

Loss of Phenotypic Plasticity Generates Genotype-Caste Association in Harvester Ants

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Summary

Caste differentiation and reproductive division of labor are the hallmarks of insect societies [1]. In ants and other social Hymenoptera, development of female larvae into queens or workers generally results from environmentally induced differences in gene expression [2–4]. However, several cases in which certain gene combinations may determine reproductive status have been described in bees [5] and ants [6–9]. We investigated experimentally whether genotype directly influences caste determination in two populations of *Pogonomyrmex* harvester ants in which genotype-caste associations have been observed. Each population contains two genetic lineages [10]. Queens are polyandrous [11, 12] and mate with males of both lineages [6, 7], but in mature colonies, over 95% of daughter queens have a pure-lineage genome, whereas all workers are of F1 interlineage ancestry [6–8]. We found that this pattern is maintained throughout the colony life cycle, even when only a single caste is being produced. Through controlled crosses, we demonstrate that pure-lineage eggs fail to develop into workers even when interlineage brood are not present. Thus, environmental caste determination in these individuals appears to have been lost in favor of a hard-wired genetic mechanism. Our results reveal that genetic control of reproductive fate can persist without loss of the eusocial caste structure.

Results and Discussion

Strong correlations between genomic composition and reproductive role have been well documented in reproductively mature colonies of two *Pogonomyrmex* populations: Hidalgo, which contains the H1 and H2 lineages, and Junction, containing the J1 and J2 lineages [10].

However, nothing is known about the factors producing such correlations because developmental flexibility of the two genotypes under different conditions has not been investigated experimentally. To address this, we first assessed whether genotype-caste associations are a characteristic feature of colonies throughout the life cycle. We genotyped the first workers produced by newly mated queens, who are limited to raising workers because their fat and muscle reserves used to nourish the brood cannot provide enough resources for reproductive offspring [13, 14]. Queens were collected in copula along with a male mate, although they had probably previously mated with one or more additional males at the time of collection. Both same-lineage and interlineage matings were collected from the mating swarms (H swarm: 47 same-lineage pairs, 30 interlineage pairs; J swarm: 44 same-lineage pairs, 44 interlineage pairs). We found that the extent of bias toward interlineage workers in founding colonies was as extreme as that in adult colonies (Figure 1). Like adult colonies [6–8, 10], most mother queens possessed a pure-lineage genotype, but virtually all worker offspring were of interlineage ancestry. This was true even for queens whose last mate was of the same lineage, for whom appropriate sperm were clearly available for pure-lineage progeny, yet none were observed as adult workers (H lineages: n = 9 workers; J lineages: n = 29 workers).

These results suggest that the lack of pure-lineage workers in adult colonies is not merely a result of preferential shunting of such progeny into queen developmental slots when available [7] but is instead a more rigid mechanism limiting the developmental pathway of each genotypic class of offspring. Indeed, in both populations, queens collected with a same-lineage male showed a significant reduction in initial productivity, consistent with the hypothesis that their pure-lineage offspring were incapable of developing as workers (Figure 2).

Pure-Lineage Brood Intrinsically Fail to Develop into Workers

Although these data suggest that genotype directly determines caste fate, several extrinsic mechanisms could also be responsible for producing such patterns. First, queens could utilize sperm selectively by inseminating eggs with alternate-lineage sperm when producing workers and same-lineage sperm when queens are produced [15]. Second, queens (and adult workers in older colonies) could discriminate between interlineage and pure-lineage brood by selectively raising interlineage females as workers [8]. Third, the association could result from competition between female larvae, with interlineage females having a higher competitive ability when resources are low and thus being more likely to develop into workers than pure-lineage brood [6]. All of these three mechanisms are nongenetic, in the sense that individuals of both genotypes retain the intrinsic capability to develop into either caste.

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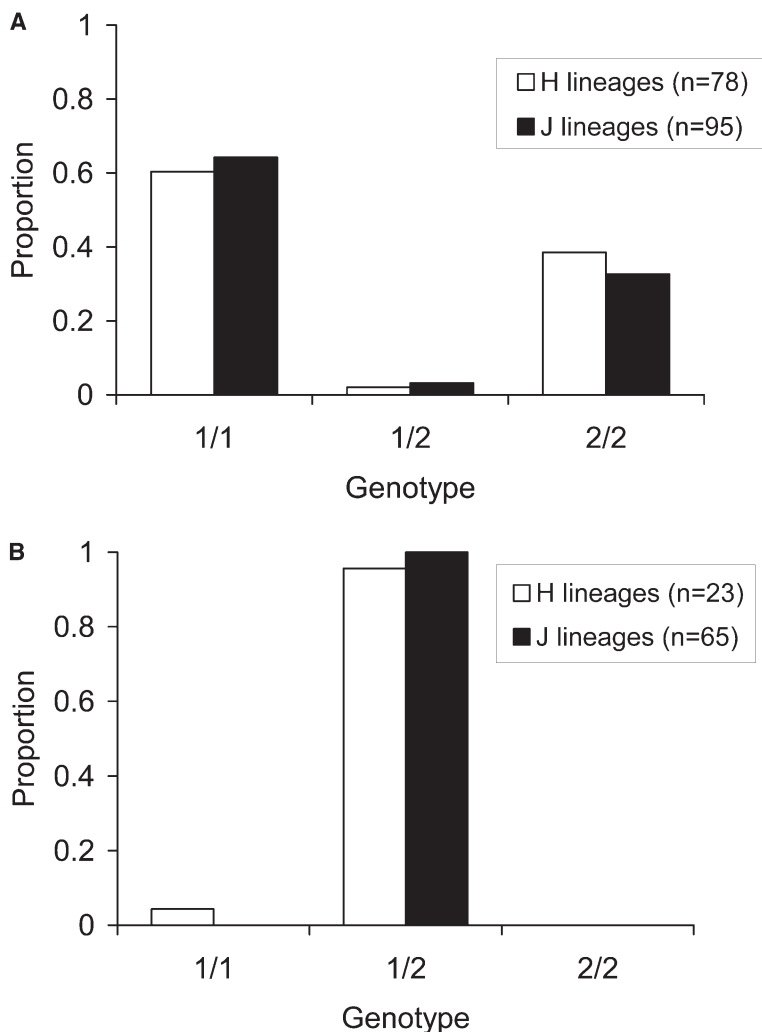


Figure 1. Genotypes of (A) Queens Collected from Natural Mating Swarms and (B) Their First Adult Worker Offspring

No brood were sampled from queens that failed to raise an adult worker in 6 weeks. The single worker successfully produced by an interlineage queen (J1/J2) was presumed to be of backcross ancestry and is not included here.

We tested these hypotheses experimentally by crossing virgin pure-lineage queens from the Hidalgo population with a single male of the same or alternate lineage to compare the worker developmental potential of interlineage and pure-lineage offspring. By mating queens singly, we removed the opportunity for selective sperm usage, preferential treatment, or larval competition. Thus, failure of pure-lineage offspring to develop into workers under these conditions is only predicted if caste were influenced directly by genotype.

Whether a queen was mated with a male of the same or of the alternate lineage strongly influenced the developmental fate of her offspring. The large majority (87%) of interlineage crosses resulted in adult workers, whereas only 17% of same-lineage crosses successfully did so (G-test, $G_1 = 24.24$, $p < 0.00001$) (Figure 3). This strong difference cannot be explained by a difference in egg laying between cross types. H1 queens mated to a same-lineage male had slightly lower initial egg production, but all queens had produced large numbers of eggs by week 3 (Figure 4A). The difference between interlineage and same-lineage crosses stemmed from a drastic difference in the subsequent developmental fate of these eggs (Figures 4B–4D). Whereas interlineage

eggs began to hatch into larvae after week 2, most H1/H1 (queen lineage/male lineage) eggs failed to hatch (Figure 4B). In fact, in 23 of the 37 (62%) H1/H1 colonies, none of the eggs laid hatched successfully throughout the experiment, and of the 14 H1/H1 colonies that did produce larvae, seven failed to produce pupae, resulting in only 19% of H1/H1 colonies producing any workers (Figure 3). Even in these colonies, productivity was significantly reduced, indicating that same-lineage offspring face difficulties in developing as workers even when the queen is willing to rear such offspring to adulthood. Successful H1/H1 crosses produced significantly fewer larvae ($F_{1,74} = 27.47$, $p < 0.0001$), pupae ($F_{1,31} = 14.59$, $p < 0.001$), and workers ($F_{1,19} = 9.68$, $p = 0.006$) than interlineage crosses, and the first adult worker from successful H1/H1 colonies eclosed significantly later ($\chi^2_1 = 9.2$, $p = 0.002$).

Same-lineage crosses between H2 queens and H2 males were also associated with developmental breakdown of the brood, but at a slightly later stage. All five H2/H2 queens produced high numbers of eggs and larvae (Figures 4A and 4B), but none of these larvae successfully developed into pupae (Figure 4C). As a result, none of the H2/H2 colonies produced any adult workers.

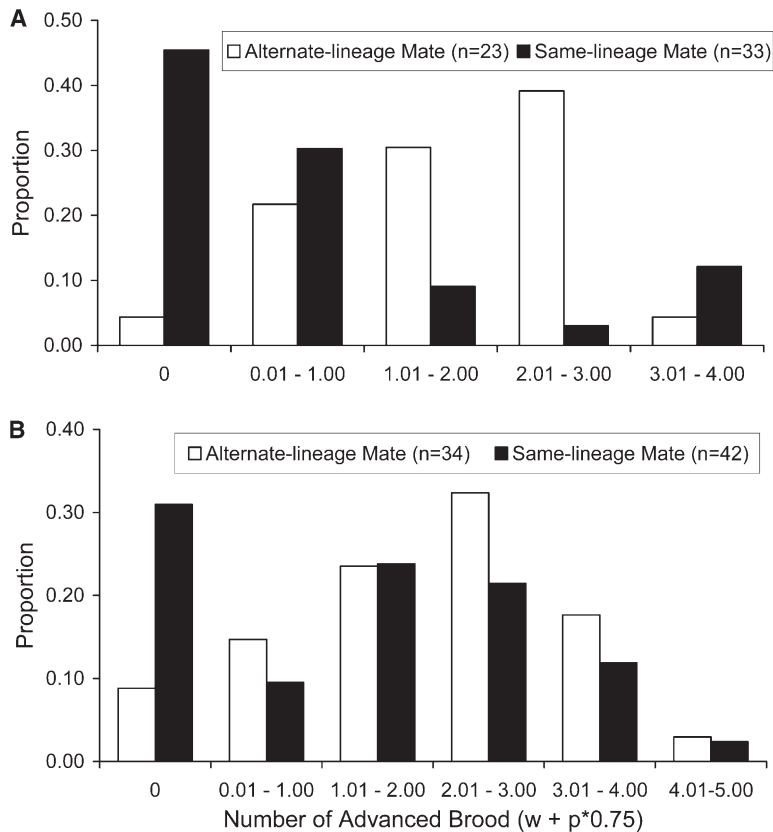


Figure 2. Productivity Distributions of Queens from Natural Mating Swarms and Whose Last Male Mate Was of the Alternate Lineage (White Bars) or the Same Lineage (Black Bars) All queens were maintained at 30°C for 6 weeks, when all advanced brood (pupae and workers) were counted for each queen. Pupae were included in the brood count to increase the number of scorable offspring but were counted at three-quarters the value of an adult worker to represent the potential for mortality before adulthood as well as their lesser value to initial colony function. (A) H1/H2 population. Distributions of brood production differed significantly between queens whose last mate was an alternate-lineage male and those whose last mate was a same-lineage male ($G_4 = 33.02$, $p < 0.0001$); queens with same-lineage mates were more likely to fail to produce advanced brood and less likely to produce 2–3 workers. (B) J1/J2 population. Again, distributions of brood production differed significantly between queens whose last mate was an alternate-lineage male and those whose last mate was a same-lineage male ($G_5 = 24.74$, $p < 0.0005$); queens with same-lineage mates were more likely to fail to produce advanced brood and less likely to have broods with high numbers of workers.

Overall, pure-lineage progeny had an extremely low probability of successful development. Given that each queen laid approximately 60 initial eggs, only 0.3% of all pure-lineage eggs developed to adulthood.

These results suggest that phenotypic plasticity in pure-lineage offspring is substantially reduced, at least during the colony founding stage. The hypothesis that queens differentially used sperm on the basis of the caste being produced cannot account for our finding because queens laid large numbers of eggs regardless

of the identity of the male with which they had mated. Similarly, it is unlikely that pure-lineage offspring were killed because the most probable agents of such behavior, adult workers, were not present during their development. It is possible that the queens themselves kill all the brood in pure-lineage clutches, but given that queens tend brood only during colony founding, when the negative consequences of removing viable brood would be the most extreme, it seems highly unlikely that such behavior should evolve in queens. Moreover, comparison of offspring numbers over time reveals that same-lineage offspring were being retained in the nest in an arrested developmental state rather than being removed by the queen. Pure-lineage colonies contained higher numbers of brood than other colony types at the specific stage at which development terminated—the egg stage for H1/H1 colonies (Figure 4A) and the larval stage for H2/H2 colonies (Figure 4B). Finally, competitive displacement of pure-lineage brood could not have occurred because each colony contained only a single offspring type. It is possible that in natural colonies, competitive displacement does prevent the small proportion of pure-lineage brood that could develop from doing so, but this is only likely to explain a small proportion of the observed genotype-caste association in the field.

As colonies mature, the quantity of resources available per offspring increases, leading to a gradual increase in worker size and, ultimately, to the production of sexuals [16]. Because only the first cohort of workers was examined here, it is not known whether worker

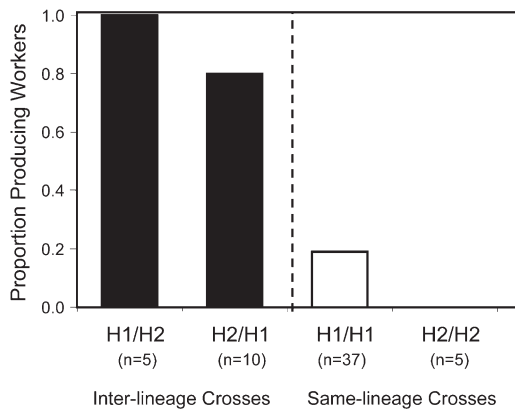


Figure 3. Proportion of Founding Queens of Each Mating Type Successfully Raising Offspring after 8 Weeks Mating types are designated by the lineage of the queen and then male (i.e., H1/H2 = H1 queen mated to an H2 male).

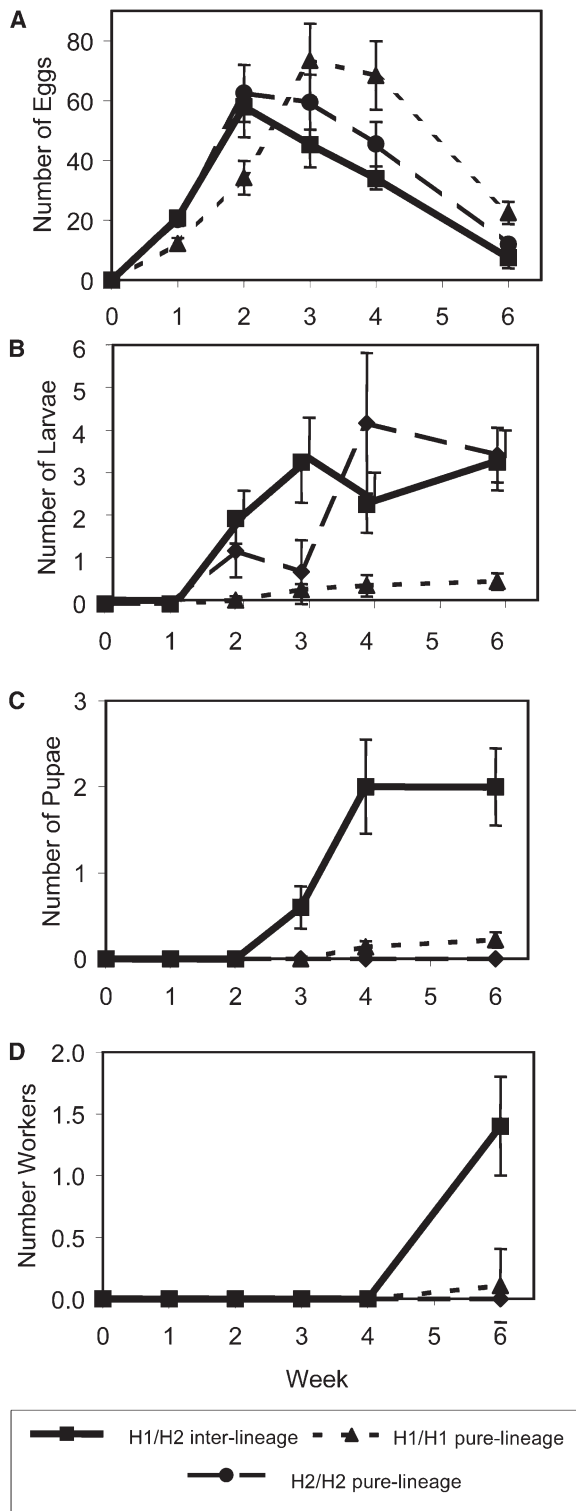


Figure 4. Brood Production (\pm the Standard Error) of H1 and H2 Queens Mated to a Single Male over the First 6 Weeks of Colony Founding

Only mating pairs from 2001 were surveyed weekly (H1/H1, $n = 35$ colonies; H1/H2, $n = 5$ colonies; H2/H2, $n = 4$ colonies). No H2/H1 crosses were obtained in 2001; thus, this treatment is not included in the figures.

(A) Number of eggs. Repeated-measures ANOVA revealed a significant mating type \times week interaction ($F_{2,42} = 3.98$, $p = 0.02$).

development would also be curtailed for pure-lineage brood under less austere resource conditions. Nevertheless, field collections suggest that such loss of worker development is typical for pure-lineage offspring at all colony stages; in total, we have genotyped over 800 workers from site H and 200 workers from other sites in the region but not a single pure-lineage worker has been discovered (S.H.C. and T.S., unpublished data). One surprising aspect of our results is that H1/H1 brood terminated in the egg stage, before the usual nutritional cues about which caste is being produced would be available. It is possible that variation in nutritional or hormonal levels in the egg, as is seen in some species with very early environmental caste determination, influences individual probability of development [17]. It is worth noting that such early termination would tend to minimize the fitness costs of a genetic mechanism because little would be invested into genetically inappropriate offspring when colonies are not raising a particular caste.

Both pure-lineage and interlineage offspring show a strong caste bias in field colonies, but it is not yet clear whether genetic predetermination also regulates the development of interlineage females. H1/H2 and J1/J2 interlineage winged queens are found at low levels ($\sim 7\%$) in the field (Figure 1; [10]), suggesting that such offspring are more totipotent than pure-lineage brood, but the relative influences of intrinsic and extrinsic factors in their development cannot yet be assessed.

Genetic Caste Determination Selects for Lineage Coexistence during Colony Foundation

The finding that pure-lineage reproductive caste is genetically determined has important consequences for the dynamics of interbreeding between cooccurring lineages. Unlike extrinsic mechanisms, which are facultative in the sense that each genotype could assume both reproductive roles if the other genotype were not present, nearly complete loss of worker potential in pure-lineage brood makes both lineages wholly reliant on interbreeding for the worker caste, without which queens cannot survive to produce reproductive offspring (Figure 3). Thus, although interbreeding is needed to produce genotype-caste associations regardless of mechanism, interbreeding is an obligate condition only when independent worker production potential is lost, either spontaneously via negative epistatic interactions between

(B) Number of larvae. Mating type had a significant main effect on larval production ($F_{2,42} = 33.78$, $p < 0.001$). In post-hoc pairwise comparisons, H1/H1 differed significantly from both H1/H2 (Tukey's test, $p < 0.001$) and H2/H2 ($p < 0.001$) colonies. Hybrid and H2/H2 colonies did not differ.

(C) Number of pupae. Mating type had a significant main effect on pupal production ($F_{2,42} = 49.59$, $p < 0.001$). H1/H2 colonies produced significantly more pupae than H1/H1 colonies ($p < 0.001$) and H2/H2 colonies ($p < 0.001$). H1/H1 and H2/H2 colonies did not differ.
 (D) Number of workers. Mating type had a significant main effect on worker production ($F_{2,42} = 24.34$, $p < 0.0001$). H1/H2 colonies produced significantly more workers than either H1/H1 colonies ($p < 0.001$) or H2/H2 colonies ($p < 0.001$). H1/H1 and H2/H2 colonies did not differ.

Table 1. Proportion of Naturally Mated Queens Successfully Producing a First Worker Cohort as a Function of the Relative Frequency of that Lineage in the Mating Swarm

| Lineage | Year | N | Relative Frequency in Swarm | Predicted Successful | Observed Successful |
|---------|------|-----|-----------------------------|----------------------|---------------------|
| H1 | 2004 | 367 | 0.53 | 0.83 | 0.75 |
| H2 | 2004 | 326 | 0.47 | 0.85 | 0.78 |
| J2 | 2001 | 74 | 0.54 | 0.83 | 0.83 |
| J1 | 2001 | 66 | 0.46 | 0.85 | 0.88 |
| J2 | 2003 | 105 | 0.68 | 0.75 | 0.91 |
| J1 | 2003 | 46 | 0.32 | 0.87 | 0.97 |
| H1 | 2004 | 47 | 0.69 | 0.74 | 0.70 |
| H2 | 2004 | 14 | 0.31 | 0.87 | 0.93 |
| H2 | 2003 | 116 | 0.77 | 0.66 | 0.94 |
| H1 | 2003 | 25 | 0.23 | 0.87 | 1.00 |
| J2 | 2004 | 142 | 0.91 | 0.41 | 0.58 |
| J1 | 2004 | 14 | 0.09 | 0.87 | 0.86 |
| H1 | 2001 | 87 | 0.94 | 0.34 | 0.49 |
| H2 | 2001 | 3 | 0.06 | 0.87 | 1.00 |

Foundress queens that had dealated were collected from the soil surface within 200 m of a mating swarm 1–2 hr after peak activity. Horizontal rules separate data from different mating swarms. Two separate mating swarms were sampled for the H lineages in 2004. N indicates the number of queens sampled. Predicted foundress success was calculated as $y = 0.87(1 - 0.83p_y^{4.71})$, where y is the proportion of foundresses predicted to produce workers, p_y is the relative frequency of the lineage, 0.87 is the proportion of queens expected to succeed in the absence of sperm limitation (from Figure 3), 0.83 is the proportion of same-lineage mated queens failing because of sperm limitation (from Figure 3), and 4.71 is the effective mating frequency [12].

genomes [10] or because of direct selection on offspring caste fate [6, 7].

This interdependence, in turn, may be a critical factor in the evolutionary stability of two-lineage populations. Worker production is the most important determinant of queen survival and reproductive success [18] because workers are responsible for all nonreproductive tasks, including raising the reproductive brood. If pure-lineage eggs are laid but cannot become workers, a significant proportion of eggs laid during periods of worker production would be effectively inviable, reducing the efficiency of worker production and potentially leading to colony failure caused by lack of a sufficient workforce. Importantly, the magnitude of this egg inviability load is dependent on the relative frequency of each lineage in the population. Queens whose sperm stores are biased toward their own lineage should produce a higher proportion of eggs that fail to develop and should therefore incur higher fitness costs. Assuming that queens do not mate assortatively [6, 7], the proportion of same-lineage sperm should increase with the relative frequency of a queen's lineage in the population. This would select against the more common lineage, promoting lineage coexistence via negative frequency-dependent selection.

To test this prediction, we collected mated queens leaving natural mating swarms that varied in the relative proportions of the two interbreeding lineages. For all seven swarms sampled, queens of the more common lineage were significantly less likely to succeed in producing workers than queens of the rarer lineage, regardless of which lineage was the more frequent (Sign test, $p < 0.02$; Table 1). When each lineage was treated as an independent data point, the proportion of queens failing to produce workers was significantly correlated with the relative frequency of the lineage (linear regres-

sion on arcsin-transformed data, $F_{1,12} = 15.60$, $p = 0.002$), with the most extreme case (94% of the mating swarm) showing over 50% failure rate of founding queens. Thus, loss of the capacity for independent worker production not only produces genotype-caste associations within colonies but also may play an important role in maintaining such associations at the population level.

Conclusions

This study suggests that the alternate genomic composition of queens and workers is primarily caused by a hardwired genetic system of caste determination, with pure-lineage individuals having almost completely lost the ability to develop into workers. The transition from an environmental to a genetic system of caste determination has functionally linked two independent genomes via the hybrid worker caste such that they can only persist as a two-lineage system [19]. This interdependence, in turn, produces negative frequency-dependent selection on queens during colony founding, which may play an important role in the stability of the system. These findings demonstrate that the mechanisms underlying caste development can have unexpectedly complex and important higher-level effects in eusocial organisms. In the same way that relatively simple individual behavioral rules can produce complex colony level behaviors [20], the loss of phenotypic plasticity at the individual level here generates a complex system of interdependence at higher levels of biological organization.

Experimental Procedures

Mating Crosses

For creation of known crosses, virgin queens and males from H1 and H2 colonies at site Hidalgo [6] were either excavated from nests

in the field (1997; $n = 10$ nests) and briefly chilled to immobility at -20°C to release mating behavior or trapped in conical wire mesh traps as they emerged for the mating flight (2001 and 2002; $n = 30$ nests each year). Reproductives were separated by sex to avoid inbreeding and released into one of four $0.75\text{ m} \times 0.75\text{ m} \times 2\text{ m}$ wire mesh mating cages containing one of the four possible combinations of queens and males. Queens generally resist copulations and mate only when engaged by multiple males simultaneously [11], resulting in relatively low sample sizes for some crosses despite repeated efforts. Cages were observed continually while the ants were active (mid-morning and late afternoon), and all copulating pairs (1997: 12 pairs; 2001: 50 pairs; 2002: 3 pairs) were removed. Queens were housed in glass tubes with water-soaked cotton at one end at 30°C . Brood was censused weekly for 6 weeks (except week 5), and worker production was monitored for an additional 2 weeks. After 8 weeks, a subset of queens that had not produced advanced offspring (larvae, pupae, or workers) were dissected to confirm insemination. All queens and advanced brood were genotyped at an enzyme (PGI; [6]) or microsatellite locus (*Pr1*; [12]) diagnostic for the two lineages to confirm ancestry.

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