Has gene expression neofunctionalization in the fire ant antennae contributed to queen discrimination behavior?

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Abstract
Queen discrimination behavior in the fire ant Solenopsis invicta maintains its two types of societies: colonies with one (monogyne) or many (polygyne) queens, yet the underlying genetic mechanism is poorly understood. This behavior is controlled by two supergene alleles, SB and Sb, with ~600 genes. Polygyne workers, having either the SB/SB or SB/Sb genotype, accept additional SB/Sb queens into their colonies but kill SB/SB queens. In contrast, monogyne workers, all SB/SB, reject all additional queens regardless of genotype. Because the SB and Sb alleles have suppressed recombination, determining which genes within the supergene mediate this differential worker behavior is difficult. We hypothesized that the alternate worker genotypes sense queens differently because of the evolution of differential expression of key genes in their main sensory organ, the antennae. To identify such genes, we sequenced RNA from four replicates of pooled antennae from three classes of workers: monogyne SB/SB, polygyne SB/SB, and polygyne SB/Sb. We identified 81 differentially expressed protein-coding genes with 13 encoding potential chemical metabolism or perception proteins. We focused on the two odorant perception genes: an odorant receptor SiOR463 and an odorant-binding protein SiOBP12. We found that SiOR463 has been lost in the Sb genome. In contrast, SiOBP12 has an Sb-specific duplication, SiOBP12b′, which is expressed in the SB/Sb worker antennae, while both paralogs are expressed in the body. Comparisons with another fire ant species revealed that SiOBP12b′ antennal expression is specific to S. invicta and suggests that queen discrimination may have evolved, in part, through expression neofunctionalization.

1 | INTRODUCTION

Great variation has evolved in animal societies. Society complexity ranges from simple cooperative pairs (Pilakouta, Richardson, & Smiseth, 2015; Riehl, 2013) to huge but loose flocks and schools (Hemelrijk & Hildenbrandt, 2012), and even to the highly hierarchical colonies of ants, bees, and wasps (Hines, Hunt, O’Connor, Gillespie, & Cameron, 2007; Richards, Wettberg, & Rutgers, 2003;
In this study, we aimed to determine which genes are associated with the queen discrimination behavior in the fire ant workers. We reasoned that identifying the genes expressed in the worker antennae may reveal important genes underlying this behavior. However, there are other behaviors that are different between monogyne and polygyne workers beside the queen discrimination behavior, for example, higher intercolony aggression in monogyne compared to polygyne colonies. Therefore, distinguishing the genes that are regulated by genetic background or social environment is critical. Here, we used RNA sequencing (RNA-Seq) to profile gene expression in the antennae of three worker classes: monogyne SB/ SB, polygyne SB/SB, and polygyne SB/Sb. Given that odorant perception genes function in chemical recognition, we further examined two odorant perception genes that were differentially expressed between SB/ SB and SB/SB workers in a comparative genomic approach. Last, we compared gene expression across major body parts between two fire ant species to study whether high antennal gene expression for one odorant perception gene, SiOBP12, was conserved across species or specific to SB/SB workers.

2 | MATERIALS AND METHODS

Detailed methods are provided in the supplementary materials (Appendix S1).

2.1 | Ant colony collection, maintenance, and genotyping

Colonies of *S. invicta* were collected from Taoyuan County, Taiwan. For *Gp-9* genotyping, an PCR/RFLP assay was conducted on pooled DNA from at least ten workers (Krieger & Ross, 2002). Colonies were maintained under standard conditions (Jouvenaz, Allen, Banks, & Wojcik, 1977). Ants were sampled at least two to three months after collection from the field.

Two monogyne colonies of the tropical fire ant, *S. geminata*, were collected in Taichung City, Taiwan. After collection, we maintained them in the laboratory in the same conditions as *S. invicta*, except with the addition of seeds used as bird food.

2.2 | Sample and tissue collection

All *S. invicta* and *S. geminata* samples were from foraging workers, which we obtained by attracting with honey. To minimize sampling differences, we selected only medium-sized (ca. 1.5 mm in length) workers.

For *S. invicta*, we sampled different body parts from three classes of workers for each bioreplicate (Figure S1): monogyne SB/SB (M_BB), polygyne SB/SB (P_BB), and polygyne SB/Sb (P_Bb). For RNA sequencing, we extracted total RNA from 36 to 55 pairs of antennae for four bioreplicates (12 total samples). For qRT-PCR experiments, we extracted total RNA from 20 to 24 antennal pairs or 15 units of a body part (head, thorax, or abdomen). For sequence trace analysis,
we extracted total RNA from the antennae, heads, or thoraces-abdomens of 10–11 SB/Sb workers. Different experiments used different colony pairs.

Body part samples of S. geminata (head, thorax, and abdomen) were from 20 and 26 individuals. Antennae samples were from 99 and 108 individuals.

2.3 | RNA extraction, amplification, and sequencing

We extracted RNA using the Direct-zol RNA MiniPrep Kit (R2050, ZymoResearch) following a modification of the manufacturer’s instructions (Zhang et al., 2013). To obtain sufficient cDNA for sequencing, we amplified each antennal RNA sample independently using the NuGEN Ovation V.2 kit (M01206, NuGEN Technologies), following the manufacturer’s instructions. All samples were sequenced on the Illumina HiSeq 2500 platform with a 150 bp paired-end protocol (at the High Throughput Genomics Core at the Biodiversity Research Center, Academia Sinica, Taiwan).

2.4 | RNA-Seq data processing

To generate an antennal reference gene list for differential gene expression analysis, we combined 15,589 genes from the fire ant official gene set (OGS) with novel transcripts within our RNA-Seq data as follows. We first controlled the quality of the sequence reads of the 12 NuGEN amplified antennal cDNA libraries by retaining reads passing a base-quality threshold of 20 and minimum length of 30 bases using cutadapt v1.16 (Martin, 2011). Next, we followed the Tuxedo pipeline (Trapnell et al., 2012) to map quality-controlled reads onto the fire ant genome Si_gnH (Privman et al., 2018) and generated an expressed gene set containing 44,521 putative genes. We subsequently retained potential protein-coding genes which encode peptides with an open reading frame of at least 50 amino acids and having similarity to genes in the NCBI nonredundant reference genomes. We aligned these six protein sequences using MEGA7 (Kumar, Stecher, & Tamura, 2016). The helices and the residues, which are likely to be involved in the bombykol binding pocket and pH-sensitive conformation change, were adopted from previous studies (Krieger & Ross, 2005; Sandler, Nikonova, Leal, & Clardy, 2000).

2.5 | Identification of the different paralogs of SiOBP12 (SiOBP12 and SiOBP12b’)

To obtain the cDNA sequence of the expressed SiOBP12 in the SB/Sb worker antennae, we used RACE assays that were subsequently confirmed by cloning and Sanger sequencing. To estimate the expression of SiOBP12 and SiOBP12b’, we mapped the merged quality-controlled reads of the four bioreplicates of the SB/Sb antennal cDNA libraries onto the Si_gnH genome and used GATK for variant calling at the SiOBP12 locus. To determine the expression of SiOBP12 and SiOBP12b’ in the SB/Sb individual antennae, heads, and bodies (i.e., thorax-abdomen), we amplified partial cDNAs of the two paralogs using shared PCR primers and Sanger-sequenced the PCR products without purification.

2.6 | Structure analysis of SiOBP12 proteins

To examine whether the amino acid differences between SiOBP12B, SiOBP12b, SiOBP12b’, and SgOBP12 may be potentially functionally important, we compared their predicted protein sequences to Gp-9 (NCBI RefSeq Q8WP90) and the pheromone-binding protein (PBP) in Bombyx mori (NCBI RefSeq NP_001037494). SiOBP12b’ was translated from RACE cDNA sequence obtained in this study (MN193778). SiOBP12B (Wurm et al., 2011), SiOBP12B (Wang et al., 2013), and SgOBP12 (Dryad) were translated from their respective genomic sequences. We aligned these six protein sequences using MEGA7 (Kumar, Stecher, & Tamura, 2016). The helices and the residues, which are likely to be involved in the bombykol binding pocket and pH-sensitive conformation change, were adopted from previous studies (Krieger & Ross, 2005; Sandler, Nikonova, Leal, & Clardy, 2000).

2.7 | Examination of SiOR463 and SiOBP12b’ in the S. invicta SB, Sb, and S. geminata genomes

To infer gene deletion or insertion, we examined the sequences of SiOR463 and SiOBP12b’ and their surrounding regions in three PacBio genome assemblies: S. invicta SB, Sb, and S. geminata. The homologous sequences were aligned and visualized using Mauve (Darling, Mau, Blattner, & Perna, 2004). For SiOR463, we also mapped low-coverage whole genome sequence of 14 males (Wang et al., 2013) onto the S. invicta SB and Sb contigs where SiOR463 is located, and visualized mapping results in IGV (Thorvaldsdóttir, Robinson, & Mesirov, 2013).

3 | RESULTS

3.1 | Differentially expressed genes in the antennae of the three fire ant worker classes

To distinguish potential genetic (SB/SB vs. SB/Sb) or social (monogyne vs. polygyne) effects on antennal gene expression differences, we examined three worker classes: monogyne SB/SB, polygyne SB/SB, and polygyne SB/Sb (hereafter as M_BB, P_BB, and P_Bb, respectively, Figure S1A). We extracted and amplified total antennal RNA from four bioreplicates from each of the three worker classes for sequencing (12 total samples). RNA-Seq yielded 47–61 million raw reads per sample, and after quality control, trimming, and filtering, 45–60 million reads were retained (see Appendix S1: Methods, Table S1). To identify differentially expressed genes (DEGs), we first estimated the expression level of each gene by mapping postfiltered
reads onto “OGS-plus,” a set of 19,230 putative protein-coding genes, using Bowtie2 and counting the mapped reads with RSEM (Li & Dewey, 2011; see Appendix S1: Methods, Figure S1D). We used transcript sequences as the mapping reference (rather than the whole genome) to minimize the effect of transcribed noncoding regions (e.g., introns) on the mapping results.

We identified 81 DEGs (posterior probability differentially expressed (PPDE) >95%; range 1.2- to 168-fold difference; Figure 1; Table S2). Of these, 36 genes were differentially expressed by social supergene genotype, with 31 and five genes being up- and downregulated, respectively, in P_Bb compared to both M_BB and P_BB. A comparison between social form samples revealed 43 genes differentially expressed with 21 and 22 being up- and down-regulated, respectively, in monogyne (M_BB) compared to polygyne (P_BB and P_Bb). Two genes, SINVm1_gene_00166 and XLOC_023347, were differentially expressed in all three comparison pairs (posterior probability of the expression pattern, >94%; PPDE >99%), possibly suggesting regulation by both genotype and social form. However, visual inspection and the Tukey HSD test suggested that genotype is the main factor regulating these two genes (Figure S2A, B). Definitive ascertainment of whether social form contributes to gene expression differences for these two genes will require greater sample sizes (Schurch et al., 2016).

The queen discrimination behavior in fire ants is linked to the social supergene (Keller & Ross, 1995; Wang et al., 2013). Of the 81 antennal DEGs, seven (8.64%) are located in the supergene and all were differentially expressed by genotype (Table S2). With respect to the 36 genes regulated by supergene genotype, this corresponds to a 6.68-fold over-representation of supergene genes (p-value = .0002, Fisher’s exact one-tailed test). In contrast to a previous study on whole worker bodies, which found more genes regulated by social environment than by genotype (Wang, Ross, & Keller, 2008), we found a similar number of DEGs due to social environment (n = 43) and genetic effects (n = 38, including SINVm1_gene_00166 and XLOC_023347) on the worker antennae (p-value = .65, exact binomial two-tailed test).

Inspection of the putative functions of all DEGs revealed 13 genes potentially involved in chemical metabolism (e.g., fatty acid synthase, cytochrome P450 enzymes, and esterase) and odorant perception (i.e., an OBP and an OR). Of these, eight genes were likely socially regulated with six and two genes being more and less expressed, respectively, in monogyne (M_BB) compared to polygyne (P_BB and P_Bb) samples. Five genes were regulated by genotype with four more highly expressed in SB/Sb (P_Bb) compared to SB/SB (M_BB and P_BB) samples.

Besides these two classes of genes, 16 transposable elements (TEs) were differentially expressed among the samples. Of these, the expression of six TEs was associated with social environment, with two and four TEs being more and less expressed, respectively, in monogyne (M_BB) compared to polygyne (P_BB and P_Bb) samples. Another ten TEs were differentially expressed by genotype, with two and eight (including SINVm1_gene_00166) genes being more and less expressed, respectively, in M_BB and P_BB compared to P_Bb samples.

To verify the RNA-Seq analysis, we conducted quantitative real-time PCR (qRT-PCR) assays on independent antennal RNA samples for four (of the seven) DEGs that are located in the supergene. We found the upregulation of all four genes in P_Bb samples compared to both M_BB and P_BB samples, confirming the RNA-Seq analysis results (Figure 2a; Figure S3). We were unable to design specific qRT-PCR primers for the remaining three genes.

3.2 | The differentially expressed odorant perception genes have copy number variation

An OR (XLOC_016888, hereafter SiOR463) and an OBP (SINVm1_gene_02111; also known as SiOBP12 (Gotzek, Robertson, Wurm, &
FIGURE 2 qRT-PCR expression assays of SgOBP12 (left), SiOBP12 (middle), and Gp-9 (right) in different worker body parts. For S. invicta samples, each data point is the fold difference (log2) between the sample and the mean of the M_BB samples of the same gene in the same body part. SiOBP12 and Gp-9 were not differentially expressed between the three worker classes in the worker heads, thoraces, and abdomens. In the worker antennae, only SiOBP12 was highly expressed in P_Bb compared to both M_BB and P_BB. The expression of SgOBP12 is relative to the M_BB samples of SiOBP12, that is, a fold difference value of 0 means that the expression level of SgOBP12 relative to two internal controls (ΔCt) was equal to the average expression of SiOBP12 relative to two orthologous internal controls (ΔCt) in M_BB in the same body part. In the head, thorax, and abdomen, the expression of SgOBP12 was similar to that of SiOBP12 in M_BB. Differential expression of the genes in different body parts was tested using the Tukey HSD test. Not shown p-values are larger than 0.1. This assay cannot distinguish between the different alleles and paralogs of SiOBP12. The S. invicta head, thorax, and abdomen samples came from the same set of workers; the antennae samples were prepared independently. Data from the same bioreplicates of S. invicta are indicated with the same point shape. Each experiment consisted of three (S. invicta) or two (S. geminata) bioreplicates. Missing data points are due to low expression (undet), data removed for technical reasons (removed), or no assay for low RNA concentration (NA). Sgem, S. geminata; M_BB, monogyne SB/SB; P_BB, polygyne SB/SB; and P_Bb, polygyne SB/Sb
FIGURE 3  Mauve alignment of the SiOBP12b′ region in the genomes of S. invicta Sb and SB as well as Solenopsis geminata. The ~30-kb region (empty red box) with SiOBP12b′ (empty red arrow) is absent in both the SB and S. geminata genomes, indicating that SiOBP12b′ likely inserted into the Sb genome after the split between the S. invicta and S. geminata lineages. This ~30-kb region also contains two pseudogenized reductase genes (blue arrowheads); other predicted genes outside this region are not shown except for a Copia transposable element (blue arrow). Blocks with the same color in each genome indicate corresponding regions among the genomes. The height of each column in the blocks indicates percent similarity among the genomes. Blocks above or below the black lines indicate inverted regions.

Shoemaker, 2011)) were among the seven DEGs located within the superfene. OBPs and ORs are involved in the very first step of odorant perception, where a chemical is carried by an OBP (or a CSP) to a chemoreceptor and thus activating signal transmission (Leal, 2013). Because of a potential role in explaining the queen discrimination behavior, we analyzed these two genes in greater detail.

We identified SiOR463 as a newly annotated Sb-specific OR gene, which has 95% nucleotide similarity to SiOR163 (NCBI RefSeq XM_026133769.1), based on a new PacBio assembly of the SB genome. SiOR463 is located between SiOR163 and an unannotated OR, SiOR462 (SiVM1_gene_13698). SiOR463 has been deleted in the Sb genome (Figures S4–S7); thus, the approximate half expression level of SiOR463 in SB/Sb compared to SB/ SB individuals may simply reflect gene dose (Figure S2C).

In contrast to SiOR463, SiOBP12 was expressed ~24-fold higher in the antennae of SB/Sb compared to SB/ SB workers. This higher expression was not from either of the Sb or SB alleles of SiOBP12 (SiOBP12B and SiOBP12A, respectively) but rather from a duplicated (paralogous) copy of SiOBP12, which we call SiOBP12b′ (corresponding to SiOBP25 (Pracana et al., 2017)), based on four lines of evidence. First, all of the mapped reads from the SB/Sb samples at the SiOBP12 locus contained mismatches, which corresponded to neither the SB (reference genome) nor Sb alleles (Table S3). Second, we conducted a rapid amplification of cDNA ends (RACE) assay on antennal expressed SiOBP12 and found that the expressed transcript has an extension of ~170 bp of the 5′-UTR compared to SiOBP12 (NCBI HQ853360). This extension mapped to a different genomic region (contig 000102F in the Sb PacBio genome). Third, copy number variation analysis indicated that there is twice the number of copies of SiOBP12 in the Sb compared to the SB genome (Table S4). Finally, based on a new Sb PacBio assembly, we confirmed that this SiOBP12 paralog is Sb-specific and located within the superfene (Figure 3; Figure S8).

The SiOBP12b′ gene could have been duplicated after the superfene was formed in an ancestor of S. invicta, and hence be specific for the Sb superfene allele, or it could have been lost from the ancestral SB superfene allele (Ross, Krieger, & Shoemaker, 2003). In the former case, the SiOBP12b′ flanking region should be adjoined, while in the latter case, we may be able to find SiOBP12b′ (or its remnants) in the genome of Solenopsis geminata, an outgroup species to the South American socially polymorphic clade of fire ants that includes S. invicta (Gotzek et al., 2010; Martins, Fernando de Souza, & Bueno, 2014). In contrast to a previous study, which suggested that OB12 in S. geminata (SgOB12) was partially degenerated (Pracana et al., 2017), we found that the 17 kbp OBP gene cluster containing Gp-9 (Gp-9, OB4P, OB1P3, and OB12) is conserved in the two species (identity >95%, blast results in Dryad). However, the SiOBP12b′ region is a complex duplication of ~30 kbp that is absent in the S. geminata genome while its 5′ and 3′ adjacent regions are conserved and separated by only a ~2 kbp unconserved fragment (Figure 3). This result suggests that SiOBP12b′ appeared in S. invicta or its ancestral lineage after divergence from the S. geminata lineage, and is compatible with its appearance after the formation of the SB and Sb superfene alleles.

3.3  Possible neofunctionalization of SiOBP12b′

The fate of a gene duplicate is typically loss of one of the copies but sometimes neofunctionalization. While the SiOBP12b′ sequence has an intact open reading frame, SiOBP12B and SiOBP12b appeared to have become nonfunctional independently because of an early stop codon caused by a C-to-T substitution at the 46th nucleotide relative to the start codon in SiOBP12B or a 17-base frame-shifting insertion in SiOBP12b (Pracana et al., 2017). The nonfunctional SiOBP12b allele is consistent with no (this study) or very low expression of this allele (1 of 32 OB12 reads; Gotzek et al., 2011; Table S5). In contrast, the predicted nonfunctional status of SiOBP12B was surprising because it is expressed and also differed from the initially published intact SiOBP12B sequence (NCBI RefSeq HQ853360; Gotzek et al., 2011). Inspection of the original RNA-Seq dataset revealed that the erroneous SiOBP12B assembly was due to the collapse of reads from both the SiOBP12B
and SiOBP12b′ alleles (Table S5). Furthermore, all SiOBP12B sequences examined from two invasive populations (seven unrelated SB males from Georgia, USA [Wang et al., 2013] and 12 families from Taiwan [Qiu et al., 2018 and this study]) have the C-to-T substitution, indicating that the premature stop codon is likely fixed in the invasive range.

The loss of SiOBP12B and SiOBP12b may be compensated (i.e., replaced) by SiOBP12b′ in which case the protein sequence of the parental SiOBP12 and SiOBP12b′ should be highly similar. Alternatively, amino acid changes at functionally important residues may indicate neofunctionalization. Although both SiOBP12B and SiOBP12b have premature stop codons, their degeneration appears to be recent because both have high nucleotide and amino acid similarities to the ortholog SgOBP12 and paralog SiOBP12b′. Thus, mutations in SiOBP12b′ can be inferred based on the patterns of substitutions in comparison among these sequences. We compared the four OBP12s to Gp-9 (NCBI RefSeq Q8WP90) and the solved structure of the B. mori pheromone-binding protein (NCBI RefSeq NP_001037494) and found 14 changes (8% of full-length protein) in OBP12b′ relative to the presumptive ancestral sequence (Figure S9). Interestingly, six of these are located in alpha helix 5 including two at potentially important positions. The R117P change is potentially located in the binding pocket, and the R121H change is potentially at the pH-sensitive conformation site. These amino acid differences may indicate that OBP12b′ has different ligand binding properties compared to OBP12B or OBP12b, rather than simply replacing the original function of OBP12.

Genes may also neofunctionalize in terms of gene expression. Prior studies have shown that many OBPs, including Gp-9 and SiOBP12, are expressed in nonantennal tissues (Morandin et al., 2016; Pracana et al., 2017; Zhang, Wanchoo, Ortiz-Urguiza, Xia, & Keyhani, 2016). We tested whether SiOBP12 (in an assay that cannot distinguish between paralogs) was also differentially expressed in the worker heads (without antennae), thoraces, and abdomens using qRT-PCR assays. Similar to the well-known Gp-9, we found no expression differences between the three worker classes (Figure 2, all Tukey’s HSD p-values >0.05). Interestingly, the expression pattern of SgOBP12 was similar to those of SiOBP12B—low expression in the antennae and at comparable levels relative to the internal reference genes in the heads, thoraces, and abdomens (Figure 2).

To determine whether the expression of SiOBP12 in SB/Sb worker bodies is also primarily from SiOBP12b′ as in the worker antennae (which has 98% from SiOBP12b′ based on RNA-Seq; Table S3), we inspected Sanger sequencing trace files of partial SiOBP12 cDNA amplicons from the antennae, heads, and bodies (i.e., thorax-abdomen). We found a similar expression level of both SiOBP12B and SiOBP12b′ in the heads and bodies while, again, the antennae showed predominantly (or even pure) signal from SiOBP12b′ (Figure S10, all three bioreplicates in Dryad). Together, these results suggest that SiOBP12b′ may have acquired a new antennal regulatory element.

4 | DISCUSSION

This study compared gene expression levels in the antennae of the fire ant workers of alternate social supergene genotypes and social forms in order to identify candidate genes that explain how SB/SB and SB/Sb workers differ in discriminating between SB/SB and SB/Sb queens. By targeting the most relevant sensory organ associated with this behavior, our study provided better sensitivity to detect tissue-specific differences compared to similar studies which used the whole bodies of workers (Wang et al., 2008) and queens (Nipitwattanaphon, Wang, Dijkstra, & Keller, 2013). Other transcriptomics experiments have been conducted in fire ants in other contexts (Calkins et al., 2018; Chen, Shen, & Lee, 2006; Morandin et al., 2016; Nipitwattanaphon et al., 2014; Qiu et al., 2018). Our results and follow-up experiments uncovered an interesting, differentially expressed OBP, SiOB12b′, whose expression patterns suggest that it may play a role in the differences between monogyne and polygyne colony forms in fire ants.

4.1 | Antennal gene expression differences between the three worker classes are mainly from transposable elements, chemical metabolism, and perception genes

The antennae are the main sensory organ of insects, and thus, the genes expressed there have a large effect on what insects can detect and consequently how they behave (Athrey et al., 2017; Chen et al., 2017; McBride et al., 2014; Zhao et al., 2016). Our analysis identified 81 DEGs in the antennae across three worker classes (monogyne SB/SB, polygyne SB/Sb, and polygyne SB/Sb). Globally, our results are consistent with previous studies of gene expression comparing different social supergene genotypes on whole bodies of queens, workers, and males, in that we also found an over-representation of DEGs located in the supergene (Nipitwattanaphon et al., 2013; Wang et al., 2008, 2013). Despite supergene over-representation, there was no overlap for the DEGs located within the supergene found by the antennal and worker whole-body experiments. Thus, many other DEGs within the supergene likely remain to be found by supergene genotype comparisons of other tissues and developmental times. Another general pattern found in whole-body worker gene expression profiles was that social environment had a stronger effect on gene expression than supergene genotype (Wang et al., 2008, 2013). However, we found that antennal expression was comparably affected by these two factors. A possible explanation for this difference is that by examining a specific organ (the antennae), we excluded many other organs which may be affected more by the social environment. For example, Thelohania solenopsae infection and gut symbiotic microbes tend to be found among polygyne colonies (Lee, Husseneder, & Hooper-Bui, 2008; Oi, Valles, & Pereira, 2004) while Wolbachia infections were higher in monogyne colonies (Shoemaker et al., 2003).
In fire ants, several TEs are presumably active based on their germline expression and many TEs have substantial expanded in the Sb haplotype (Lee & Wang, 2018; Stolle et al., 2019; Wang et al., 2013). Our study identified ten TEs that were differentially expressed based on genotype. This general expression pattern could be explained either by supergene allele-specific gene regulation or by copy number variation of TEs between SB and Sb genomes. The general consensus is that most TE insertions are neutral or slightly deleterious; however, TEs that cause adaptive phenotypes have been described in many organisms, including insects (van’t Hof et al. 2016; Jangam, Feschatte, Betrán, 2017; Li et al. 2018). Thus, it would be interesting to determine whether the differential expression of these TEs is just nonfunctional noise or adaptive gene regulation, especially for those associated with social form. Similarly, identifying the exact TE copy (or copies) that might contribute to the queen discrimination behavior would be an interesting avenue for future study.

Of the remaining DEGS, 13 encode plausible candidates that may explain how SB/SB and SB/Sb workers differ in discriminating between the SB/SB and SB/Sb queen chemical cues. The candidates include genes for both chemical metabolism (fatty acid synthase, cytochrome P450 enzymes, and esterase) and odorant perception (i.e., an OBP and an OR) (Table S2). Workers rely on the semiochemical cues displayed on the queen’s cuticle to recognize a queen’s genotype (Eliyahu et al., 2011; Keller & Ross, 1998; Tribble & Ross, 2016). Therefore, the presence of DEGs that detect or metabolize chemicals was expected, due to their potential roles in a chemical sensing pathway, starting from chemical transport, continuing to receptor binding, and ending with chemical degradation. A genetic change at any step could affect insect behavior through a change in chemical sensitivity (transport and binding steps) or chemical modification (degradation), which in turn could affect receptor availability (Van den McBride et al., 2014; Montague, Mathew, & Carlson, 2011; Nakagawa, Sakurai, Nishioka, & Touhara, 2005; Ozaki et al., 2005; Pelletier et al., 2010; Berg & Ziegelberger, 1991). For example, OBPs (and CSPs) have roles in filtering and maximizing the signal through odorant solubilization (Leal, 2013; Van den Berg & Ziegelberger, 1991). Similarly, metabolism genes (e.g., esterases and cytochrome P450 enzymes) were found to degrade sex pheromones and volatile environmental chemicals possibly to reset receptor sensitivity in the antennae of moth and beetles, respectively (Cano-Ramírez et al., 1991). The OBP Gp-9 is a classic, although disputed, candidate that may have this function with regard to regulating social form differences (Gotzek & Ross, 2009; Gotzek, Shoemaker, & Ross, 2007; Keller & Ross, 1998; Krieger & Ross, 2002; Leal & Ishida, 2008). Our study showed that Gp-9 is not differentially expressed in the antennae or in different body parts between the three worker classes (Figure 3; see also Pracana et al., 2017). Previous studies showed that Gp-9 was highly expressed in polygyne queens compared to monogyne queens (Morandin et al., 2016; Pracana et al., 2017). Thus, differential expression of Gp-9 could possibly be important in queens but is unlikely in workers; functional differences in workers, if any, would have to be mediated by protein sequence differences.

Although any of the 13 differentially expressed chemical metabolism and odorant perception genes has the potential to mediate the queen discrimination behavior, we suggest that SiOBP12b’ may be the strongest candidate among these to explain the sensitivity of SB/Sb workers to introduced queens (Keller & Ross, 1998; Ross & Keller, 2002; Tribble & Ross, 2016). Our results indicated that SiOBP12b’, an Sb-specific gene, arose as a duplicate after the lineage leading to S. invicta split from that of S. geminata. Therefore, this gene could plausibly be involved in the social organization polymorphism in S. invicta and possibly the other socially polymorphic South American fire ants (Krieger & Ross, 2005). Based on the distinct and high expression in the antennae of SB/Sb individuals, we speculate that SiOBP12b’ may provide a simple mechanistic explanation for the queen discrimination behavior (Figure 4). In our model, during the evolution of the supergene in the fire ant, the SB/Sb queens gained an “Sb-cue” (e.g., possibly unsaturated hydrocarbons, polar biomolecules or Gp-9 (Eliyahu et al., 2011; Tribble & Ross, 2016)) while the SB/Sb workers gained an “Sb-cue” detector: SiOBP12b’. Binding between the “Sb-cue” and SiOBP12b’ would form a complex, which would subsequently activate an unknown OR to transfer the signal of “Acceptance.” The SB/SB queens without the “Sb-cue” fail to activate this “Acceptance” signal. In a polygyne colony, SB/SB workers, which do not have SiOBP12b’, respond by either ignoring introduced queens or following the “decision” of SB/Sb workers (Gotzek & Ross, 2008; Keller & Ross, 1998). In a monogyne colony, SB/SB workers accept only the dominant queen, which is their own queen. Future functional genetic experiments will be needed to test this model.

4.4 | SiOBP12b’: Neofunctionalization, adaptation, or demography?

Our analysis also revealed that SiOBP12b’ has gained a new antennal regulatory element. While expression in the head, thorax, and abdomen for OB12 occurs in both S. geminata and S. invicta, expression in the antenna occurs only in S. invicta workers, with SiOBP12b’ being the predominantly expressed paralog. A previous study indicated that SiOBP12b’ has experienced positive selection (Pracana et al., 2017). Examination of the protein sequence also revealed multiple amino acid site differences, including two affecting potential functional sites, which may affect OB12b’ ligand binding efficiency, regulation, and/or target identity. These changes may indicate neofunctionalization of a duplicated gene. Alternatively, this pattern might reflect a bottleneck in forming the invasive population coupled with adaptation. Further investigation of this gene in related species and functional confirmation of
the amino acid changes between the ancestral OBP12 and SiOBP12b’ will help reveal the evolutionary history of these paralogs.

The contribution of neofunctionalized after gene duplication in the evolutionary divergence between non-recombining supergene alleles is poorly understood. Supergenes and their evolution have many parallels with sex chromosomes (Graves, 2006; Liu, 2018). Neofunctionalization has been appreciated in the sex chromosome evolution literature. For example, many new sex chromosomes have evolved by the acquisition of a duplicate copy and subsequent neofunctionalization of the "trigger" genes in terrestrial vertebrates and fish (Matsuda et al., 2002; Smith et al., 2009). Additionally, several examples in both vertebrate and invertebrate sex chromosomes have demonstrated duplication and then neofunctionalization of genes for other processes on the Y (Ellegren & Parsch, 2001; Liu, 2018). If confirmed, SiOBP12b’ would represent one of the rare examples for nonsex chromosomes.

Does SiOBP12b’ also have a function in the rest of the body? We found expression of SiOBP12b’ in tissues outside of the antennae of the SB/Sb workers, and its expression in whole bodies of queens in other studies presumably also reflects at least some nonantennal gene expression (Morandin et al., 2016; Pracana et al., 2017). This adds to the growing list of OBPs and CSPs found to be expressed in nonantennal tissues (Calvello et al., 2003; Guo et al., 2011; Jacquin-Joly, Vogt, François, & Nagnan-Le, 2001; Ozaki, Morisaki, Idei, Ozaki, & Tokunaga, 1995). The potential functions of OBPs/CSPs in nonantennal body parts are still poorly understood, although there are some data. For example, OBPs have been found associated with leg regeneration in the American cockroach (Kitabayashi, Arai, Kubo, & Natori, 1998; Nomura, Kawasaki, Kubo, & Natori, 1992), visual pigment transportation in Lepidoptera (Zhu et al., 2016), and larval molting in S. invicta (Cheng, Lu, Zeng, Liang, & He, 2015). Our comparative expression results suggest that SiOBP12b’ has at least two functions: chemical perception in the antennae and an unknown, possibly ancestral, function in other tissues (e.g., replacement of the degenerated parental SiOBP12). Additionally, because SiOBP12b’ is only present in SB/Sb individuals, its nonantennal functional could...
also affect phenotypic differences between monogyne and polygyne colonies. The precise roles of this gene remain to be investigated.

5 | CONCLUSIONS

In summary, we have profiled gene expression of the fire ant worker antennae and found that supergene genotype and social environment equally affected antennal gene expression. We identified 81 DEGs including 13 putative metabolism and odorant perception genes that may be involved in queen discrimination. Of these, we found that SiOBP12b′ is a particularly interesting candidate because it is an Sb-specific paralog that has acquired high expression in the antennae. These genes, and especially SiOBP12b′, will be the subject of further behavioral genetic analyses.

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CONFLICT OF INTEREST

None declared.

AUTHORS’ CONTRIBUTIONS

V.D.D. and J.W. conceived and designed the experiments. V.D.D. conducted experiments. All authors contributed to bioinformatic analyses. V.D.D. and J.W. wrote the manuscript. All authors gave final approval for publication.

DATA AVAILABILITY STATEMENT

The GenBank accession for the raw and processed RNA sequence data is GSE126684 and that for the SiOBP12b′ cDNA sequence is MN193778. The accession numbers of the fire ant PacBio genomes are SAMN11869237 (SB) and SAMN11869238 (Sb). Additional auxiliary data are deposited in Dryad at https://doi.org/10.5061/dryad.qn4d68k.

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REFERENCES

Athey, G., Cosme, L. V., Popkin-Hall, Z., Pathikonda, S., Takken, W., & Slotman, M. A. (2017). Chemosensory gene expression in olfactory organs of the anthropophilic Anopheles coluzzii and zoophilic Anopheles quadriannulatus. BMC Genomics, 18(1), 751. https://doi.org/10.1186/s12864-017-4122-7
Bethesda (MD). National Center for Biotechnology Information (NCBI). Retrieved from https://www.ncbi.nlm.nih.gov/
Bourke, A., & Franks, N. R. (1961). Social evolution in ants. Princeton, NJ: Princeton University Press.
Calkins, T. L., Chen, M. E., Arora, A. K., Hawkings, C., Tamborindeguy, C., & Pietrantonio, P. V. (2018). Brain gene expression analyses in virgin and mated queens of fire ants reveal mating-independent and socially regulated changes. Ecology and Evolution, 8(8), 4312–4327. https://doi.org/10.1002/ece3.3976
Calvello, M., Guerra, N., Brandazza, A., D’Ambrosio, C., Scaloni, A., Dani, F. R., ... Pelosi, P. (2003). Soluble proteins of chemical communication in the social wasp Polistes dominulus. Cellular and Molecular Life Sciences, 60(9), 1933–1943.
Cano-Ramírez, C., López, M. F., Cesar-Ayala, A. K., Pineda-Martínez, V., Sullivan, B. T., & Zúñiga, G. (2013). Isolation and expression of cytochrome P450 genes in the antennae and gut of pine beetle Dendroctonus rhizophagus (Curculionidae: Scolytinae) following exposure to host monoterpenes. Gene, 520(1), 47–63. https://doi.org/10.1016/j.gene.2012.11.059
Chen, J. S. C., Chen, C.-H., & Lee, H.-J. (2006). Monogynous and polygynous red imported fire ants, Solenopsis invicta Buren (Hymenoptera: Formicidae), in Taiwan. Molecular Ecology and Evolution, 35(1), 167–172.
Chen, Q., Pei, D., Li, J., Jing, C., Wu, W., & Man, Y. (2017). The antenna transcriptome changes in mosquito Anopheles sinensis, pre- and post-blood meal. PLoS ONE, 12(7), e0181399.
Cheng, D., Lu, Y., Zeng, L., Liang, G., & He, X. (2015). Si-CSP9 regulates the integument and moulting process of larvae in the red imported fire ant, Solenopsis invicta. Scientific Reports, 5, 9245. https://doi.org/10.1038/srep09245
Darling, A. C. E., Mau, B., Blattner, F. R., & Perna, N. T. (2004). Mauve: Multiple alignment of conserved genomic sequence with rearrangements. Genome Research, 14(7), 1394–1403.
Eliyahu, D., Ross, K. G., Haight, K. L., Keller, L., & Liebig, J. (2011). Venom alkaloid and cuticular hydrocarbon profiles are associated with social organization, queen fertility status, and queen genotype in the fire ant Solenopsis invicta. Journal of Chemical Ecology, 37(11), 1242–1254.
Ellegren, H., & Parsch, J. (2007). The evolution of sex-biased genes and sex-biased gene expression. Nature Reviews Genetics, 8(9), 689–698.
Gotzek, D., Clarke, J., & Shoemaker, D. (2010). Mitochondrial genome evolution in fire ants (Hymenoptera: Formicidae). BMC Evolutionary Biology, 10(1), 300.
Gotzek, D., Robertson, H. M., Wurm, Y., & Shoemaker, D. (2011). Odorant binding proteins of the red imported fire ant, Solenopsis invicta: An example of the problems facing the analysis of widely divergent proteins. PLoS ONE, 6(1), e16289.
Gotzek, D., & Ross, K. G. (2008). Experimental conversion of colony social organization in fire ants (Solenopsis invicta): Worker genotype manipulation in the absence of queen effects. Journal of Insect Behavior, 21(5), 337–350.
Gotzek, D., & Ross, K. G. (2009). Current status of a model system: The gene Gp-9 and its association with social organization in fire ants. PLoS ONE, 4(11), e7713.

Gotzek, D., Shoemaker, D. D. W., & Ross, K. G. (2007). Molecular variation at a candidate gene implicated in the regulation of fire ant social behavior. PLoS ONE, 2(11), e1088.

Graves, J. A. M. (2006). Sex chromosome specialization and degeneration in mammals. Cell, 124(5), 901–914.

Guo, W., Wang, X., Ma, Z., Xue, L., Han, J., Yu, D., & Kang, L. (2011). CSP and Takeout genes modulate the switch between attraction and repulsion during behavioral phase change in the migratory locust. PLoS Genetics, 7(2), e1001291.

Hemelrijk, C. K., & Hildenbrandt, H. (2012). Schools of fish and flocks of birds: Their shape and internal structure by self-organization. Interface Focus, 2(6), 726–737. https://doi.org/10.1098/rsfs.2012.0025

Hines, H. M., Hunt, J. H., O’Connor, T. K., Gillespie, J. J., & Cameron, S. A. (2007). Multigene phylogeny reveals eusociality evolved twice in vespid wasps. Proceedings of the National Academy of Sciences, 104(9), 3295–3299.

Hölldobler, B., & Wilson, E. O. (1990). The ants. Berlin, Germany: Springer-Verlag.

Jacquin-Joly, E., Vogt, R. G., Francois, M. C., & Nagnan-Le, M. P. (2001). Functional and expression pattern analysis of chemosensory proteins expressed in antennae and pheromonal gland of Mamestra brassicae. Chemical Senses, 26(7), 833–844. https://doi.org/10.1093/chemseb/26.7.833

Jangam, D., Feschotte, C., & Betrán, E. (2017). Transposable element domestication as an adaptation to evolutionary conflicts. Trends Genet, 33(11), 817–831.

Jouveza, D. P., Allen, G. E., Banks, W. A., & Wojcik, D. P. (1977). A summary of the current knowledge of fire ants. Florida Entomologist, 60(4), 275–279. https://doi.org/10.2307/3493922

Keeling, C. I., Henderson, H., Li, M., Dullat, H. K., Ohnishi, T., & Bohlmann, J. (2013). CYP345E2, an antenna-specific cytochrome P450 from the American cockroach. Insect Biochemistry and Molecular Biology, 43(12), 1142–1151. https://doi.org/10.1016/j.ibmb.2013.10.001

Keller, L., & Ross, K. G. (1995). Gene by environment interaction: Effects of a single gene and social environment on reproductive phenotypes of fire ant queens. Functional Ecology, 9(4), 667–676. https://doi.org/10.2307/2390159

Keller, L., & Ross, K. G. (1998). Selfish genes: A green beard in the red fire ant. Nature, 394(6693), 573–575. https://doi.org/10.1038/29064

Kitabayashi, A. N., Arai, T., Kubo, T., & Natori, S. (1998). Molecular cloning of cDNA for p10, a novel protein that increases in the regenerating legs of Periplaneta americana (American cockroach). Insect Biochemistry and Molecular Biology, 28(10), 785–790. https://doi.org/10.1016/S0965-1748(98)00058-7

Kocher, S. D., Mallarino, R., Rubin, B. E. R., Yu, D. W., Hoekstra, H. E., & Pierce, N. E. (2018). The genetic basis of a social polymorphism in halictid bees. Nature Communications, 9(1), 4338.

Krieger, M. J. B., & Ross, K. G. (2002). Identification of a major gene regulating complex social behavior. Science, 295(5553), 328–332.

Krieger, M. J. B., & Ross, K. G. (2005). Molecular evolutionary analyses of the odorant-binding protein gene Gp-9 in fire ants and other Solenopsis species. Molecular Biology and Evolution, 22(10), 2090–2103.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Lahn, B. T., Pearson, N. M., & Jegalian, K. (2001). The human Y chromosome, in the light of evolution. Nature Reviews Genetics, 2(3), 207–216.

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. Nature Methods, 9(4), 357–359.

Langmead, B., Wilks, C., Antonescu, V., & Charles, R. (2019). Scaling read aligners to hundreds of threads on general-purpose processors. Bioinformatics, 35(3), 421–432. https://doi.org/10.1093/bioinformatics/bty648

Leal, W. S. (2013). Odorant reception in insects: Roles of receptors, binding proteins, and degrading enzymes. Annual Review of Entomology, 58, 373–391. https://doi.org/10.1146/annurev‐ento‐120811‐153635

Leal, W. S., & Ishida, Y. (2008). GP-9s are ubiquitous proteins unlikely involved in olfactory mediation of social organization in the red imported fire ant, Solenopsis invicta. PLoS ONE, 3(11), e3762.

Lee, A. H., Husseneder, C., & Hooper-Büi, L. (2008). Culture-independent identification of gut bacteria in fourth-instar red imported fire ant, Solenopsis invicta Buren, larvae. Journal of Invertebrate Pathology, 98(1), 20–33.

Lee, C.-C., & Wang, J. (2018). Rapid expansion of a highly germline-expressed mariner element acquired by horizontal transfer in the fire ant genome. Genome Biology and Evolution, 10(12):3262–3278.

Leng, N., Dawson, J. A., Thomson, J. A., Ruotti, V., Rissman, A. I., Smits, B. M. G., … Kendziorski, C. (2013). EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. Bioinformatics, 29(8), 1035–1043.

Li, B., & Dewey, C. N. (2011). RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics, 12(1), 323.

Li, Z. W., Hou, X. H., Chen, J. F., Xu, Y. C., Wu, Q., Gonzalez, J., & Guo, Y. L. (2018). Transposable elements contribute to the adaptation of Arabidopsis thaliana. Genome Biol Evol, 10(8), 2140–2150.

Liu, W. S. (2018). Mammalian sex chromosome structure, gene content, and function in male fertility. Annual Review of Animal Bioscience, 7(1), 103–124.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBNet Journal, 17(1), 10–12.

Martins, C., Fernando de Souza, R., & Bueno, O. C. (2014). Molecular characterization of fire ants, Solenopsis spp., from Brazil based on analysis of mtDNA gene cytochrome oxidase I. Journal of Insect Science, 14(50), 1–17.

Matsuda, M., Nagahama, Y., Shinomiya, A., Sato, T., Matsuda, C., Kobayashi, T., … Sakaiizumi, M. (2002). DMY is a Y-specific DM-domain gene required for male development in the medaka fish. Nature, 417(6888), 559–563.

Matsuo, T., Sugaya, S., Yasukawa, J., Aigaki, T., & Fuyama, Y. (2007). Odorant-binding proteins OBPs57d and OBPs57e affect taste perception and host-plant preference in Drosophila sechellia. PLoS Biology, 5(5), e118.

McBride, C. S., Baier, F., Omondi, A. B., Spitzer, S. A., Lutomiah, J., Sang, R., … Vosshall, L. B. (2014). Evolution of mosquito preference for humans linked to an odorant receptor. Nature, 515(7526), 222–227.

Montague, S. A., Mathew, D., & Carlson, J. R. (2011). Similar odorants elicit different behavioral and physiological responses, some super-sustained. Journal of Neuroscience, 31(21), 7891–7899.

Morandin, C., Tin, M. M. Y., Abril, S., Gómez, C., Pontieri, L., Schiøtt, M., … Mikheyev, A. S. (2016). Comparative transcriptomics reveals the conserved building blocks involved in parallel evolution of diverse phenotypic traits in ants. Genome Biology, 17, 43.

Nakagawa, T., Sakurai, T., Nishio, K., & Touhara, K. (2005). Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. Science, 307, 1638–1642. https://doi.org/10.1126/science.1106267
Nipitwattanaphon, M., Wang, J., Dijkstra, M. B., & Keller, L. (2013). A simple genetic basis for complex social behaviour mediates widespread gene expression differences. Molecular Ecology, 22(14), 3797–3813.

Nipitwattanaphon, M., Wang, J., Ross, K. G., Riba-grognuz, O., Wurm, Y., Khurewathanakul, C., & Keller, L. (2014). Effects of ploidy and sex-locus genotype on gene expression patterns in the fire ant Solenopsis invicta. Proceedings of the Royal Society B-Biological Sciences, 281, 1–8.

Nomura, A., Kawasaki, K., Kubo, T., & Natori, S. (1992). Purification and localization of p10, a novel protein that increases in nymphal regenerating legs of Periplaneta americana (American cockroach). International Journal of Developmental Biology, 36(3), 391–398.

Oí, D. H., Valle, S. M., & Pereira, R. M. (2004). Prevalence of Thelohania solenopsae (Microsporidia: Thelohaniidae) infection in monogyne and polygyne red imported fire ants (Hymenoptera: Formicidae). Environmental Entomology, 33(2), 340–345.

Ozaki, M., Morisaki, K., Idei, W., Ozaki, K., & Tokunaga, F. (1995). A putative lipophilic stimulant carrier protein commonly found in the taste and olfactory systems - A unique member of the pheromone-binding protein superfamily. European Journal of Biochemistry, 230(1), 298–308. https://doi.org/10.1111/j.1432-1033.1995.tb02981.x

Ozaki, M., Wada-Katsumata, A., Fujikawa, K., Iwasaki, M., Yokohari, F., Satoji, Y., ... Yamaoka, R. (2005). Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. Science, 309(5732), 311–314.

Pelletier, J., Guidolini, A., Syed, Z., Cornell, A. J., & Leal, W. S. (2010). Knockdown of a mosquito odorant-binding protein involved in the sensitive detection of oviposition attractants. Journal of Chemical Ecology, 36(3), 245–248.

Pilakouta, N., Richardson, J., & Smiseth, P. T. (2015). State-dependent cooperation in burrowing beetles: Parents adjust their contribution towards care based on both their own and their partner’s size. Journal of Evolutionary Biology, 28(11), 1965–1974. https://doi.org/10.1111/jeb.12712

Pracana, R., Levantis, I., Martinez-Ruiz, C., Stolle, E., Priyam, A., & Wurm, Y. (2017). Fire ant social chromosomes: Differences in number, sequence and expression of odorant binding proteins. Evolution Letters, 1(4), 199–210.

Privman, E., Cohen, P., Cohanim, A. B., Riba-Grognuz, O., Shoemaker, D., & Keller, L. (2018). Positive selection on sociobiological traits in invasive fire ants. Molecular Ecology, 27(15), 3116–3130. https://doi.org/10.1111/mec.14767

Purcell, J., Breilsford, A., Wurm, Y., Perrin, N., & Chapuisat, M. (2014). Convergent genetic architecture underlies social organization in ants. Current Biology, 24(22), 2728–2732.

Qiu, B., Larsen, R. S., Chang, N. C., Wang, J., Boomsma, J. J., & Zhang, G. (2018). Towards reconstructing the ancestral brain gene-network regulating caste differentiation in ants. Nature Ecology and Evolution, 2(11), 1782–1791.

Reinhard, J. (2004). Insect chemical communication. Chemosene, 6(4), 2–6.

Richards, M. H., von Wettberg, E. J., & Rutgers, A. C. (2003). A novel social polymorphism in a primitive eusocial bee. Proceedings of the National Academy of Sciences, 100(12), 7175–7180.

Riehl, C. (2013). Evolutionary routes to non-kin cooperative breeding in birds. Proceedings of the Royal Society B, 280(1772), 20132245.

Ross, K. G. (1997). Multilocus evolution in fire ants: Effects of selection, gene flow and recombination. Genetics, 145(4), 961–974.

Ross, K. G., & Keller, L. (1998). Genetic control of social organization in an ant. Proceedings of the National Academy of Sciences, 95(24), 14232–14237.

Ross, K. G., & Keller, L. (2002). Experimental conversion of colony social organization by manipulation of worker genotype composition in fire ants (Solenopsis invicta). Behavioral Ecology and Sociobiology, 51(3), 287–295. https://doi.org/10.1007/s00265-001-0431-5

Ross, K. G., Krieger, M. J. B., & Shoemaker, D. D. (2003). Alternative genetic foundations for a key social polymorphism in fire ants. Genetics, 165(4), 1853–1867.

Ross, K. G., & Shoemaker, D. (2018). Unexpected patterns of segregation distortion at a selfish supergene in the fire ant Solenopsis invicta. BMC Genetics, 19(1), 1–22.

Sandler, B. H., Nikonova, L., Leal, W. S., & Clardy, J. (2000). Sexual attraction in the silkworm moth: Structure of the pheromone-binding-protein-bombykol complex. Chemistry & Biology, 7(2), 143–151.

Schurch, N. J., Schofield, P., Gierliński, M., Cole, C., Sherstnev, A., Singh, V., ... Barton, G. J. (2016). How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use? RNA, 22(6), 839–851.

Shanbhag, S., Müller, B., & Steinbrecht, R. (2000). Atlas of olfactory organs of Drosophila melanogaster 1. Types, external organization, innervation and distribution of olfactory sensilla. Arthropod Structure and Development, 29(1999), 211–229.

Shoemaker, D. D., Ahrens, M., Sheill, L., Mescher, M., Keller, L., & Ross, K. G. (2003). Distribution and prevalence of Wolbachia infections in native populations of the fire ant Solenopsis invicta (Hymenoptera: Formicidae). Environmental Entomology, 32(6), 1329–1336.

Smith, C. A., Roeszler, K. N., Ohnesorg, T., Cummins, D. M., Farlie, P. G., Doran, T. J., & Sinclair, A. H. (2009). The avian Z-linked gene DMRT1 is required for male sex determination in the chicken. Nature, 461(7261), 267–271.

Stolle, E., Pracana, R., Howard, P., Paris, C. I., Brown, S. J., Castillo-Carrillo, C., ... Wurm, Y. (2019). Degenerative expansion of a young supergene. Molecular Biology and Evolution, 36(3), 553–561.

Taber, S. W. (2000). Fire ants. 1st ed. College Station, TX: Texas A&M University Agriculture. Series; no. 3.

Thorvaldsdóttir, H., Robinson, J. T., & Mesirov, J. P. (2013). Integrative Genomics Viewer (IGV): High-performance genomics data visualization and exploration. Briefings in Bioinformatics, 14(2):178-192.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D. R., ... Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nature Protocols, 7(3), 562–578.

Trible, W., & Ross, K. G. (2016). Chemical communication of queen supergene status in an ant. Journal of Evolutionary Biology, 29(3), 502–513.

Van den Berg, M. J., & Ziegelbeger, G. (1991). On the function of the pheromone binding protein in the olfactory hairs of Antheraea polyphemus. Journal of Insect Physiology, 37(1), 79–85. https://doi.org/10.1016/0022-1910(91)90022-R

van’t Hof, A. E., Campagne, P., Rigden, D. J., Yung, C. J., Lingley, J., Quail, M. A., ... & Saccheri, I. J. (2016). The industrial melanism mutation in British peppered moths is a transposable element. Nature, 534(7605), 102–105.

Vogt, R. G., & Riddiford, L. M. (1981). Pheromone binding and inactivation by moth antennae. Nature, 293(5828), 161–163. https://doi.org/10.1038/293161a0

Wang, J., Ross, K. G., & Keller, L. (2008). Genome-wide expression patterns and the genetic architecture of a fundamental social trait. PLoS Genetics, 4(7), e1000127. https://doi.org/10.1371/journal.pgen.1000127

Wang, J., Wurm, Y., Nipitwattanaphon, M., Riba-Grognuz, O., Huang, Y.-C., Shoemaker, D., & Keller, L. (2013). A Y-like social chromosome segregates at a selfish Y-locus genotype in fire ants. Nature, 493(7434), 664–668. https://doi.org/10.1038/nature11832

Wojtasek, H., & Leal, W. S. (1999). Degradation of an alkaloid pheromone from the pale-brown chafer, Phillopertha diversa (Coleoptera: Scarabaeidae), by an insect olfactory cytochrome P450. FEBS Letters, 458(3), 333–336.

Wurm, Y., Wang, J., Riba-Grognuz, O., Corona, M., Nygaard, S., Hunt, B. G., ... Keller, L. (2011). The genome of the fire ant Solenopsis invicta. Proceedings of the National Academy of Sciences, 108(14), 5679–5684.
Zhang, H., Finiguerra, M., Dam, H. G., Huang, Y., Xu, D., Liu, G., & Lin, S. (2013). An improved method for achieving high-quality RNA for copepod transcriptomic studies. *Journal of Experimental Marine Biology and Ecology*, 446, 57–66. https://doi.org/10.1016/j.jembe.2013.04.021

Zhang, W., Wanchoo, A., Ortiz-Urquiza, A., Xia, Y., & Keyhani, N. O. (2016). Tissue, developmental, and caste-specific expression of odorant binding proteins in a eusocial insect, the red imported fire ant, *Solenopsis invicta*. *Scientific Reports*, 6, 35452. https://doi.org/10.1038/srep35452

Zhao, Y., Wang, F., Zhang, X., Zhang, S., Guo, S., Zhu, G., ... Li, M. (2016). Transcriptome and expression patterns of chemosensory genes in antennae of the parasitoid wasp *Chouioia cunea*. *PLoS ONE*, 11(2), e0148159. https://doi.org/10.1371/journal.pone.0148159

Zhu, J., Iovinella, I., Dani, F. R., Liu, Y.-L., Huang, L.-Q., Liu, Y., ... Wang, G. (2016). Conserved chemosensory proteins in the proboscis and eyes of Lepidoptera. *Int. J. Biol. Sci.*, 12(11), 1394–1404.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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