomyrmex badius*: gynes (reproductives before mating and dispersal), minor workers, and major workers. This species is the only *Pogonomyrmex* in North America with three female castes (Cole 1968). Our goal in this manuscript is to integrate data on nutritional, social, and genetic factors in caste determination in a social insect with three discrete female castes. Thus, we aim to outline how different proximate factors of caste determination interact to generate diversity in form and function within a social insect colony. To accomplish this aim, we (1) examine qualitative differences in nutrition received by each caste using stable isotope analysis, (2) examine how changes in colony composition affect individual size across all three female castes, and (3) test whether genetic variation exists for both caste and size.

Methods

Species and Site Descriptions

Pogonomyrmex badius occurs across the southeastern United States in areas with sandy soils, a low water table, and relatively high amounts of solar radiation. Foragers collect seeds and insects in nearly equal numbers, though seeds account for ~75% of dry biomass collected (C. R. Smith, unpublished data). Larvae feed directly on food brought into the nest. Colonies can contain up to 10,000 adult workers and always have a single, multiply mated queen (Rheindt et al. 2004). Both worker castes, minor and major, occur in colonies of all sizes at a nearly constant ratio, ~20 : 1 minors to majors (Tschinkel 1998). Only colonies reaching a size of about 700 workers reproduce, and once they reach reproductive size, the total reproductive output is proportionate with colony size (Smith and Tschinkel 2006). Mating typically occurs at the onset of seasonal summer rains, normally in early/mid-June. Sexu- als are produced as a single pulse in spring, followed by continuous worker production until November. Adults of all three female castes co-occur in colonies only after gynes eclose in late May before the onset of nuptial flights. Developing stages (larvae and pupae) of all three female castes co-occur in colonies before gyne eclosion.

All colonies described in this study were collected in the Apalachicola National Forest, approximately 16 km from Tallahassee, Florida (30°21'N, 84°25'W). Collections were

made from two populations separated by several kilometers, clear-cut (CC) and ant heaven (AH). Full site descriptions can be found in an article by Smith and Tschinkel (2005).

Larval Diet Differences between Castes

To quantify differences in diet among larvae of the three female castes, we analyzed $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, %N, %C, and C : N. We hypothesized that both $\delta^{15}\text{N}$ and %N would be greater and C : N lower in larger castes, reflecting feeding from a higher relative trophic position (DeNiro and Epstein 1981; see the appendix in the online edition of the *American Naturalist*). No a priori prediction was made regarding the direction of difference for $\delta^{13}\text{C}$ and %C, but these values may reflect different seed sources in diet (DeNiro and Epstein 1978).

Pupae of all female castes were sampled from two colonies from CC in 2003 and from one colony from CC and one colony from AH in 2007. Pupae were chosen for analyses because they are closed systems and represent the sum of all assimilated material from larval growth, when caste determination occurs. All samples were collected in early June before nuptial flights. Samples were killed by freezing for 6–12 h and then were dried at 60°C for at least 36 h and stored dry. Five pupae of each caste from each colony were weighed whole on a microbalance (UMX2, Mettler-Toledo) to the nearest microgram and pulverized using sterile forceps (excluding the abdomen because of the presence of stored food), and 1,500–2,200 μg was weighed into sterile tin capsules. All analyses were performed at the University of California Davis Stable Isotope Facility using a Europa Hydra 20/20 continuous-flow isotope-ratioing mass spectrometer. Two-way ANOVA with colony and caste as factors was used to analyze differences in $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, %N, %C, and C : N. A priori contrasts were used to evaluate differences between castes if the overall analysis was statistically significant. To control for multiple comparisons across the dependent variables, we adjusted the tablewide α using the standard Bonferroni correction (Sokal and Rohlf 1995).

Colony Size Effects on Caste Size

Seventeen whole colonies were excavated at CC in May/June 2003 following the protocol of Tschinkel (1998). In each female caste, 10–20 mature adult individuals were randomly sampled, and head widths were measured using a stage micrometer (Semprex) accurate to 0.01 mm. The effect of colony size on caste size was examined by regressing the average head width per caste against colony size (the total number of adult workers).

Table 1: Sample size of individuals genotyped in each caste for each colony and mating frequency statistics

Colony	Minor	Major	Gyne	k_{obs}	m_e	m_{ecq}
2	60	71	36	36	7.0	7.3
5	52	52	48	11	6.1	6.3
6	36	32	44	26	6.5	6.8
7	47	36	39	14	6.4	6.8
8	54	38	45	23	10.2	10.9
9	57	47	43	23	11.5	12.4
10	55	54	54	14	5.6	5.7
11	50	32	48	16	8.6	9.2
Average	51.4	45.3	44.6	20.4	7.7	8.2
Total	411	362	357	163

Note: k_{obs} = the observed number of mates, m_e = the effective mate frequency, and m_{ecq} = the effective mate frequency corrected for sampling (Pamilo 1993).

Patriline Effects between and within Castes

Sample Collection, DNA Extraction, and Microsatellite Genotyping. Genotyped workers were from eight of the colonies described by Smith (2007) from the CC site. Whole colonies were collected with queens, thus making it possible to directly determine the matriline of all individuals. Colonies were exclusively monogyne (Tschinkel 1998; Rheindt et al. 2004). Approximately 50 individuals in each caste were collected directly into 95% ethanol (table 1). Minors and majors can be effectively assigned to caste by sight: if the occipital region of the head is concave, they are majors, and if not, they are minors (Smith et al. 2007). This assignment process assigns castes conforming to the definition of castes in *P. badius* used by Tschinkel (1998).

We extracted DNA as follows: (1) the ant was taken out of alcohol, briefly dried, and its gaster and head (for the majority of individuals) were removed; (2) the ant was pulverized with a pestle; (3) the crushed ant was then dissolved in $\sim 100 \mu\text{L}$ 10% Chelex 100 (minors = 75 μL , majors = 100 μL , and gynes = 150 μL) and 1 μL 20 mg/mL proteinase K; (4) the mixture was incubated at 57°C for 1 h and subsequently heated for 5 min at 95°C; and (5) the mixture was centrifuged at 14,000 rpm for 15 min, and the supernatant containing the DNA was extracted. Polymerase chain reactions (PCRs) for each locus followed the protocol described by Gadau et al. (2003) for Pr2 and by Volny and Gordon (2002) for Pb5 and Pb6 using 2 μL of the extracted DNA in solution. Allele length was detected by running denatured PCR product (1 : 1 with formamide/EDTA) on a 6.5% polyacrylamide gel in a LiCor 4300 DNA Analysis System and using SAGA^{GT} software for microsatellite analysis. Additional gels were run to homologize alleles and confirm allele lengths.

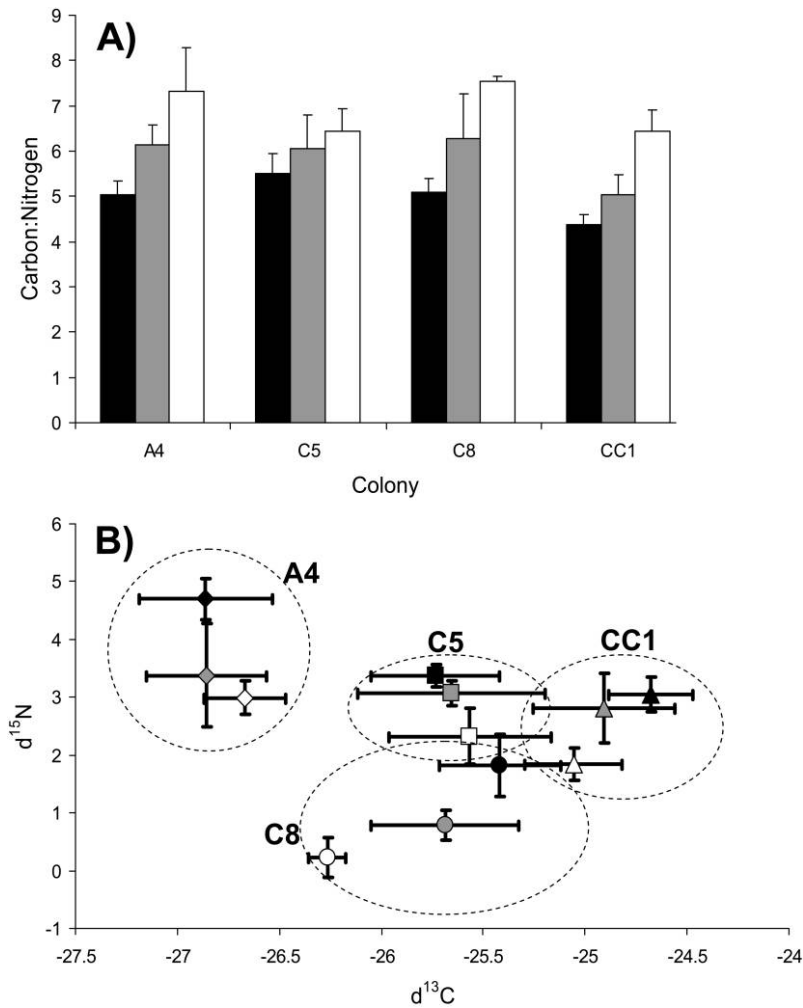


Figure 1: Summary of elemental analyses by caste and colony. In both graphs, the different colors represent different castes (*black* = gynes, *gray* = majors, and *white* = minors). Error bars are 95% confidence intervals. Significant predictors ($P < .01$) of variation in colonies and castes in two-way ANOVAs were C:N, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$. However, values of $\delta^{13}\text{C}$ were not uniform across castes and colonies (significant colony \times caste interaction), though C:N and $\delta^{15}\text{N}$ were consistent. In A, each group of bars represents one colony and each bar the average of at least five individuals. B, Variation in the isotope ratios of N and C by caste and colony. Colonies are represented by different symbols (*diamonds* = A4, *squares* = C5, *circles* = C8, and *triangles* = CC1).

Determining Allele Frequencies and Parentage. Allele frequencies were estimated using the method of Kronauer et al. (2006). First, we ran a patriline assignment analysis in MATESOFT, version 1.0 (Moilanen et al. 2004), using even allele frequencies for all loci. Next, the inferred male genotypes were diploidized and queen genotypes were doubled to obtain unbiased estimates of allele frequencies using FSTAT, version 2.9.3.2 (Goudet 1995). Finally, we entered these allele frequencies into MATESOFT and reran the patriline assignment analysis. All individuals of ambiguous parentage or of a foreign matriline (nine individuals) were discarded from future analyses. After the assignment of individuals to patrilines, we calculated mate

frequency statistics according to Pamilo (1993): the number of observed patrilines (k_{obs}), the effective mate frequency (m_q), and the effective mate frequency corrected for sampling effort (m_{ecq}).

Assessing Patriline Effects on Caste. To ascertain whether patrilines had biased caste ratios, we used a G-test for heterogeneity (Sokal and Rohlf 1995) for each colony separately. Because this test has increased Type I error with low sample sizes, we constricted our analysis to patrilines containing at least 10 individuals. The expected values for each caste were calculated from the number of individuals of each caste that were genotyped for each colony. Many

Table 2: Results of diet analyses

	Colony (df = 3, 48)	Caste (df = 2, 48)	Colony × caste (df = 6, 48)
$\delta^{15}\text{N}$	102.1***	49.9***	1.9
%N	6.6**	41.8***	2.6*
$\delta^{13}\text{C}$	86.8***	8.2**	6.4***
%C	2.5	4.0*	1.9
C : N	7.6**	46.9***	1.6

Note: *F* values from two-way ANOVAs with colony and caste as factors for five measures of differences in larval nutrition (measured as the material assimilated at the pupal stage). Only values with $P < .01$ are considered statistically significant with the Bonferroni adjustment for multiple comparisons.

* $P < .01$.

** $P < .001$.

*** $P < .0001$.

patrilines produced no individuals of a particular caste, and because zeros interfere with the computation of the *G*-test, we added one individual to all castes (tables and figures represent actual numbers before adding the one individual). A patriline could be biased in any of six directions, where each of the three castes could be either overrepresented or underrepresented. We had no explicit hypotheses regarding the direction of caste bias because all of the potential outcomes have been observed previously in other taxa.

If sperm are clumped within the spermatheca, then some patrilines may be overly represented in a caste if caste production is temporal, which is true in *P. badius*, where gynes are all of a single cohort but adult workers comprise many cohorts (Smith and Tschinkel 2006). We examined whether sperm clumping biased our results by comparing the relative numbers of workers (minors and majors) and gynes produced by patrilines using a *t*-test; if sperm is clumped, then gynes are expected to be produced from only a fraction of those patrilines represented among workers.

Genetic Differences among and within Castes. We measured the head width of 842 of the genotyped individuals (230 gynes, 177 majors, and 309 minors) that were definitively assigned to patrilines, on average four or five individuals per patriline per colony, using a stage micrometer (Semprex). Although our study has the potential to measure heritability of head width in each of the castes (sire within dam design as in Bargum et al. 2004; Fjerdingstad 2005; Schwander et al. 2005), we have low within-colony, within-caste, within-patriline representation, which suggests that our estimates are of questionable validity. Moreover, *P. badius* may not meet the assumptions of such an analysis because effective population sizes may be small (Strehl and Gadau 2004) and mating is not likely random (Rheindt et al. 2004). Therefore, we do not explicitly calculate her-

itability but present the means for its calculation through the following analysis. To assess whether there is a genetic effect on size within castes, we used nested ANOVA (colony and patriline nested within colony were both random factors) on each caste separately and then together.

To evaluate whether patrilines affected size similarly across castes, we performed correlations. For these correlations, we used the average size of individuals within a patriline and caste and performed correlations between castes; thus, each point in the correlation represents a single patriline. Because of low within-colony sample size of patrilines, we pooled across colonies; pooling should make this a more conservative analysis because we did not account for between-colony differences. To control for multiple comparisons, we adjusted α using the standard Bonferroni method (Sokal and Rohlf 1995). A significant correlation between castes would indicate that some patrilines commonly produce smaller or larger individuals regardless of which caste their sperm is used to produce.

Results

Larval Diet Differences between Castes

Pupal mass was different between castes (means \pm 95% confidence intervals in mg: minor workers, 2.5 ± 0.2 ; major workers, 8.3 ± 1.6 ; gynes, 11.9 ± 0.9). We found a significant effect of colony for all diet measures ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, %N, %C, and C : N) except %C. All diet measures showed significant differences among castes (table 2; fig. 1); only %N and $\delta^{13}\text{C}$ had significant colony \times caste interactions. Larger castes had greater values for both %N and $\delta^{15}\text{N}$, which suggests that they fed on resources with higher nitrogen content (%N) and from higher relative trophic positions ($\delta^{15}\text{N}$). The colony \times caste interaction in %N was driven by majors; in two colonies they had levels intermediate between minors and gynes, in one colony they were more similar to gynes, and in another they were more similar to minors. In all colonies, majors were still intermediate between minors and gynes, and each caste differed significantly from all others in a priori contrasts. Not all colonies showed consistent patterns of $\delta^{13}\text{C}$, which drove the significant colony \times caste interaction; in two colonies, $\delta^{13}\text{C}$ was higher in larger pupae, while in the other two, the pattern was reversed. The C : N measure showed consistent results across all colonies such that smaller pupae had higher C : N (fig. 1). In general, significant results were driven by differences between minor workers and gynes, and major workers were intermediate.

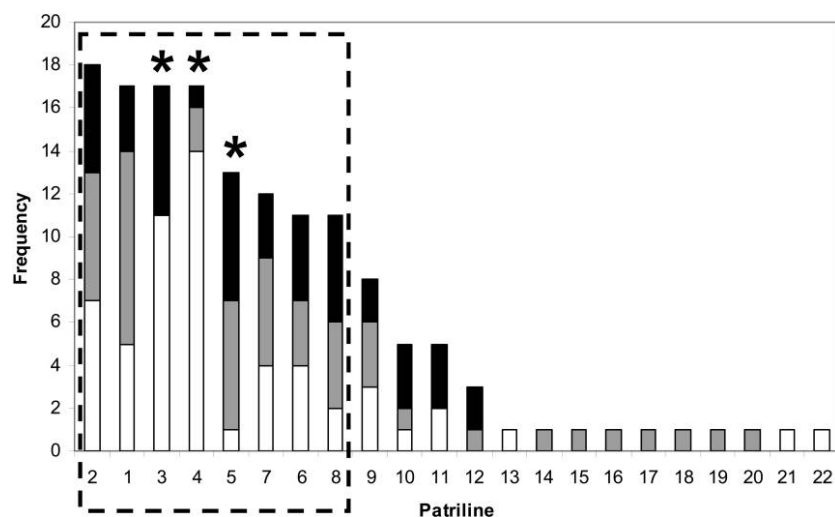


Figure 2: The frequency distribution of females by patriline and caste for colony 9. The numbers on the X-axis represent patriline identities and are ranked by the number of females produced (not by their identity). The colors within the bars represent female castes (*white* = minor, *gray* = major, and *black* = gyne). The dashed line encircles only those patrilines with ≥ 10 females, which were those included in the analysis of patriline bias. An asterisk above a patriline indicates a significant caste bias ($P < .05$ in a G-test for heterogeneity). Although queens mate with many males (22 in this case), the effective mate frequency (m_e ; table 1), which accounts for relative abundance, is much lower (7.7) because of the skew in relative abundance.

Colony Size Effects on Caste Size

Colony size, the population of adult workers, was a significant predictor of minor worker head width ($F = 7.9$, $df = 1, 16$, $P = .01$, $r^2 = 0.33$) but not major ($F = 0.09$, $df = 1, 16$, $P = .76$) or gyne ($F = 0.005$, $df = 1, 14$, $P = .94$) head widths.

Patriline Effects between and within Castes

All loci showed high degrees of polymorphism, with nine, 13, and 14 alleles for each Pr2, Pb5, and Pb6, respectively. Of the individuals assigned to an unambiguous patriline, 1,112 were successfully genotyped for Pr2, 1,113 for Pb5, and 637 for Pb6. In the following analyses, we used 603 individuals genotyped at all three loci, 526 at two loci, and a single individual genotyped at one locus, for a grand total of 1,130 individuals (table 1). We detected a total of 163 patrilines, an average of 20.4 per colony, with a maximum observed mate number of 36 (table 1). The number of individuals per patriline was highly variable and skewed such that a minority of patrilines accounted for the majority of female offspring. On average, each father sired only 5% of the females in the nest. Colony 9 represents most of the variation found in the data for all colonies (fig. 2). Of the 22 unique patrilines in colony 9, only eight produced at least 10 females, and 10 produced no more than one female. Furthermore, the most common patriline in this colony produced only 12% of all females. Across

all colonies, although the observed number of matings was rather high (20), the effective mate frequency is less than half, approximately eight or nine (table 1). The small difference between the raw and the corrected effective mate frequencies indicates that our sampling of patrilines was nearly complete.

We detected no signature of sperm clumping. The relative proportions of workers and gynes produced by a patriline were not different ($t = -0.02$, $n = 48$, $P = .98$).

Of the 167 patrilines, only 48 contained at least 10 females and were used in an analysis of within-patriline caste bias (table 3; fig. A1 in the online edition of the *American Naturalist*). A significant patriline bias was found in 18.8% of patrilines but in only four of the eight colonies tested. Of the nine biased patrilines, the direction of bias was highly variable (fig. 2; appendix), where two produced an excess of minors, one produced an excess of majors, two produced an excess of gynes, two were biased against minor production, two were biased against major production, and none was biased against gyne production. Of the eight colonies, only two showed a significant G statistic at the colony level, which indicates an overall caste \times patriline association. A third colony had a marginally significant P value (.08).

Patriline (within colony) had a significant effect on head size in both minor workers and gynes but not majors (table 4). Colony had a significant effect on size in all castes.

Table 3: Number of patriline that showed a significant bias in the caste ratios produced and total number of patrilines tested per colony

Colony	No. biased	No. tested	Percent biased
2	3	5	40.0
5	0	6	.0
6	2	5	40.0
7	0	5	.0
8	0	7	.0
9	3	8	37.5
10	0	5	.0
11	1	7	14.3
Total	9	48	18.8

Note: Significant bias was determined as significant skew in a heterogeneity G -test.

When all castes were analyzed together, patriline (within colony) still explained a significant, although small (7%), amount of variation in head size. The average size of individual produced by a patriline was significant between the major worker and the gyne castes ($r = 0.30$, $P < .0001$; fig. 3) but not between major and minor workers ($r = 0.04$, $P = .25$) or between gynes and minor workers ($r = 0.01$, $P = .45$).

Discussion

Qualitative and quantitative differences in the nutrition that an individual assimilated during larval growth are strong predictors of caste. We found that minor worker size increased with colony size, but major worker and gyne size did not. This may be because major workers and gynes are at a maximum physiological size (i.e., increased food no longer translates into increased growth). Our results also indicate that maximal size in majors and gynes is influenced by patriline identity, but minor workers vary in accord with available resources. That is, genetic differ-

ences between individuals contribute to variation in the largest of individuals, but, otherwise, differences in the social environment govern size variation; larval nutrition is a candidate factor in governing the differentiation of developmental pathways and explains a large portion of size variation regardless of caste.

Caste Differences in Larval Nutrition

Despite the wealth of literature on honeybees and the causal nature of larval nutrition on the differentiation of the queen and worker developmental pathways, few data exist on differences in larval diet across reproductive and worker castes. The measures %N, $\delta^{15}\text{N}$, and C : N were the best predictors of caste, where the former two increased and the latter decreased with increasing caste size. Increased %N and $\delta^{15}\text{N}$ typically correspond to feeding at a higher trophic level (DeNiro and Epstein 1981; Tillberg et al. 2007; appendix), which in the case of *Pogonomyrmex badius* is likely increased insect consumption relative to seeds. The variation in $\delta^{15}\text{N}$ across all colonies and castes was $>3\%$, which is typically associated with a difference of one trophic level, for example, seeds to insects (DeNiro and Epstein 1981; Tillberg et al. 2006). The %N assimilated within castes across colonies was similar, while the $\delta^{15}\text{N}$ was much more variable. Thus, individuals of colonies that fed at a high trophic level did not likely feed as much to acquire sufficient N. The trophic position of food an individual is fed is not likely causal in caste determination but instead probably reflects the role of N in developing larger size. Increased N may be necessary for reproductive development (Brown 2001; Brent and Traniello 2002), which differs among all castes in *P. badius*; minor workers have five ovarioles, majors 10, and queens 35 (Smith et al. 2007).

Table 4: Breakdown of explained variance in individual size

Effect	Proportion of variance explained			
	Minor ($n = 309$)	Major ($n = 177$)	Gyne ($n = 230$)	Combined ($n = 716$)
Colony	.42*	.11*	.30***	.07**
Patriline within colony	.15***	.05	.30***	.07*
Within patriline	.43	.85	.40	.86

Note: The proportion of variance in size (head width) explained by colony and patriline within colony and level of significance in nested ANOVAs for each caste and then all females together. Significant colony effects indicate significant effects of the environment, differences in patriline within colony are significant genetic effects, and within-patriline effects are unexplained variance. Statistically significant differences between or within colonies (between patrilines) are indicated.

* $P < .05$.

** $P < .001$.

*** $P < .0001$.

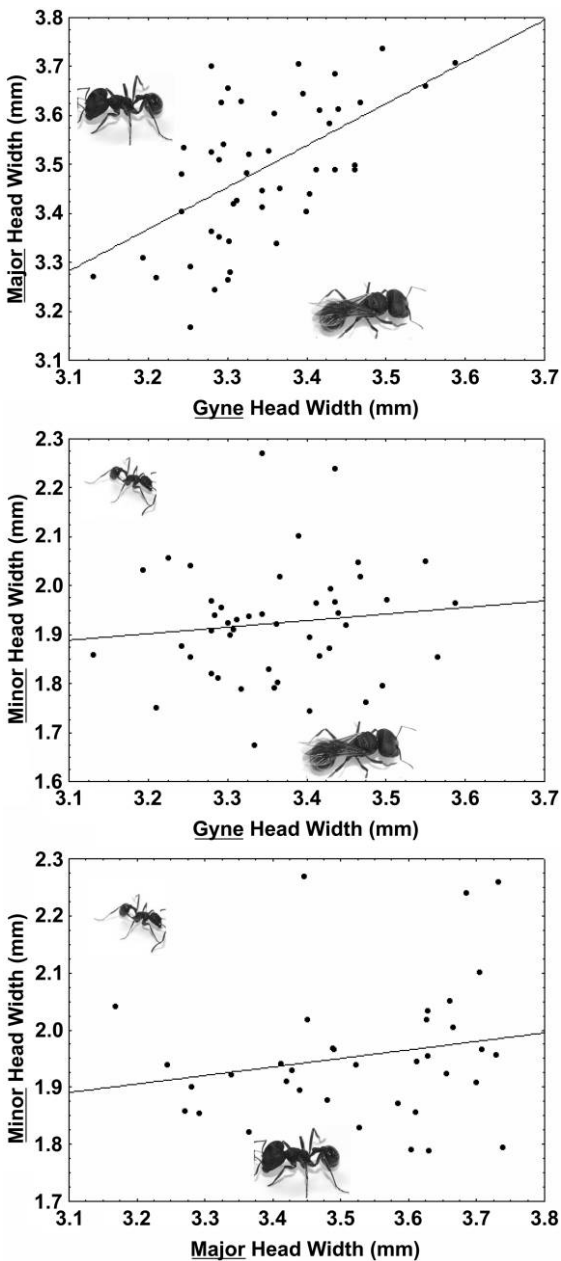


Figure 3: Correlations of the average head width of each caste produced by a patriline. Each point on the graphs represents a patriline. Only the top panel, major versus gyne, is statistically significant ($r = 0.30$, $P < .0001$). Thus, patrilines that produce larger major workers also tend to produce larger gynes, suggesting a genetic component to size within castes but not for all castes (i.e., minors; their size is, however, influenced by colony size). Photographs by Adrian A. Smith.

Colony Size and Caste Size

Variation in worker size is often affected by colony ontogeny in ants (Oster and Wilson 1978; Hölldobler and Wilson 1990). Generally, as colonies grow, they add castes

and increase the average size of individuals in each caste (Oster and Wilson 1978). Variation in worker size may increase division of labor (Wilson 1971, 1984; Oster and Wilson 1978). In *P. badius*, minor workers outnumber major workers by 20 : 1 regardless of colony size (Tschinkel 1999). A constant ratio may be adaptive if the majors perform a specialized task and the demand for this task grows in proportion to colony size (Oster and Wilson 1978; Ferster et al. 2006). Major workers likely use their large muscle-packed heads to mill seeds (Ferster et al. 2006).

Tschinkel (1999) documented larger colonies having disproportionately more energetic resources than smaller colonies of *P. badius*. In this study, we confirmed that minor workers are the only female caste that increases in size with colony size, likely as a response to the increasing nutritional resources of a colony (Smith 2007). By converting their additional resources to make larger minor workers, colonies may increase the range of tasks the workers can manage. Although forager size does not correspond to load size in *P. badius* (Traniello and Beshers 1991), larger minor workers may be more capable seed millers and may be essential for transporting large reproductive brood within the nest.

Genetic Differences among and within Castes

High levels of polyandry have evolved multiple times within and between lineages of eusocial Hymenoptera (Fuchs and Moritz 1999; Villesen et al. 2002; Schlüns et al. 2005; Brady et al. 2006; Holbrook et al. 2007; Kronauer et al. 2007). In *Pogonomyrmex* harvester ants, it appears to have evolved a single time in the transition between the smaller-bodied *Ephebomyrmex* subgenus and *Pogonomyrmex* sensu stricto (Holbrook et al. 2007). As expected, we documented a very high degree of polyandry in *P. badius*, confirming the results of Rheindt et al. (2004). In fact, the average numerical mating frequency of our study, 20, was double that of Rheindt et al.'s (2004), although because of high skew in patriline frequencies among offspring, the effective mate frequencies of the two studies are similar (eight or nine). The observed number of matings was likely higher in our study because of much greater sampling (150 vs. 20 individuals), but the similarity in effective mate frequency suggests that additional sampling primarily adds rare patrilines.

Similar to other studies investigating genetic contributions to caste (e.g., Hughes et al. 2003; Rheindt et al. 2005; Jaffe et al. 2007; Hughes and Boomsma 2008), our study documented a bias in caste production in 19% of patrilines. The direction of caste bias (over- or underproduction of a particular caste) was highly variable, and the majority of patrilines produced all castes (figs. 2, A1).

Thus, we conclude that there is genetic variation in caste determination, though it is unclear how this variation affects development. Patriline (within colony) explained significant variance in both gyne and minor worker size, suggesting a genetic basis for size in both of those castes. Although we did not detect a genetic effect on the size of major workers, the average head sizes of major workers and gynes produced by patrilines are correlated (fig. 3), suggesting a genetic basis for size in both castes. Genetic differences that affect gyne size may correspond to differential fitness among patrilines; gyne size does predict founding success in *Pogonomyrmex occidentalis* (Wiernasz and Cole 2003). However, selection on gyne size may be balanced by selection on worker traits; for example, producing excessively large major workers may be an energetic load on colonies.

Insights on Caste Determination

Major workers likely result from an increase in critical size relative to minor workers, possibly because of a change in juvenile hormone (JH) threshold (Wheeler 1986). The qualitative differences in minor and major worker nutrition suggest a means for altering JH levels, similar to the mode of action of royal jelly in honeybees (Michener 1974); it is now understood that JH levels are connected with nutrition via the insulin signaling pathway (Tu et al. 2005). Why *P. badius* is the only North American *Pogonomyrmex* with significant size variation among workers may be due to changes in diet accompanied by changes in the molecular connection between nutrition and endocrinology. Because there is only one other *Pogonomyrmex* species with worker size variation similar to that in *P. badius* (*Pogonomyrmex coarctatus* in South America; Kusnezov 1951), comparisons between the species are likely to shed light on how morphological variation evolves in social insect societies.

Evidence of genetic caste determination has now been described from at least four different origins of eusociality (stingless bees [Kerr 1950], honeybees [Robinson and Page 1988], ants [Helms Cahan et al. 2002], and termites [Hayashi et al. 2007]). However, studies manipulating caste ratios (Hughes and Boomsma 2007) and colony demography (Chapman et al. 2007) have revealed genotype \times environment interactions, suggesting that despite genetic effects on caste determination, development is still plastic. Although the debate over caste determination has typically been presented as a dichotomy between genetic and environmental factors (Light 1943; Hölldobler and Wilson 1990), evidence across many social insect taxa support that it is a result of nature \times nurture interactions. Here we show that larval nutrition can predict castes but that genetic differences among individuals may either make them

more likely to become a particular caste or affect their final size once caste has been determined. By having genetic specialists among workers, colonies may increase division of labor efficiency and/or be better able to respond to changing environmental conditions (Fjerdingstad and Crozier 2006; Oldroyd and Fewell 2007), but by maintaining environmental mechanisms capable of overriding genetic predispositions, colonies are able to functionally adjust caste ratios.

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