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GENETIC CASTE DETERMINATION IN HARVESTER ANTS: POSSIBLE ORIGIN AND MAINTENANCE BY CYTO-NUCLEAR EPISTASIS

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Abstract. While reproductive caste in eusocial insects is usually determined by environmental factors, in some populations of the harvester ants, *Pogonomyrmex barbatus* and *P. rugosus*, caste has been shown to have a strong genetic component. This system of genetic caste determination (GCD) is characterized by between-caste nuclear variation and high levels of mitochondrial haplotype variation between alternative maternal lineages. Two previous genetic models, involving a single nuclear caste-determining locus or interactions between two nuclear loci, respectively, have been proposed to explain the GCD system. We propose a new model based on interactions between nuclear and mitochondrial genes that can better explain the co-maintenance of distinct nuclear and mitochondrial lineages. In our model, females with coevolved cyto-nuclear gene complexes, derived from intra-lineage mating, develop into gynes, while females with disrupted cyto-nuclear complexes, derived from inter-lineage mating, develop into workers. Both haplodiploidy and inbreeding facilitate the buildup of such coevolved cyto-nuclear complexes within lineages. In addition, the opportunity for both intra-lineage and inter-lineage mating in polyandrous populations facilitates the accumulation of gyne-biasing genes. This model may also help to explain the evolution of workerless social parasites. We discuss similarities of GCD and cytoplasmic male sterility in plants and how worker production of males would affect the stability of GCD. Finally, we propose experiments and observations that might help resolve the origin and maintenance of this unusual system of caste determination.

Key words: cyto-nuclear coevolution; genetic caste determination; hybrid breakdown; inbreeding; social parasitism.

Introduction

In most social Hymenoptera, diploid female offspring can become either sterile workers or reproductive queens (gynes; Brian 1956), depending upon the social environment, whereas males are haploid and develop from unfertilized eggs. Recent research has shown that some populations of the harvester ant species, *Pogonomyrmex barbatus* and *P. rugosus*, are exceptions to this pattern. Molecular data indicate that female caste is associated with level of zygosity at some nuclear markers, with reproductive gynes being homozygous and workers being heterozygous, suggesting that female caste within colonies is genetically determined (Volny and Gordon 2002, Helms Cahan et al. 2002, Julian et al. 2002, Helms Cahan and Keller 2003, Parker 2004, Anderson et al. 2006). In order for a homozygous founding queen to produce a successful colony with both heterozygous workers and homozygous gynes, she must mate multiply, with males of her own genotype and with males of an alternate genotype. Polyandry is common in this genus, with some species having an average of more than six patrilines per colony (Parker and Rissing 2002, Wiernasz et al. 2004).

Some mitochondrial sequence data suggest that hybridization between *P. barbatus* and *P. rugosus* may have given rise to the genetic caste determination (GCD) system (Julian et al. 2002, Helms Cahan and Keller 2003), while other data suggest that GCD arose within *P. barbatus* and subsequently introgressed into *P. rugosus* (Anderson et al. 2006). Still other data suggest that these two species are part of a larger multispecies complex, with "insufficient time for speciation to be complete" (Cole 1968, Parker and Rissing 2002). A genetic model involving incompatibilities between nuclear genes manifest with hybridization has been proposed to describe the underlying cause of the GCD system (Helms Cahan and Keller 2003). We suggest an alternative model based upon interactions between cytoplasmic and nuclear genes that might better account for some aspects of the genetic data.

We first review the genetic and geographic data gathered for this system (Volny and Gordon 2002, Julian et al. 2002, Helms Cahan and Keller 2003, Anderson et al. 2006). We review two previous genetic models for GCD (Volny and Gordon 2002, Helms Cahan and Keller 2003), then we present a model of

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cyto-nuclear epistasis that could explain the GCD system. We discuss the evolutionary origin of the GCD system and factors that could affect the maintenance of the system. Lastly, we propose experimental studies that could shed additional light on the origin and maintenance of this interesting system.

The Genetic and Geographic Data

Within colonies with GCD, workers are associated with heterozygosity at one or more nuclear molecular markers and gynes are associated with homozygosity (Volny and Gordon 2002, Julian et al. 2002, Helms Cahan and Keller 2003, Parker 2004, Anderson et al. 2006). This suggests that there are two nuclear lineages within colonies, with hybrids (either hybrids between species, or hybrids between lineages within a species) developing into workers but with same lineage forms developing into gynes. Each colony appears to consist of a single mitochondrial lineage and is derived from a single queen.

Some genetic data are consistent with GCD being associated with between-species hybridization, either directly as a result of an ancient hybridization event (Helms Cahan and Keller 2003), of ongoing hybridization, or some sort of introgression (Anderson et al. 2006). Evidence for hybridization includes high levels of within-population diversity of mitochondrial haplotypes, with some haplotypes sharing sequence similarity with haplotypes from each of the putative parental species, *P. rugosus* and *P. barbatus*. However, some populations have colonies with the GCD phenotype (i.e., a homozygous queen and gynes but heterozygous workers) but mitochondrial haplotypes from only one of the parental species, *P. barbatus*. In these cases, there is no evidence from mitochondrial data for hybridization (Anderson et al. 2006). These data suggest that GCD arose within *P. barbatus* and secondarily manifests itself in some hybrid zones of regional sympatry with *P. rugosus*. That is, there is evidence that GCD occurs in the absence of hybridization between *P. barbatus* and *P. rugosus*, and may predate hybridization instead of resulting from it (Anderson et al. 2006). Hybridization appears to be common throughout the range of the *P. barbatus* complex (Parker and Rissing 2002), making it difficult to definitively resolve the importance of hybridization in the origin of GCD.

All scenarios, whether or not they involve between-species hybridization, require that populations retain dual matrilineal nuclear lineages. Because both nuclear lineages are required within a population to produce workers and gynes within colonies, frequency-dependent selection at the colony level could favor the rarer lineage at each generation (Parker 2004). In addition, the genetic data suggest that two mitochondrial lineages are maintained in populations with GCD. In fact, mitochondrial haplotype appears to be a better predictor of GCD at the lineage level than is genotype at nuclear markers (Anderson et al. 2006). As a result of maternal inheritance of mitochondria, frequency-dependent selection results in the maintenance of dual mitochondrial lineages, depending on the degree of mixing of nuclear lineages across mitochondrial lineages.

Next, we review previous genetic models for GCD, and then suggest a new one based on a simple model of cyto-nuclear interaction. The genetic data that these models must explain are (1) the association of gynes with homozygosity and workers with heterozygosity at some molecular markers (i.e., the diagnostic GCD phenotype); (2) the origin of the GCD phenotype; (3) the maintenance of the dual nuclear lineages; and (4) the maintenance of dual mitochondrial lineages and the accumulation of diversity between mitochondrial haplotypes.

Genetic Models for GCD

Two previous genetic models have been proposed to explain GCD. The first simply suggests that caste is determined by a single genetic factor, the "caste-determining locus," such that individuals homozygous at the caste-determining locus develop into gynes, and individuals heterozygous at this locus develop into workers (Volny and Gordon 2002). Nuclear genetic markers used to diagnose caste in *Pogonomyrmex* populations with GCD are presumed to be in linkage disequilibrium with this caste determining locus (Volny and Gordon 2002, Anderson et al. 2006). This model explains the association of caste and nuclear heterozygosity, and it explains the maintenance, if not the origin, of two distinct nuclear lineages. However, if any heterozygous individuals develop into gynes (i.e., if caste is not determined solely by genotype), the mixing of nuclear and mitochondrial lineages can occur, so that the two mitochondrial lineages are not likely to remain distinct and associated with the nuclear heterozygosity. Over time, recombination between the marker and the caste determining locus is expected to diminish the association between the nuclear genes as well as that between the marker and the two mitochondrial lineages.

Helms Cahan and Keller (2003) suggested a more complex model, explaining both the origin and maintenance of GCD. It is based on the idea that GCD is caused by incompatibilities between two interacting nuclear loci brought together by interspecific hybridization (cf. Dobzhansky 1937, Muller 1942). In this model (see Fig. 1), two species hybridize to produce an F1 generation, the F1 generation produces an F2, and the F2 produces an F3. Two of the several genotypic lineages that characterize the F3 then stabilize and persist as the hybrid lineages responsible for GCD. The two stable hybrid lineages are fixed for alleles from each of the parental species, one and two, at two different loci (i.e., one lineage has queens with genotype $A_1A_1B_2B_2$ and the other lineage has queens with genotype $A_2A_2B_1B_1$, see Fig. 1). Under this model, both loci must have alleles derived from the same species to enable

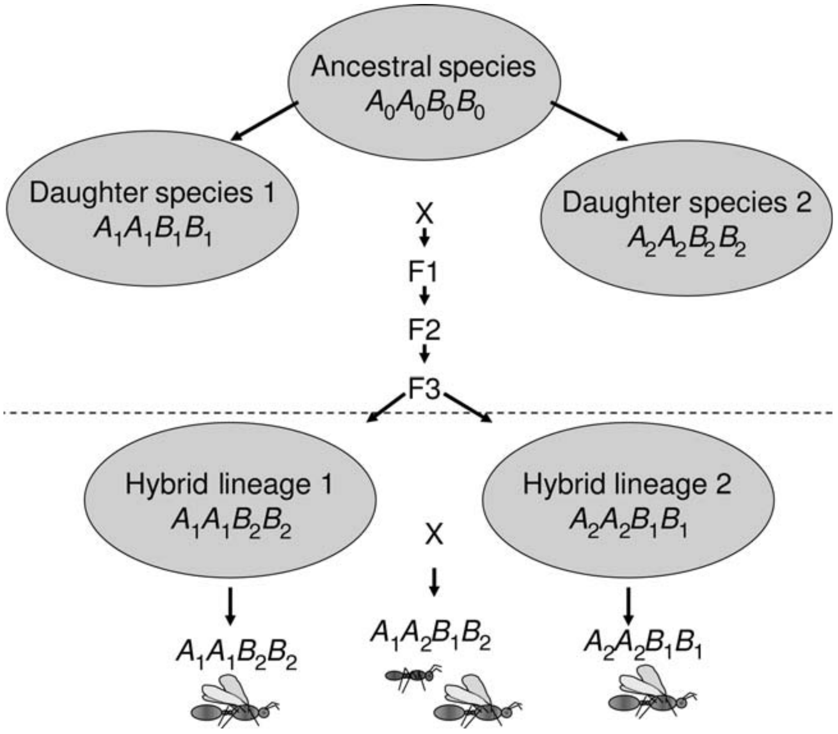

FIG. 1. Diagrammatic representation of the two-locus epistatic nuclear model of genetic caste determination (Helms Cahan and Keller 2003). The ancestral species is fixed for allele A_0 at the nuclear locus *A*, and for allele B_0 at the nuclear locus *B*. Daughter species 1 and 2 are derived from the ancestor by fixation of alternate alleles at the *A* and *B* loci. Crosses between species 1 and 2 create an F1 generation, crosses between F1 individuals create an F2, and crosses between F2 individuals create an F3. Two of the possible F3 genotypes then stabilize as hybrid lineages 1 and 2, each fixed for alleles derived from a different species at the loci *A* and *B*. Under this model, female offspring must possess alleles at both loci that are derived from the same species (i.e., A_1-B_1 or A_2-B_2) to develop into workers, while female offspring with alleles at each locus from a different species can only develop as gynes. Colonies from lineages below the horizontal dotted line express GCD, while those above the dotted line express environmental caste determination. Female offspring derived from matings between males and females from one of the stable hybrid lineages only develop into gynes, as indicated by the winged gyne pictures. Female offspring derived from matings between males and females from different lineages can develop into gynes or workers, as indicated by the pictures of winged gynes and workers, although these offspring usually develop into workers.

worker development (Helms Cahan and Keller 2003). Female offspring derived from matings between males and gynes from the same lineage (i.e., $A_2A_2B_1B_1$ or $A_1A_1B_2B_2$) have both alleles at each locus from a different parent species, and are restricted to gyne development. Female offspring derived from matings between males and gynes from different lineages are heterozygous for parental alleles at both loci (i.e., $A_1A_2B_1B_2$). These individuals have one allele from each parent species at both loci, so that worker development is possible. Under this model, these double heterozygote females are bi-potential (as is necessary for the F1s), but almost always develop into workers because double homozygote females only develop into gynes and monopolize colony resources, or otherwise prevent most heterozygotes from developing into gynes (Helms Cahan and Keller 2003).

This two-locus nuclear model provides an explanation for the origin of GCD. It also explains the association of nuclear heterozygosity with the worker phenotype, to the degree that double heterozygotes (e.g., $A_1A_2B_1B_2$) do not develop into gynes. However, double heterozygotes must be capable of developing into gynes in this model because they have the same genotype as all the F1, half of the F2, and one quarter of the F3 hybrid females, which precede or coincide with the stable F3 hybrid lineages. Whenever a heterozygote develops into a gyne and successfully initiates a colony, mixing between nuclear and mitochondrial lineages can occur, so that the two mitochondrial lineages will not remain distinct and in linkage disequilibrium with the GCD loci, making them poorer rather than better predictors of GCD.

Thus, neither the one-locus nor the two-locus nuclear models of GCD account for the maintenance and accumulation of diversity of the mitochondrial haplotypes. Below, we suggest an alternative simpler genetic model based on the interactions between nuclear and mitochondrial genes. Because cyto-nuclear interactions are directly involved in caste determination under this model, alternative cyto-nuclear combinations are actively maintained by selection. As a consequence, the dual nuclear and mitochondrial lineages remain associated and accumulate variation.

Interactions between nuclear and cytoplasmic genes

The interaction of nuclear and mitochondrial genomes is essential for eukaryotic cellular function. For example, in animals, mitochondrial function requires the coordinated expression of hundreds of nuclear-encoded genes and 37 mitochondrial genes (Sackton et al. 2003, Rand et al. 2004). Crosses between species or lines often result in decreased mitochondrial function, demonstrating the breakup of coadapted cyto-nuclear complexes (Sackton et al. 2003). In the eusocial Hymenoptera, there is evidence that cyto-nuclear interactions directly influence caste determination. In honeybees, queen-destined larvae have a higher respiratory rate than worker-destined larvae, and have increased expression of both nuclear and mitochondrial genes encoding protein components of cyto-nuclear complexes, possibly enabling the higher respiratory rates (Eder et al. 1983, Corona et al. 1999, Evans and Wheeler 2000). Given this biological background, a straightforward scenario for GCD involves females with coadapted cyto-nuclear complexes developing into gynes and those with disrupted cyto-nuclear complexes developing into workers. Within a colony with a single common mitochondrial type, this kind of cyto-nuclear epistasis would appear as nuclear GCD.

Cyto-nuclear gene interactions cause cytoplasmic male sterility (CMS) in many plant species, and result in a gender polymorphism, called gynodioecy, in which some cyto-nuclear gene combinations develop into hermaphrodites and others develop into females (Stadler and Delph 2002, Jacobs and Wade 2003). Since females produce more seed than the hermaphrodites, they enjoy an ovular fitness advantage that can be balanced by pollen limitation or other factors. Similarly, *Pogonomyrmex* colonies with GCD may enjoy a fitness advantage through the production of more gynes, relative to colonies with environmental caste determination, but this advantage can be balanced if GCD colonies do not produce enough workers. A genetic feature of plant systems with gynodioecy is a high level of heterogeneity of both cytoplasmic and nuclear genes (cf. review in Jacobs and Wade 2003, Stadler and Delph 2002) similar to that reported for *Pogonomyrmex*. In addition, the CMS system involves marginal frequency-dependent selection similar to that hypothesized for the *Pogonomyrmex* GCD system.

Next, we discuss in detail possible scenarios for the evolutionary origin of GCD, and parallels to cytoplasmic male sterility (CMS) in plants. Finally, we discuss factors, including worker production of males, which might affect the stability of systems with GCD.

Cyto-nuclear coevolution and GCD

For either inter-lineage or interspecific hybrids, a simple cyto-nuclear model can produce partial inviability or sterility of the reciprocal F1 genotypes, $A_1A_2c_1$ and $A_1A_2c_2$, under the hypothesis that the interactions, A_2c_1 and A_1c_2, between nuclear and mitochondrial alleles are unbalanced and deleterious (Fig. 2). Within colonies, this will appear as nuclear genetic caste determination with homozygous gynes and heterozygous workers, because, on the uniform cytoplasmic background of a single queen, genotype at the nuclear locus determines whether an offspring becomes a worker ($A_1A_2c_1$ or $A_1A_2c_2$) or has the capacity to develop into a gyne ($A_1A_1c_1$ or $A_2A_2c_2$). This fulfills model requirements 1 and 2 listed in *The genetic and geographic data*. Because interactions between nuclear and mitochondrial genotypes determine caste in this model, mitochondrial and nuclear lineages are co-maintained, also fulfilling requirements 3 and 4.

If the two F1 genotypes are fully sterile, producing no male or gyne offspring, and if gynes must mate with males of both types to produce viable colonies, then the system can be stable. If F1 genotypes rarely develop into gynes, as essential to the model of Helms Cahan and Keller (2003), their offspring have either coevolved parental cyto-nuclear combinations ($A_1A_1c_1$ and $A_2A_2c_2$) and can develop into workers or gynes but may be biased towards gyne development, the F1 cyto-nuclear combinations ($A_1A_2c_1$ and $A_1A_2c_2$) and are biased to develop as workers, or completely mismatched cyto-nuclear combinations ($A_1A_1c_2$ and $A_2A_2c_1$) and are sterile or inviable. Thus, under this model, no mixing of mitochondrial lineages can occur and they remain associated with the nuclear alleles with which they coevolved.

How would such a cyto-nuclear system originate? Both haplodiploidy and within-colony mating facilitate the coevolution of cyto-nuclear gene combinations. Haplodiploidy facilitates the evolution of cyto-nuclear combinations that are beneficial for females because two out of every three copies of nuclear genes are in females (i.e., females are diploid and males are haploid) and only females pass on mitochondrial genes (Rand et al. 2001, Wade 2001). Nuclear-mitochondrial interactions are involved in hybrid breakdown of females and males in the haplodiploid parasitoid *Nasonia*, suggesting the importance of cyto-nuclear interactions in speciation in haplodiploids (Breeuwer and Werren 1995). Within-colony mating to any degree facilitates the evolution of cyto-nuclear combinations because it guarantees that both the mitochondrial genotype and nuclear genotype are inherited together from parents to offspring (Wade and Goodnight 2006). With outcrossing, nuclear alleles are randomly distributed across mitochondrial haplotypes, reducing the heritability of nuclear-cytoplasmic combinations and favoring nuclear alleles that are "good mixers," functional on almost any mitochondrial background. Selection among maternal lineages practicing within-colony mating would favor mitochondrial and nuclear genotypes that interact to produce the favored proportion of gynes and workers. Intermediate

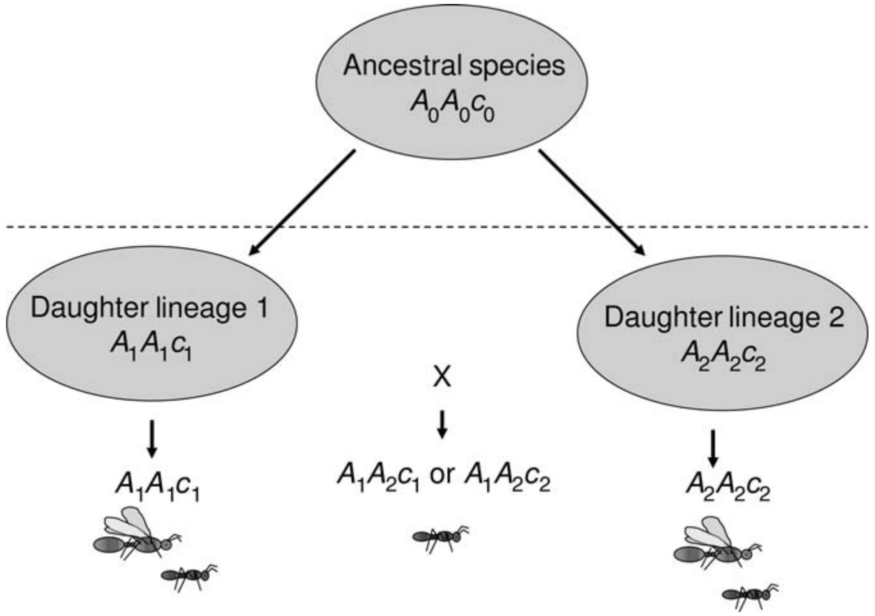

Fig. 2. Diagrammatic representation of the cyto-nuclear epistatic model of genetic caste determination. The ancestral species is fixed for allele A_0 at the nuclear locus A, and for allele c_0 at the mitochondrial locus c. Daughter lineage 1 is derived from the ancestor by fixation of the allelic combination, A_1c_1, which is introduced by mutation into the population and whose subsequent fixation by selection and random genetic drift is facilitated by inbreeding. Similarly, daughter lineage 2 is derived by fixation of the allelic combination A_2c_2. Colonies from lineages below the horizontal dotted line express GCD, while those above the dotted line express environmental caste determination. Females derived from inter-lineage crosses have disrupted gene combinations (A_1-c_2 or A_2-c_1) and develop into workers. Females derived from matings between males and females from the same lineage possess coevolved cyto-nuclear gene combinations (A_1-c_1 or A_2-c_2) and can develop into gynes or workers but may have gyne-biased development.

levels of within-colony mating are known to occur in *P. occidentalis* (Cole and Wiernasz 1997), and high levels of within-colony mating occur in the workerless inquilines, *P. colei* (Johnson et al. 1996) and *P. anergismus*, both sister taxa arising from *P. barbatus* (Parker and Rissing 2002), suggesting the potential for within-colony mating to have played a role in the evolution of GCD in *Pogonomyrmex* lineages.

We propose that a combination of within-colony (or within-lineage) mating and subsequent outcrossing between colonies or lineages could lead to colonies expressing GCD. Outcrossing tends to break up coadapted cyto-nuclear gene combinations, leading to female offspring that develop as workers in our model. In contrast, female offspring resulting from within-colony (or within-lineage) matings have coadapted cyto-nuclear gene combinations and can develop into gynes or workers, but may be biased towards gyne development. When queens mate multiply, and both within- and between-colony (or lineage) mating occurs, both workers and gynes can be produced by a single queen. If very few colonies in a region produce reproductives in a given year, then the probability that a polyandrous queen mates with males from her own colony is high despite the absence of within-colony mating sensu strictu.

The evolution of workerless social parasites from their host species by sympatric speciation is also thought to be facilitated by within-colony mating (Buschinger 1986, 1990). Lineages with GCD could be considered a type of workerless social parasite, with the "host" being the alternate lineage or species, because workers are provided when the queen outcrosses to the host lineage or species (Julian et al. 2002, Umphrey 2006). Indeed, GCD in *P. barbatus* may be a preadaptation that has facilitated the evolution of the two species of workerless inquilines from this lineage (Parker and Rissing 2002).

The disruption of coevolved cyto-nuclear complexes by out-crossing or hybridization could manifest itself as a nuclear genetic system of caste determination in much the same way that disruption of coevolved cyto-nuclear complexes manifests itself as nuclear determination of gender in CMS systems (Jacobs and Wade 2003). In the CMS system, combinations of dominant nuclear restorers and CMS cytotypes determine hermaphrodites, while homozygous recessives and CMS cytotypes become females (Bailey and McCauley 2005). Different populations appear to have different combinations of CMS cytotypes and nuclear restorers (Bailey and McCauley 2005) and crosses between distant populations produce more daughters. Notably, mitochondrial lineages for CMS species exhibit high levels of haplotype diversity, owing to the accumulation of neutral mutational variations within haplotypes and sequence divergence between haplotypes over time (e.g., Stadler and Delph 2002).

Similar to systems with CMS, nuclear alleles causing gyne-biased development, through either enhanced or diminished sensitivity to social or environmental cues, could be segregating in parental populations. If expressed during larval development, these alleles would be favored by within-colony selection as "selfish" caste-biasing alleles, getting disproportionately into gynes rather than sterile workers. They might be disfavored by among-colony selection, however because the production of too few workers could lead to colony failure (Bourke and Ratnieks 1999). Among-colony selection for normal, nongenetic caste determination could lead to the spread of mitochondrial modifier alleles that removed the effect of the gyne-biasing alleles and restored normal caste determination. Parental populations would then have segregating nuclear gyne-biasing alleles as well as restorer mitochondrial alleles. Genetic influences on caste determination may be discovered to be less rare than previously assumed. Alleles causing a slight bias towards gyne development may be able to sweep to fixation, while alleles causing a stronger bias may most commonly be held at low frequency by among-colony selection favoring environmental caste determination.

However, polyandry and the potential for mating both within and between colonies or lineages (as is found in the *Pogonomyrmex* GCD populations) may reduce the constraint on the spread of gyne-biasing alleles by weakening opposing among-colony selection. Between-lineage mating provides workers, so that offspring derived from within-lineage mating may have gyne-biased development without incurring fitness penalties at the colony-level due to a lack of workers (Julian et al. 2002, Umphrey 2006). Thus, polyandry and the coexistence of multiple lineages in mating swarms may facilitate the initial accumulation of alleles which strongly bias caste. The stronger the genetic influence on caste in these lineages, the more they would become codependent on each other for the production of both gynes and workers. Some lineages could effectively break this cycle if they had completely gyne-biased development with only intra-lineage mating. Gynes from these workerless parasite lineages would then be dependent on other, host lineages to provide colonies with a workforce. The evolution of GCD may thus be influenced by complex patterns of selection within colonies, selection among colonies, and selection among lineages.

Comparison of models for GCD

All three genetic models described here explain the association of heterozygosity with workers and homozygosity with gynes, but differ in their accounts of its origin and maintenance, and in accounting for the observed high levels of mitochondrial haplotype diversity. Only the cyto-nuclear model explains all four features of the GCD system.

Both the one- and two-locus nuclear models allow mixing of mitochondrial and nuclear lineages, depending on the frequency with which heterozygotes (double heterozygotes in the two locus model) develop into gynes. In contrast, in the cyto-nuclear model, even if heterozygotes rarely develop into gynes, their only offspring that have the potential to develop into gynes are those with coevolved cyto-nuclear parental genotypes, preventing mixing of nuclear and mitochondrial lineages. Note that recombination in these offspring of rare heterozygote gynes can contribute to low levels of gene flow at loci unlinked to the nuclear locus involved in cyto-nuclear interactions. The genetic data suggest that distinct mitochondrial lineages with GCD have been maintained for long periods of time, with low levels of gene flow at unlinked nuclear loci (Anderson et al. 2006). These data are most consistent with our cyto-nuclear model.

STABILITY OF SYSTEMS WITH GCD

With the one- and two-locus nuclear models for GCD, queens must mate with males from both their own lineage and the alternate lineage in order to produce both workers and gynes. The cyto-nuclear model is more permissive of only intra-lineage mating, although as already explained, intra-lineage mating may be associated with strong gyne-biased development such that coevolved cyto-nuclear combinations develop into gynes (Fig. 2). In these cases, for the GCD system to persist indefinitely, males from each of the two lineages must be produced each year in nearly equal frequencies. By analogy with Fisher's arguments for sex ratio in diploid organisms (Fisher 1930), selection is expected to lead to a 1:1 ratio of investment in the two male lineages across the population (Seger and Stubblefield 2002). This implies that frequency-dependent selection maintains nearly equal frequencies of the two homozygous nuclear maternal lineages.

There is considerable opportunity for random genetic drift to lead to the loss of one lineage in the *Pogonomyrmex* populations with GCD. In a given year, the total number of reproductives emerging from the colonies in a local region is small. More than one-third of all colonies, independent of age, produce no reproductives at all and approximately one-third of all reproductives are produced by only 3% of the colonies. Colony reproductive rates are also correlated across years, so that "a few, older colonies dominate the pool of reproductives year after year" (Wagner and Gordon 1999:175). On average, a reproducing colony produces only eight gynes and 19 males per year (Wagner and Gordon 1999). Colony failure rates at founding are very high (~99%) in these species (Gordon and Kulig 1996, Gordon and Wagner 1997, Johnson 1998). The among-colony variation in reproductive rate, the correlation in reproductive rate from year to year, and the high failure rate of new colonies offer a significant opportunity for random genetic drift to affect allele frequencies without strong balancing selection. The apparent long-term maintenance of GCD (Anderson et al. 2006) is puzzling.

One factor that may strongly affect the maintenance of GCD systems is the production of haploid males by workers. Such worker reproduction occurs commonly in ants, especially following queen loss (Bourke 1988), although no evidence for worker reproduction has been found in the *Pogonomyrmex* populations with GCD. Worker reproduction facilitates the maintenance of both nuclear lineages. If workers are produced by inter-lineage mating, and all males are derived from workers, then males of each nuclear lineage are produced in equal frequencies each year regardless of the lineage frequencies in the population. In contrast, the frequency of each mitochondrial lineage each year reflects the relative success of the two kinds of queens in producing gynes, not males, because mitochondria are passed on only through females. Worker production of males can accelerate the loss of mitochondrial lineages by drift when males of both lineages are produced in equal frequencies, so that all gynes that mate multiply can produce both workers and gynes, regardless of the lineage frequency of gynes. Thus, worker reproduction facilitates the maintenance of both nuclear lineages but it exacerbates the loss by drift of one mitochondrial lineage. However, if males must also have cyto-nuclear matches to be viable or fertile (Breeuwer and Werren 1995), then worker production of males would be effectively the same as gyne production of males because only those worker-derived males that had coevolved cyto-nuclear combinations would contribute to the next generation. More generally, worker reproduction is a factor likely to have a strong impact on the stability of other possible systems of GCD.

Future Work

Although breeding ants is not always feasible, crosses between geographically distant *Pogonomyrmex* populations or species could be used to produce synthetic hybrids, as well as backcrosses to parent populations, to investigate the genetic basis of GCD. Although the observed patterns of coinheritance of alternative nuclear and cytoplasmic alleles (Anderson et al. 2006) are consistent with a model of cyto-nuclear epistasis, empirical proof would be furnished by the artificial creation of nuclear-cytoplasmic types not observed to date in natural populations. We would expect that, if the GCD system observed in *P. barbatus* depends upon cyto-nuclear interactions, then inter-population crosses within this species would reveal the local fixation and coevolution of nuclear and cytoplasmic alleles. Similarly, in *P. rugosus*, crosses between genetically distant populations might reveal a predisposition toward a GCD system. More generally, crosses in other ant populations may reveal more widespread genetic influences on caste.

If trait combinations from both parent species are important for exploiting certain ecological habitats, then the rearing of colonies of interspecific hybrids and the parent species across a range of experimentally controlled environments or field transplants could reveal conditions under which the hybrids have higher fitness (Julian and Helms Cahan 2006). If segregating backcrosses or F2s can be made, then the selective pressures acting on composite traits and on the associated microsatellite markers can also be assessed (Rieseberg et al. 2003) to elucidate the selective forces at work and their phenotypic targets. In addition, such work might make it possible to document the variations in fertility and viability associated with different genotypes that are postulated in the Helms Cahan and Keller (2003) model and in our model.

Although the evidence supporting the claim of hybrid origin for GCD is weak, given the findings of Anderson et al. (2006), it appears that neither *P. barbatus* nor *P. rugosus* is monophyletic (Cole 1968, Parker and Rissing 2002). Within this complex, distinct morphotypes can be observed, suggesting the possibility of local ecotypes. More data are needed to evaluate the selective forces acting locally and regionally on this complex. This will require ecological studies as well as laboratory studies using controlled crosses, to monitor the fitness of particular genotypes.

The single-locus nuclear model and the cyto-nuclear model suggest that the developmental fate of a female larva is strongly influenced by its genotype. These genetic effects may influence caste by affecting the physiological response of larvae to the nutritional environment or the solicitation behavior of larvae. For example, gyne-biasing alleles could be associated with a lower threshold for gyne development and increased begging behavior. Besides these larval traits, reproductive caste is also likely influenced by traits expressed by the colony queen, workers, and larval nestmates. For example, genes expressed in workers may affect nutritional provisioning to brood which in turn affects caste development. Thus, both direct effect genes expressed in developing larvae, and indirect effect genes expressed in nestmates are likely to influence caste determination (Linksvayer and Wade 2005). In the two-locus nuclear model, females derived from intra-lineage mating are restricted to gyne development whereas females derived from inter-lineage mating are bipotential, but almost always develop into workers because of the presence of intra-lineage gyne-destined larvae (Helms Cahan and Keller 2003). If resource limitation causes most between-lineage larvae to develop into workers (e.g., if intra-lineage larvae monopolize colony resources), then resource supplementation of field colonies, or providing laboratory colonies with unlimited resources, should diminish the association between genotype and caste. Further study of the importance of the social environment on GCD, for example with cross-fostering, would be very informative (see Linksvayer and Wade 2005).

It may be possible to gain insight into the mechanisms underlying genetic caste determination by studying the size distributions of workers and gynes from mature colonies. One simple approach would be to compare the size distribution of workers and gynes in populations exhibiting GCD and populations exhibiting environmental caste determination. Under environmental caste determination, females (workers plus gynes) often show a bimodal size distribution, with the workers in the lower peak and gynes in the higher peak. In colonies with GCD, both small (i.e., worker-destined) and large (i.e., gyne-destined) larvae may be produced regardless of genotype, but the genotype of the female may "short-circuit" the final developmental steps of caste determination. Under this scenario, populations with GCD would have workers and gynes both with a bimodal size distribution. That is, some individuals genetically constrained to be workers would be similar to gynes in the nutrition they receive and thus their adult size, while other individuals genetically constrained to be workers would be smaller because they received the nutrition normally given to worker-destined larvae. In contrast, in parental populations with environmental caste determination, gynes and workers would each have a unimodal size distribution. Alternatively, if genes involved in caste determination are widespread in their effects, e.g., if such genes influence the amount of nutrition provisioned by workers, then populations with genetic caste determination would display the same unimodal size distributions for gynes and workers as parental populations with environmental caste determination.

Long-term studies are needed that evaluate lineage frequencies, and their effect on the success of newly mated queens in founding colonies. In one population of *P. barbatus* with GCD, it appears that the sex ratio is approximately 1:1, and there is no evidence that certain colonies tend to produce only males or females (Gordon and Wagner 1997, Wagner and Gordon 1999) as is common in many other ant species (Boomsma and Nachman 2002). There are few data yet on lineage ratios in any of the *Pogonomyrmex* populations with GCD. Preliminary data (D. M. Gordon, *unpublished data*) on the same population of *P barbatus* indicates that the lineage ratio of existing colonies is approximately 1:1. Further study of lineage ratios over time will help to elucidate factors involved in the maintenance of GCD in these populations.

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