Ant Genetics: Reproductive Physiology, Worker Morphology, and Behavior

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Abstract

Many exciting studies have begun to elucidate the genetics of the morphological and physiological diversity of ants, but as yet few studies have investigated the genetics of ant behavior directly. Ant genomes are marked by extreme rates of gene turnover, especially in gene families related to olfactory communication, such as the synthesis of cuticular hydrocarbons and the perception of environmental semiochemicals. Transcriptomic and epigenetic differences are apparent between reproductive and sterile females, males and females, and workers that differ in body size. Quantitative genetic approaches suggest heritability of task performance, and population genetic studies indicate a genetic association with reproductive status in some species. Gene expression is associated with behavior including foraging, response to queens attempting to join a colony, circadian patterns of task performance, and age-related changes of task. Ant behavioral genetics needs further investigation of the feedback between individual-level physiological changes and socially mediated responses to environmental conditions.

Contents

INTRODUCTION

Social insect colonies provide compelling examples of collective behavior without central control. The study of collective behavior in ants seeks to explain how interactions among individuals allow colonies to regulate their behavior in response to changing conditions. Recent methodological advances have opened many new opportunities for studying the genetics of ant behavior. All known species of ants are eusocial, consisting of one or more reproductive females and a large number of sterile, female workers. Many recent studies have investigated the genetics of reproductive physiology and of morphological diversity in ants. Here we review the literature on ant genetics and suggest that despite many recent exciting results, few studies have investigated the genetics of ant behavior directly.

The review is divided into sections according to the phenotypic traits considered. We first consider studies that are not concerned directly with behavior: comparing families of hymenopterans, species of ants, males and females, molecular differences associated with female reproductive status, and workers that differ in body size. In the final section, we discuss genetic studies that investigate individual behavioral traits directly, including task performance and circadian activity patterns.

Within each section, we discuss a variety of methods. These include quantitative genetic approaches that examine heritability due to genetic, epigenetic, or environmental sources (Visscher et al. 2008; reviewed in Linksvayer 2015); phylogenetic analyses of DNA sequence homology (reviewed in Libbrecht et al. 2013); transcriptomic studies that measure patterns of cellular RNA abundance, corresponding to gene expression (e.g., Linksvayer et al. 2012); epigenetic studies of the regulatory mechanisms underlying activation and repression of specific regions of chromatin (reviewed in Yan et al. 2014, Glastad et al. 2015); and finally, physiological studies of traits such as enzyme activity (Noble et al. 2014).

Heritability: Resemblance between parent and offspring with regard to some phenotypic trait. Heritability can include nongenetic mechanisms of intergenerational transmission

COMPARISONS AMONG SPECIES

The genomic analysis of the evolution of colonial living and eusociality in social insects is a rapidly growing area of research (reviewed in Toth & Robinson 2007, Johnson & Linksvayer 2010, Linksvayer et al. 2012, Rittschof & Robinson 2014, Rehan & Toth 2015). Most eusocial insect species are within the order Hymenoptera, a large group with two suborders: Symphyta (sawflies) and Apocrita (ants, bees, and stinging wasps). Genomic studies have sampled broadly across the order, allowing for phylogenetic comparisons among taxa (e.g., Gadau et al. 2012, Johnson et al. 2013, Libbrecht et al. 2013, Nygaard & Wurm 2015). The genomes of eusocial hymenopteran species are marked by exceptionally high rates of gene turnover (Simola et al. 2013a, Rappoport & Linial 2015). Ant workers are either permanently or temporarily sterile, depending on the species. The last common ancestor of all ants was clearly a queen-like reproductive, in the same way that the last common ancestor of all cells in a tissue was a stem cell and not a somatic cell. Because queens and workers share the same diploid genome, researchers thus hypothesize that the innovation of the sterile worker caste of ants was related initially to temporal changes in expression of reproduction-related genes (Linksvayer & Wade 2005) associated with cooperative brood care (Rehan & Toth 2015). The transition to distinct reproductive and sterile females appears to be related to changes in conserved developmental networks involved in feeding, growth, and reproduction (Toth & Robinson 2007, Chittka et al. 2012). This evolutionary process has involved novel genes, changes in the coding sequences of existing genes, and developmental changes in gene regulation (Johnson & Tsutsui 2011, Jasper et al. 2014, Sumner 2014, Rehan & Toth 2015).

Gene turnover: Patterns of gains and losses of protein-coding genomic loci over evolutionary time

Genomic studies related to behavior in Hymenoptera have focused primarily on honey bees, so the study of ant genetics draws heavily on tools developed in honey bee research. Honey bee genetics drew initially on the genetic tools and databases of the vinegar fly, *Drosophila melanogaster* (functional genomic resources reviewed in Mohr et al. 2014). Because honey bees have been under artificial selection by humans for more than 10,000 years, the genetics of honey bee behavior may not reflect the outcome of the diverse paths that evolution has taken in shaping ant behavior.

The study of molecular variation among ant species (reviewed in Nygaard & Wurm 2015) began with the first ant genome sequence, published in 2010 (Bonasio et al. 2010), and was followed quickly by many more publicly accessible genome sequences. The first seven species whose genomes were published were *Harpegnathos saltator*, *Camponotus floridanus*, *Acromyrmex echinatior*, *Linepithema humile*, *Pogonomyrmex barbatus*, *Atta cephalotes*, and *Solenopsis invicta* (Bonasio et al. 2010, Nygaard et al. 2011, C.D. Smith et al. 2011, C.R. Smith et al. 2011, Suen et al. 2011, Wurm et al. 2011). These were followed quickly by others, and there are now at least 24 ant species with publicly accessible genomes, transcriptomes, or both (**http://www.antgenomes.org**, accessed Jan. 21, 2015).

The seven ant species whose genomes were published initially all share a common ancestor from approximately 90 million years ago and have been analyzed in the context of a large, 18-species, arthropod phylogeny with a common ancestor from about 510 million years ago (Rappoport & Linial 2015). Roux et al. (2014) found that ants show relaxed selection on immunity genes and increased selection on neurogenesis- and olfactory-related genes. These changes may be due to the importance of social processes related to immunity (Meunier 2015).

Ants rely on olfactory cues to gather information about their environment and to communicate, and they have large and complex gene repertoires involved in lipid synthesis and olfaction. Comparisons of ant genomes show strong evolutionary change in the content of genes associated with cuticular hydrocarbons (CHCs), oily glandular secretions that are used in nestmate recognition (Tsutsui 2013); microbiome regulation; and desiccation resistance (Howard & Blomquist 2005). Over the course of the diversification of ants, the gene families associated with CHC synthesis, including desaturases, elongases, and fatty acid synthases (e.g., Badouin et al. 2013, McKenzie et al. 2014, Helmkampf et al. 2014 on desaturases), have expanded. CHCs and other environmental semiochemicals (Vander Meer 2012) are perceived by binding to chemoreceptors, classified into categories of odorant receptors, gustatory receptors, and ionotropic receptors (Tsutsui 2013). Several studies have found evidence of selection acting on chemosensory gene families and patterns of tissue-specific expression in transcriptomic data (Zhou et al. 2012, Koch et al. 2013, Kulmuni et al. 2013, McKenzie et al. 2014, Zhou et al. 2015).

***cis*-Regulatory element:** Noncoding DNA region influencing the level, timing, or amount of RNA expression of a nearby or distant gene

Some olfactory perception genes have conserved sex- or tissue-specific roles, whereas others are more labile. Ant species differ in their expression of homologous chemosensory genes, presumably reflecting ecological differences in the odors they detect. Zhou et al. (2012) used RNA sequencing (RNA-seq) to examine the expressed chemosensory repertoire in the antennae and bodies of *C. floridanus* and *H. saltator* and found that some chemosensory genes displayed consistent sexually dimorphic expression within these species; others had no sexual dimorphism or appeared to switch the direction of overexpression between species. McKenzie et al. (2014) used RNA-seq on the antennae and bodies of the raider ant *Cerapachys biroi*, *C. floridanus*, and *H. saltator* and found interspecies differences in expression of the chemosensory repertoire, as well as one conserved, antennal-specific chemoreceptor.

Ants show remarkable diversity in morphological and sexual phenotypes (Chittka et al. 2012), radically diverged from their solitary, wasp-like ancestor (Ward 2014). This evolutionary process has left significant, genome-wide patterns in gene content, gene order along the chromosome, and *cis*–regulatory element composition, showing strong conservation of some noncoding regulatory elements, enriched near genes with a role in nervous system regulation (Simola et al. 2013a). A comparison of the genome sequences of four ant species (*A. echinatior*, *S. invicta*, *C. floridanus*, and *H. saltator*) found that they all share the same set of neuropeptide genes (Nygaard et al. 2011), and they share the loss of a RYamide receptor that is present in all other arthropod genomes (Caers et al. 2012).

Ant species also differ in changes, associated with developmental stage, in the expression of genes related to neurological differentiation and sensory perception. Ometto et al. (2011) compared the whole-body transcriptome of larval and adult males, gynes (unmated reproductive females), and workers in *S. invicta* and *Solenopsis richteri* and found the most interspecies transcriptomic differences in adult workers they also observed that developmental stage influenced transcriptome content more than species identity or sex. Analysis of small RNAs in *C. floridanus* and *H. saltator* revealed that the downstream targets of small regulatory RNAs are enriched for processes of neuron differentiation, neurogenesis, and steroid signaling (Bonasio et al. 2010, Simola et al. 2013a).

MALES AND FEMALES

Genetic studies show transcriptomic differences between male and female ants (Nipitwattanaphon et al. 2014 in *S. invicta*). Females, both reproductive gynes and sterile workers, are more similar to each other than to males (Ometto et al. 2011). This may be explained partially by genome ploidy: Female ants are diploid, and males are haploid. Haploidy at the complementary sex-determination locus in honey bees causes male development (Beye et al. 2003), although it is not clear if all ant species use the same mechanism of sex determination. Reanalysis of the data sets of Bonasio et al. (2010, 2012) showed a strong, sexually dimorphic link between DNA methylation and histone posttranslational modifications in *C. floridanus* (Glastad et al. 2015). Zhou et al. (2012) found that males and females differ in the expression of chemosensory proteins in the antennae of *C. floridanus* and *H. saltator*. Male ants are very short-lived and do not participate in the activities of the workers. Little is known about their behavior (reviewed in Beani et al. 2014).

REPRODUCTIVE AND STERILE FEMALES

Studies comparing reproductive and worker females have focused on genes such as *vitellogenin* (*Vg*) associated with reproductive physiology in other insects (reviewed in Heinze & Schrempf 2008, Libbrecht et al. 2013). Reproductive female ants tend to perform few tasks other than laying eggs, so most genetic studies of the differences between reproductive and sterile females investigate reproductive physiology rather than behavior. The first seven published ant genomes all contain three *Vg*-like genes in addition to *Vg*, although there are also a few lineage-specific duplications and deletions (Morandin et al. 2014). Gene expression analyses of the four *Vg* family genes across seven *Formica* species showed consistent worker-specific expression of *Vg*-like-C, but expression levels at the other paralogous loci did not display a consistent direction of expression bias between queens and workers (Morandin et al. 2014). Oxley et al. (2014) found that in the cyclically reproductive raider ant *C. biroi*, the head and abdomen expression of the *Vg* paralog *Vgq* was upregulated during the reproductive phase of the lifecycle, whereas another (*Vgw*) was upregulated during the brood care phase. This is the opposite trend from that found by Corona et al. (2013), who used reverse transcription quantitative polymerase chain reaction (rt-qPCR) to compare expression of *Vg* genes in laboratory colonies of *P. barbatus*. They quantified gene expression in queens, nurses (defined as "young ants interacting with the brood in the nest tube") and foragers (defined as "any ant handling a food item in the foraging area") (Corona et al. 2013, p. 7). They found that some *Vg* family members are upregulated in foragers, whereas others are upregulated in queens and nurses, a pattern also observed by Feldmeyer et al. (2014) in *Temnothorax*.

Gene ontology (GO): Controlled-vocabulary system that describes the biological function, relevant processes, and cellular localization associated with a given gene

RNA editing: Process by which mRNA nucleotide molecules are modified enzymatically, leading to an mRNA with information that differs from the genome

Several transcriptomic studies have demonstrated that there are few gene expression differences between reproductive and sterile females. Morandin et al. (2015) used RNA-seq on whole bodies of the adult and pupal stages of *Formica exsecta* queens and workers and found that the samples clustered more by developmental stage than by reproductive status. Genes differentially expressed between queens and workers were not enriched consistently across developmental time points for any functional annotations, although each stage-specific comparison displayed enrichment in several gene ontology (GO) categories. Transcriptomic comparisons of queens and workers showed few differences for *Pogonomyrmex* and *Vollenhovia* host/parasite species dyads (Smith et al. 2015). Ometto et al. (2011) showed that the whole-body transcriptome of *S. invicta* workers is closer to that of queens than that of males.

Other studies have indicated interesting expression differences between reproductive and sterile females. Li et al. (2014) found differences between gynes and workers in transcriptomic patterns of RNA editing in heads from the leaf-cutter ant *A. echinatior*. Differentially edited transcripts came from genomic loci that were enriched in GO categories relevant to neurotransmission, circadian rhythm, and carboxylic acid biosynthesis. They also found A-to-I RNA editing sites that differ between gynes and workers, including in the genes *TBPH* and *wah*, which have been implicated in locomotor behavior in *D. melanogaster* (Li et al. 2014). Feldmeyer et al. (2014), using RNA-seq on pooled whole-bodies of queens, foragers, fertile brood-carers, and sterile brood-carers in *Temnothorax longispinosus*, found that genes that were differentially expressed between queens and workers tended to be poorly annotated and had low overlap with genes identified in other species. This suggests that genes differentially expressed between reproductive and sterile females tend to be of recent evolutionary origin and hence cannot be annotated from the honey bee (*Apis mellifera*) or *D. melanogaster* genomes. This supports the idea, predicted by Linksvayer & Wade (2005), that rapid evolution is occurring in genes associated with workers (see Johnson & Tsutsui 2011 and Sumner 2014 for theoretical perspectives; for experimental studies, see Harpur et al. 2014 on honey bees and Ferreira et al. 2013 on wasps).

Other studies comparing female reproductives and sterile workers investigate the remarkable longevity of female reproductives relative to workers. Using whole-body transcriptomes, Bonasio et al. (2010) found that relative to workers, long-lived *H. saltator* reproductives had upregulation of genes known to play a role in longevity in other species, including humans (e.g. genes encoding telomerase and sirtuin deacetylase). In both *H. saltator* and *C. floridanus*, the expression of epigenetic regulators such as miRNAs and histone methyltransferases, as well as those related to neuronal function and chemical communication, was associated with reproductive status. Highly methylated genes tended to be expressed at medium levels in eggs, larvae, males, gamergates (reproductively active worker ants), and workers of *C. floridanus* and *H. saltator* (Bonasio et al. 2012), as in the honey bee (Foret et al. 2009). Both species showed the same pattern between queens and workers of differential methylation of genes associated with reproductive biology, telomere maintenance, and noncoding RNA metabolism. However, a recent study of the relationship between DNA methylation and reproductive status in *C. biroi* found no genes that were differentially methylated between ants in the worker-like or queen-like phases (Libbrecht et al. 2016). Their comparison of statistical techniques suggests that other results showing such differences may be misleading, owing to insufficient sample size.

In some ant species, there seems to be a genetic association with the development of queens or sterile workers. Early microsatellite studies showed that some populations of harvester ants in the genus *Pogonomyrmex* have two dependent interbreeding lineages. Females are diploid and produced by a mated queen; her matings within a mitochondrial lineage produce reproductive females, whereas matings between mitochondrial lineages produce workers (Volny & Gordon 2002, Helms Cahan & Keller 2003). Evidence is mixed about the possibility of a hybrid origin of these systems (Anderson et al. 2006, Schwander et al. 2007, Sirviö et al. 2011, Smith et al. 2012); further genetic studies are needed to resolve this (Mott et al. 2015). Eggs are laid in the ratio of the queen's mates, but reproductive eggs are consumed by workers in young colonies (Clark et al. 2006), and reproductives are produced when a colony is about five years old (Gordon 1995). Thus, a female's reproductive status depends not only on the genetic lineage of her parents but also on the behavior of the workers (Linksvayer et al. 2006, Volny et al. 2006). The population ratio of the two lineages tends to be asymmetric. For example, a long-term study of a dependent-lineage population of *P. barbatus* shows that the asymmetric lineage ratio has persisted for more than 20 years (Gordon et al. 2013), and there was no evidence of any behavioral or ecological differences between the lineages in foraging activity, nest size, or other measures, suggesting that the asymmetric ratio must be maintained by sex ratio differences (Gordon et al. 2013).

QUEEN NUMBER

The first investigation of a link between ant behavior and genetics examined the processes that determine the number of reproductives or queens in colonies of the fire ant *S. invicta*. In some ant species, colonies are monogynous, with a single queen, whereas others are polygynous, with more than one queen per colony. In some species, such as *S. invicta*, colonies can be either monogynous or polygynous (Fletcher et al. 1980). Electrophoretic gel surveys of allozyme diversity in a population of *S. invicta* revealed that the genotype at a single locus encoding the enzyme phosphoglucomutase 3(*Pgm-3*), was associated with queen number (Ross 1992). All combinations of the two alleles at the *Pgm-3* locus (*a* and *b*) were observed in queens in monogynous colonies (*aa*, *ab*, *bb*), whereas polygynous queens were never of the *aa* genotype. A nearby polymorphic genetic element was determined to be a better predictor of colony queen number, marked by two alleles (*B* and *b*) at the general protein 9 (*Gp-9*) locus (Keller & Ross 1999). In polygynous colonies, daughter gynes are sometimes recruited as new queens (DeHeer et al. 1999). Queens of the *BB* genotype introduced to existing colonies were rejected and ultimately killed by workers (Keller & Ross 1998). Monogynous colonies of *S. invicta* are often founded with multiple queens, which then fight amongst each other until only one queen remains, a system called pleometrosis. Whole-body transcriptomic differences between winner and loser queens include 43 genes in GO categories related to the metabolism of lipids and hormones (Manfredini et al. 2013). The Gp-9 protein is a putative member of the odorant binding protein family, which suggests that odor may be related to aggressive interactions between workers and queens (reviewed in Krieger 2005).

Subsequent work showed that many genes are associated with queen number in *S. invicta*. Wang et al. (2008) performed whole-body transcriptomes of *BB* and *Bb* workers from polygynous colonies using a custom cDNA microarray and showed a significant role for indirect genetic effects (reviewed in Linksvayer 2015). There were 39 genes differentially expressed between the two genotypes of workers in polygynous colonies and 91 genes differentially expressed in *BB* workers from monogynous versus polygynous colonies (Wang et al. 2008). Wang et al. (2013) then found that the association of *Gp-9* genotype with queen number was due to a large (13-Mb), nonrecombining genomic inversion that includes the *Gp-9* locus and 615 other genes of mainly unknown function. The inversion is enriched for the genes that were differentially expressed in the study by Wang et al. (2008). Recent work suggests that this inversion may alter the chemical scent of an ant carrying it, as well as altering the response of that ant to others (Trible & Ross 2015).

Indirect genetic effects: Components of phenotypic variance attributable to the genotype of another, usually conspecific, individual

Queen number is also associated with a genomic inversion in the Alpine silver ant, *Formica selysi*, in which some populations also contain both monogynous and polygynous colonies (reviewed in Rosset & Chapuisat 2006). Queen number is a labile trait at the colony level in *F. selysi* (Purcell & Chapuisat 2013). Purcell et al. (2014), using linkage mapping and genome sequencing, found that a large genomic inversion with suppressed recombination segregates between the two forms and contains hundreds of genes. No homology was observed between the genomic inversions of *S. invicta* and *F. selysi*.

WORKER BODY SIZE

The body size of workers is associated with differences in gene expression and DNA methylation. About 15% of ant genera have adult workers showing marked differences in size within a colony (Oster & Wilson 1978, Gouws et al. 2011). The larger workers are sometimes called majors and the smaller ones minors. Evidence suggests flexibility of task, rather than specialization according to body size (Wilson 1984, Patel 1990, Brown & Traniello 1998, Sempo & Detrain 2004). Differences among workers in size do not necessarily reflect differences in behavior.

Several studies show distinctive patterns of gene expression in major and minor workers of *C. floridanus*. Major and minor *C. floridanus* do not differ in their CHC profile or in their observed frequency of resting behavior (Endler et al. 2007). Simola et al. (2013b) examined differences in histone modifications and whole-body gene expression in males, majors, and minors of *C. floridanus* and found that majors and minors differed in the patterns of several histone modifications across 18% of the genome, linked with loci that had been associated previously with reproductive status in honey bees (Spannhoff et al. 2011) and with learning and memory in mice (Peleg et al. 2010). These differences in histone modifications were associated with RNA expression changes in genes enriched for GO terms related to, for example, muscle development, which was enriched in majors, and synaptic transmission, which was enriched in minors.

A recent study of *C. floridanus* indicates that differences in the activity of large and small workers may be due to differences in sensitivity to stimuli, mediated by changes in the regulation of sensitivity-modulating genes (Simola et al. 2016). The long-term stability associated with these epigenetic transformations is reminiscent of the cell-type model of ant phenotypic differentiation (figure 2 in Chittka et al. 2012). Other studies show epigenetic differences related to body size in *C. floridanus*. Alvarado et al. (2015) found that the epidermal growth factor receptor (*Egfr*) locus was hypermethylated and downregulated in larger workers. *Egfr* also plays a role in establishing size polyphenism in *A. mellifera* (Kamakura 2011). Taken together, the work of Alvarado et al. (2015) and Simola et al. (2016) has demonstrated experimental epigenetic control over the intermorphological differences in body size and activity in *C. floridanus*.

BEHAVIOR

Heritability of Task Performance

Genetic markers reveal parental effects on worker morphology and behavior. Using mitochondrial and microsatellite markers to determine matrilines and patrilines, studies of laboratory colonies of *Temnothorax* ants partitioned morphological variation into components of species, individual genotype, genotype of nestmates, and maternal environment (Linksvayer 2006 in *Temnothorax curvispinosus*, Linksvayer 2007 in three species of *Temnothorax* ants), revealing a role for each in phenotypic variability.

Many studies suggest heritability of behavioral variation from parent reproductive to workers within a colony, and from parent reproductive to the offspring reproductives that found new colonies. For example, wild colonies of *P. barbatus* resemble their mothers with regard to the regulation of foraging in poor conditions (Gordon 2013, Ingram et al. 2013). Patrilines of wild *Formica argentea* colonies displayed biases in their frequency of nest patrolling, nest maintenance, and queen guarding (Snyder 1992). Wiernasz et al. (2008) found that wild colonies of *Pogonomyrmex occidentalis* founded by queens with more mates (observed range: 3–10) tended to begin foraging earlier in the morning and foraged for longer during the day.

Quantitative genetic studies show heritable differences in task performance. In laboratory colonies of *Pogonomyrmex californicus*, workers of different matrilines differed in task performance, with a consistent trade-off between brood care and waste management (Holbrook et al. 2013). Workers in laboratory colonies of *F. argentea* had an allozyme polymorphism that was associated with patterns of nestmate grooming and leaving the nest but not with worker-queen interactions (Snyder 1993). In composite laboratory colonies of *Acromyrmex versicolor*, workers from different matrilines differed in task performance and in the age of initiation of foraging (Julian & Fewell 2004). In composite laboratory colonies of *Leptothorax rudis*, workers from different wild colonies demonstrated a bias in the frequency of foraging (Stuart & Page 1991).

Worker Behavior in the Absence of a Queen

Whether workers are in contact with a queen affects both behavior and gene expression (Feldmeyer et al. 2014 and references therein). Manfredini et al. (2014) compared the whole-body transcriptomes of workers collected from inside the nest box or out in a foraging area of laboratory colonies of *S. invicta* and found several hundred genes to be differentially expressed. Sampling location explained 57% of the variance in gene expression, and differentially expressed genes were enriched in GO terms related to musculature and energetic metabolism. Differentially expressed genes between inside and outside workers of *S. invicta* were not the same as those reported previously in honey bees and in the wasp *Polistes metricus*. When a colony fragment had been separated from the queen, more reproductive larvae were allowed to survive, and conspecific aggression decreased. In the queenless fragments, the transcriptomic differences between inside and outside workers disappeared. This suggests that behavior related to reproduction and aggression is associated with ongoing shifts in gene expression. The behavior associated with worker treatment of reproductive larvae provides an opportunity to investigate these interactions.

Age-Related Changes in Gene Expression

Ants tend to move through a series of tasks as they grow older, from work inside the nest to outside (Gordon 2015), and recent work shows that this is associated with changes in gene expression. Younger ants working inside the nest, for example, caring for the eggs, larvae, and pupae, differ in gene expression from older ants working outside the nest, for example, foraging for resources. Mikheyev & Linksvayer (2015) used whole-body RNA-seq to investigate gene expression associated with differences in worker behavior in laboratory colonies of *Monomorium pharaonis*. Nurse ants interacted with brood in the nest. At the age of 9–12 days, ants tended to become foragers, defined as those observed on a food or water source, or carrying food. Nurse-upregulated genes were enriched for GO categories related to metabolism and chromatin modifications. Compared to nurse-upregulated genes, forager-upregulated genes tended to be more connected in gene regulatory networks and had higher levels of evolutionary conservation. A similar trend is observed in honey bees, in which genes with low network connectedness have higher rates of coding sequence change, which, along with lineage-specific genes, is hypothesized to play a role in phenotypic diversification (Jasper et al. 2014). Age-related changes in behavior in workers, known as age polyethism, are widespread in social insects (Gordon 2015, Rehan & Toth 2015), and these recent studies have begun to provide insight into the molecular mechanisms associated with ontogenetic changes in behavior.

Circadian Rhythms and the Foraging Gene

The expression of genes involved in circadian rhythms is associated with temporal patterns of worker activity. Ingram et al. (2012) examined gene expression in laboratory colonies of *S. invicta* and found that eight genes involved in circadian rhythms had oscillatory dynamics throughout the day, similar to those observed in *A. mellifera* (Ingram et al. 2012), and resembled mammalian patterns more than those of *D. melanogaster* (Tataroglu & Emery 2014). De Bekker et al. (2015) analyzed the transcriptomes of *Camponotus castaneus* workers during an infection with *Ophiocordyceps unilateralis sensu lato*, a fungal parasite that induces a fatal sequence of ant behavior at a certain time of the day. RNA-seq on the brains of infected ants revealed alterations in circadian gene oscillation, biogenic amine signaling, and immune response.

The foraging gene (*foraging*) is associated with activity in many species, and has been studied in ants, with diverse results. Sokolowski (1980) first studied this gene in *D. melanogaster*. Rover larvae move around more than sitters in both laboratory and wild populations, and this difference persists into adulthood (Sokolowski & Bauer 1989). Molecular analysis identified the foraging gene as a cyclic guanosine monophosphate (cGMP)–dependent protein kinase (PKG) (Osborne et al. 1997), and associations between movement and PKG enzymatic activity have been demonstrated in many species (reviewed in Kaun & Sokolowski 2009). Honey bee forager heads show multifold higher expression of *foraging* (known as *Amfor* in this species) relative to nurses. Brain PKG activity measured higher in foragers, as quantified with an in vitro enzyme assay. Treatment with 8Br-cGMP, which elevates PKG activity, induced precocious foraging (Ben-Shahar et al. 2002). In situ RNA hybridization of *Amfor* in the honey bee brain showed gene expression in the lamina of the optic lobes and the mushroom bodies (Ben-Shahar et al. 2002), suggesting that increased expression of *Amfor* may induce maturation and foraging behavior via an increase in positive phototaxis (Ben-Shahar 2003).

Patterns of expression of the *foraging* gene in ants differ from those found in honey bees. Lucas & Sokolowski (2009) compared *foraging* enzymatic activity in major and minor workers in *Pheidole pallidula* (Wilson 1984, Patel 1990, Brown & Traniello 1998, Sempo & Detrain 2004). Major worker brains had more PKG enzymatic activity, and one isoform of *for* showed protein expression in more neurons. When increased PKG activity in vivo was induced with 8Br-cGMP, both sizes of workers were less likely to interact with a foraging stimulus (a live mealworm), and majors, but not minors, were more likely to respond to a defense stimulus (the introduction of 200 minor and 25 major workers from another nest of *P. pallidula*). Untreated majors were more likely to forage and equally as aggressive as untreated minor workers.

The expression of the foraging gene is related to circadian rhythms. Using rt-qPCR, Ingram et al. (2005) found in both laboratory and field colonies of *P. barbatus* that foraging, traveling outside the nest to search for food, is associated with lower expression of *PbFor* than work inside the nest. This pattern is similar to the ant species *S. invicta* (Lucas et al. 2015) and *C. biroi* (Oxley et al. 2014) and opposite that observed in *D. melanogaster*, *A. mellifera*, and *Caenorhabditis elegans*. Expression levels of *period*, a known circadian gene, differed according to worker task in laboratory colonies of *P. occidentalis*. Ingram et al. (2009) used rt-qPCR to show that *P. occidentalis* foragers, defined as ants that touched food in the foraging area, have strong, seasonally influenced circadian fluctuations in *period* expression, whereas interior nest workers, identified as ants that never entered the foraging arena, are arrhythmic. Foragers, but not nest workers, from laboratory colonies of *P. barbatus* showed circadian fluctuations in the level of *foraging* gene expression (Ingram et al. 2011). Foragers exhibit cyclic patterns of *PbFor* expression, peaking at midday, whereas interior workers are arrhythmic.

CONCLUSIONS

The social organization of ant colonies arises from behavioral interactions among individuals. Observational studies of behavior will be needed to examine the genetics of individual ant behavior and determine how these combine to produce the collective behavior of ant colonies. Genetic changes in physiological processes related to responses to interactions are likely to influence colony behavior (e.g., Gordon 2014, Simola et al. 2016). For example, changes in neurophysiological processes in ants influence social interactions and task performance (Kamhi & Traniello 2013, Katz & Lillvis 2014). Transcriptomic analysis is identifying candidate genes, for example, in reproductive physiology, that may have important effects on social interactions and thus on collective behavior.

To investigate ant behavior genetically, we will need to be aware of how different variables, such as the developmental stage, sampled tissue, and time of day, affect results. For example, studies across developmental time points have found interstage inconsistencies in patterns of differential gene expression (e.g., Morandin et al. 2015, Smith et al. 2015). It will be important to extend the notion of simple associations between genes and traits, as in GO annotations, to understand the many relational genetic processes that contribute to development and behavior. In addition, many studies use whole-body samples for transcriptomic studies, which may conceal tissue-specific differences in variation or detect false positives between biological groups owing to tissue allometry (discussed in Johnson et al. 2013). Brain- or at least head-specific gene expression studies in ants are encouraging and have found applicable results (e.g., Zhou et al. 2012 on antennae, Li et al. 2014 on heads, de Bekker et al. 2015 and Simola et al. 2016 on brains). The time of day of tissue sampling and behavioral testing can also radically alter results.

Future studies may be able to harness techniques such as RNA interference (RNAi) and CRISPR (Barrangou et al. 2015) to examine the genetics of ant behavior. RNAi has now been used in ants to examine reproductive physiology (Lu et al. 2009); immune response (Ratzka et al. 2013); and the development of sexually dimorphic pigmentation (Miyazaki et al. 2014), pheromone biosynthesis (Choi et al. 2012), chemosensory protein function (Cheng et al. 2015), and worker activity (Simola et al. 2016). The application of CRISPR to ants has not yet been published and will be an exciting technological advance in ant genomics.

The next step in the study of the genetics of ant behavior will be to learn about the feedback between individual-level physiological changes and socially mediated responses to environmental conditions. What genetic mechanisms regulate social interactions, and how do these produce variation among colonies upon which natural selection can act? The answers to these questions are likely to differ greatly among species. The enormous ecological diversity of ants has led to great diversity in behavior. Studies of behavioral genetics are needed that investigate the interplay of behavior and environment in the collective behavior of ant colonies.

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