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Social environment affects sensory gene expression in ant larvae

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Abstract

Social insects depend on communication to regulate social behaviour. This also applies to their larvae, which are commonly exposed to social interactions and can react to social stimulation. However, how social insect larvae sense their environment is not known. Using RNAseq, we characterized expression of sensory-related genes in larvae of the ant *Formica fusca*, upon exposure to two social environments: isolation without contact to other individuals, and stimulation via the presence of other developing individuals. Expression of key sensory-related genes was higher following social stimulation, and larvae expressed many of the same sensory-related genes as adult ants and larvae of other insects, including genes belonging to the major insect chemosensory gene families. Our study provides first insights into the molecular changes associated with social information perception in social insect larvae.

Keywords: social insects, transcriptome, communication, chemosensory proteins, odorant binding proteins, odorant receptors.

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Introduction

Social insects rely on chemical communication to maintain colony cohesion and to manage cooperative tasks. They do this using signature mixtures on the cuticle (cuticular hydrocarbons, CHCs) (Sharma *et al*., 2015; Neupert *et al*., 2018; Ferguson *et al*., 2020), and gland secretions that act in specific contexts (Le Conte *et al*., 1990; Czaczkes *et al*., 2014). The major insect chemosensory gene families include odorant binding proteins (OBPs), chemosensory proteins (CSPs), olfactory receptors (ORs), gustatory receptors (GRs) and ionotropic receptors (IRs) (Sánchez-Gracia *et al*., 2009; Joseph and Carlson, 2015). OBPs and CSPs bind and carry odorants (Steinbrecht, 1998; Pelosi *et al*., 2005) whereas the receptors (ORs, GRs, IRs) detect odorants (Missbach *et al*., 2014; Fleischer *et al*., 2018). In ants, several chemosensory-related gene families have undergone large expansions (Engsontia *et al*., 2015; McKenzie *et al*., 2016; McKenzie and Kronauer, 2018) and some genes have evolved specific functions related to social behaviour. For example, CSPs play an important role in nestmate recognition (Ozaki *et al*., 2005; Kulmuni and Havukainen, 2013) and some ORs respond to specific CHCs (Pask *et al*., 2017; Slone *et al*., 2017).

The larvae of social insects are largely protected from environmental fluctuations and pressures such as foraging and predation, factors that shape the lives of solitary insect larvae. However, social insect larvae are constantly exposed to social interactions with adults and other developing individuals and can react to social stimulation by showing developmental responses or by adjusting their behaviour (Schultner *et al*., 2017). How social insect larvae sense their social environment is not well understood. In particular, nothing is known about how larval sensory gene expression is affected by changes in the social environment, although individual recognition abilities may be primed during development (Isingrini *et al*., 1985; Signorotti *et al*., 2014).

Here, we present the first characterization of sensory-related gene expression in larvae of a social insect. We use the common black ant, *Formica fusca*, a species that exhibits precise discrimination abilities (Helanterä and Sundström, 2007; Helanterä and Ratnieks, 2009;

Chernenko *et al*., 2011; Helanterä *et al*., 2011; Martin *et al*., 2011). In *Formica* ant colonies, brood (eggs, larvae, pupae) are kept in piles, which allows larvae to consume eggs. Surprisingly, egg consumption is not random: larvae consume foreign eggs more often than familiar eggs (Schultner *et al*., 2013; 2014), and social parasite eggs more often than nonparasite eggs (Pulliainen *et al*., 2019). As *Formica* eggs carry CHC odour profiles, which can vary depending on traits such as maternity, colony origin and species (Schultner *et al*., 2013; Helanterä and D'Ettorre, 2014), it is likely that larvae use chemical information to adjust their egg consumption behaviour. The underlying recognition processes are predicted to be modulated by the expression of sensory genes. To address this question, we identified genes involved in larval sensory perception by comparing gene expression of larvae subjected to two social environments: isolation without contact to others and stimulation via the presence of other developing individuals. Gene expression was compared with particular focus on candidate sensory-related genes identified from the literature. We predict that ant larvae express many of the same sensory-related genes as adults, and that gene expression is affected by the social environment larvae encounter. In particular, we assume that expression of sensory-related genes is higher in larvae subjected to stimulation compared with larvae kept in isolation. Our results provide insights into the molecular machinery of social cue perception in social insect larvae.

Results

Across all samples, 13 169 unigenes were expressed. Of the 10 unigenes with the highest read count in each treatment, eight overlapped between the two treatments (Tables S3 and S4), and were enriched for the gene ontology (GO) terms ribosome, structural constituent of cuticle, structural constituent of ribosome and ribosomal small subunit assembly.

Of the 527 candidate sensory gene sequences identified from the literature, we found matches for 73 (13.8%). Seventeen sequences received single hits, whereas 15 sequences received hits from multiple candidate sequences (2–8 hits each, 56 candidate sequences in total). Of these 56 candidate sequences, 28 were copies or isoforms of the same gene and the remaining 27 represented genes from the same gene family with similar sequences. From the 15 sequences that received multiple hits, we picked the best hit according to match percentage and sequence length, resulting in 32 candidate sensory genes expressed in our samples (Fig. 1, Table S5).

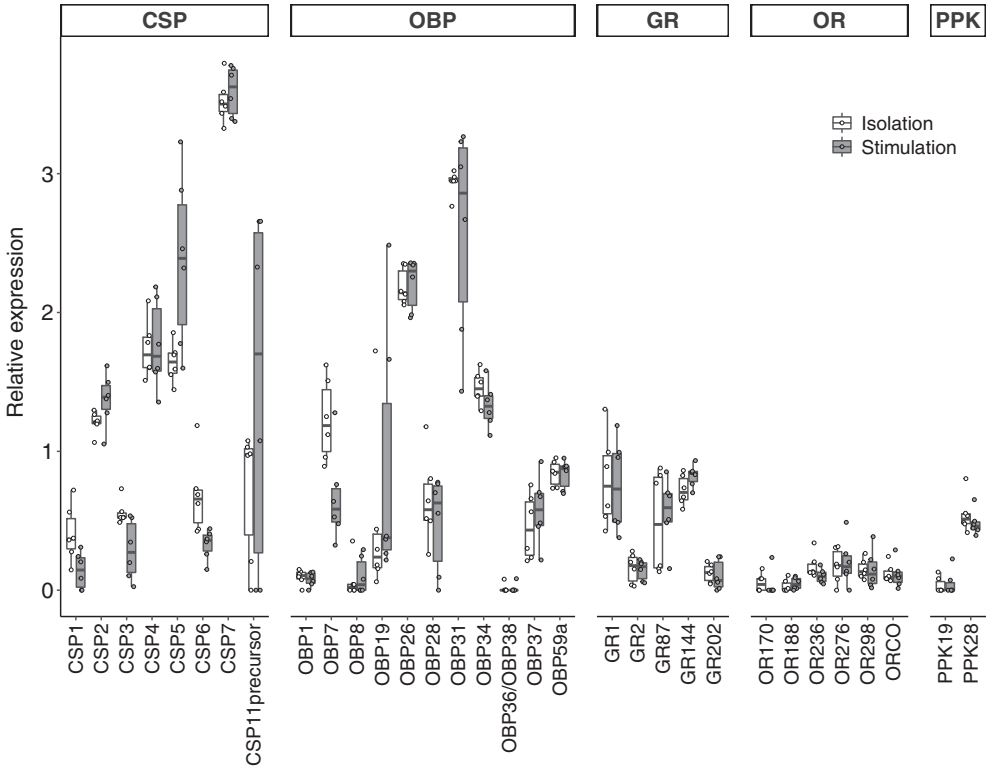

Figure 1. Candidate sensory genes from five gene families expressed in *Formica fusca* ant larvae subjected to two social treatments. For better visualization, expression is given as log(fragments per kilobase million FPKM +1). CSP, chemosensory proteins; GR, gustatory receptors; OBPs, odorant binding proteins; OR, odorant receptors; PPK, pickpocket proteins.

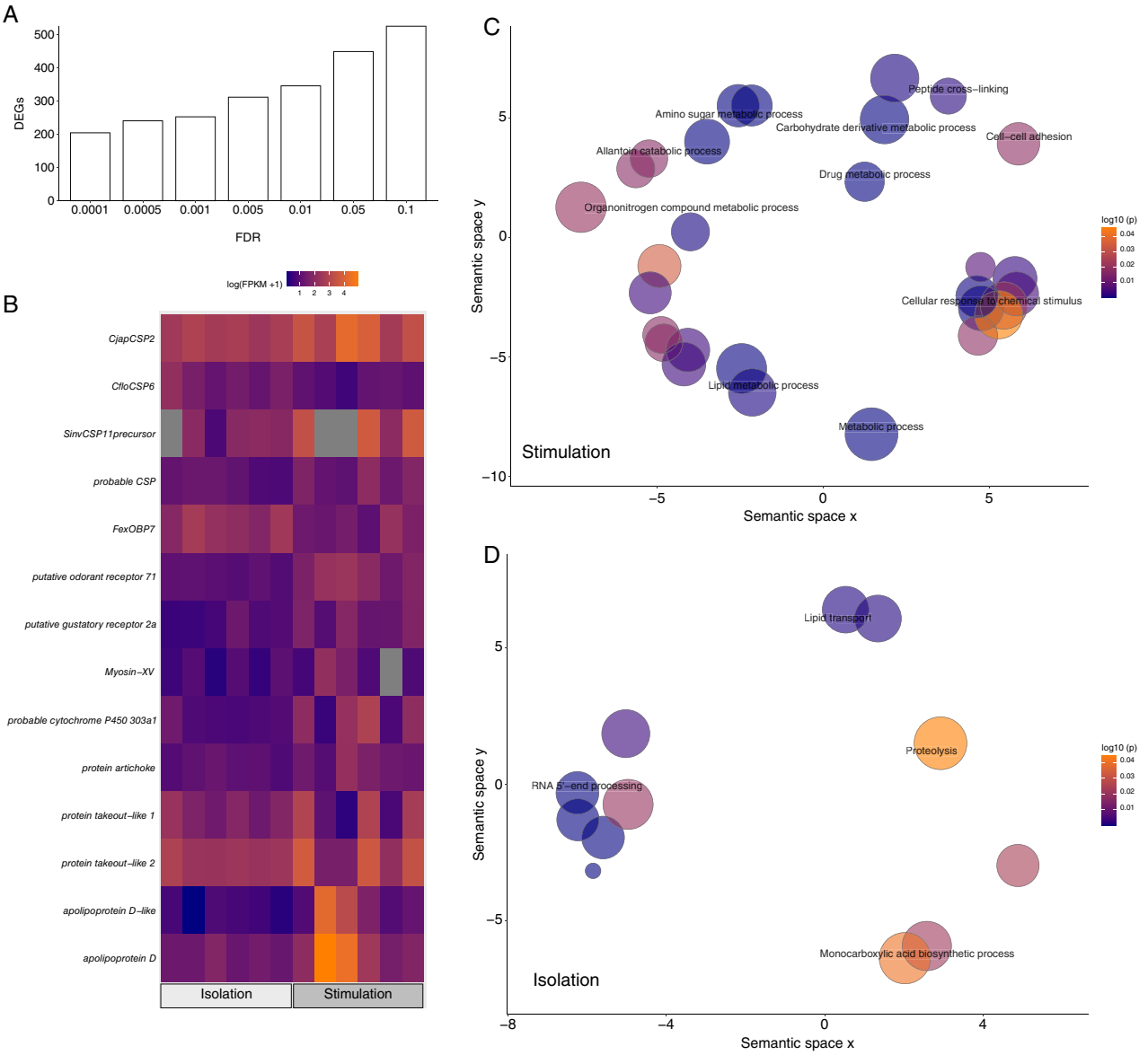

Figure 2. Differential gene expression in *Formica fusca* ant larvae subjected to two social treatments. (A) Number of differentially expressed genes (DEGs) between treatments according to false discovery rate (FDR), (B) Heat map of differentially expressed candidate sensory genes (selected from the literature, or because they were among the sensory genes showing the strongest expression differences between treatments; grey squares depict samples where FPKM = 0, ie, samples in which the gene was not expressed), (C) Gene ontology terms enriched in transcriptomes of *F. fusca* larvae subjected to stimulation treatment, (D) Gene ontology terms enriched in transcriptomes of *F. fusca* larvae subjected to isolation treatment.

Compared with the mean expression levels across all unigenes (isolation: mean = 26 FPKM, range = 0–34 178; stimulation: mean = 27, range = 0–50 142), the 32 candidate sensory genes were expressed at higher levels on average, but maximum expression levels were much lower (isolation: mean = 150, range = 0–6233; stimulation: mean = 192, range = 0–6011).

We found 448 unigenes that were differentially expressed (DEGs) between the two treatments at false discovery rate (FDR) < 0.05. This number changed marginally when applying more or less stringent statistical criteria and is thus representative for the data set (Fig. 2A). 19.9% (89/448) of DEGs were associated with genes of unknown function and 6.3% (23/448) received no significant BLAST hit. Of the 448 DEGs, 382 (2.9% of 13 169 total expressed unigenes) were overexpressed in stimulated larvae compared with isolated larvae while 66 unigenes (0.5%) were overexpressed in isolated larvae (see Table S6 for a full list of DEGs). Overexpressed unigenes in stimulated larvae were enriched for GO terms relating to chemosensory

perception, including cellular response to chemical stimulus (Fig. 2C, see Table S7 for a full list of GO terms). GO terms of overexpressed genes in isolated larvae showed enrichment for lipid transport and RNA 5′-end processing (Fig. 2D).

Among the chemosensory unigenes overexpressed in stimulated larvae, we found three belonging to the CSP gene family (*CSP5*, *CSP11precursor*, *probable CSP*), one putative OR (*putative odorant receptor 71a*) and one putative GR (*putative gustatory receptor 2a*). Other unigenes that are known to be involved in sensory perception were also overexpressed in stimulated larvae, including *apolipoprotein d*, *apolipoprotein d-like*, *Myosin-XV*, *probable cytochrome P450 303a1*, *protein artichoke* and two *protein takeout-like* genes (Fig. 2B). Isolated larvae showed overexpression of one CSP (*CSP6*) and one OBP (*OBP7*) (Fig. 2B). Four of the differentially expressed sensory genes overlapped with those identified from the literature (*CSP5*, *CSP6*, *OBP7*, *CSP11precursor*).

Comparison of expression of 40 sensory genes across insects (Table S2) revealed that 45% (18/40) were exclusively expressed in *F. fusca* larvae. Conversely, 32.5% (13/40) were expressed in larvae of all three ant species, including four CSPs (*CSP2*, *CSP5*, *CSP7*, *CSP11precursor*), two OBPs (*OPB7*, *OBP26*), three GRs (*GR1*, *GR87*, *GR202*) and two ORs (*OR170*, *ORCO*). An additional five genes were commonly expressed in *F. fusca* and *Monomorium pharaonis* larvae, whereas four others were commonly expressed in both *F. fusca* and *Cardiocondyla obscurior* larvae. Only 10% (4/40) of genes were expressed across Hymenoptera and a single gene – *ORCO* – was expressed in larvae from all species.

Discussion

To date, few studies have investigated larval gene expression in ants. We found that *F. fusca* larvae express roughly the same number of genes as larvae of three other ant species [*Camponotus floridanus*: 15 631 genes (pooled adults and larvae), (Gupta *et al*., 2015); *C. obscurior*: 10 012 genes, (Schrader *et al*., 2015); *M. pharaonis*: 10 446 genes, (Warner *et al*., 2019)] and adult *F. fusca* ants [9859 genes, (Morandin *et al*., 2016)]. The most highly expressed genes across all samples were related to ribosome and cuticle structure and function, which agrees with results from other transcriptome studies of insect larvae (Harrison *et al*., 2015).

As predicted, overexpressed genes in stimulated larvae were enriched for GO terms relating to chemosensory perception. Three genes from the CSP gene family, known to bind odorant compounds in social insects (Ishida *et al*., 2002; Kulmuni and Havukainen, 2013; McKenzie *et al*., 2014; Hojo *et al*., 2015), were overexpressed in stimulated larvae (*CSP5*, *CSP11precursor*, *probable CSP*). *CSP5* is expressed in the antennae of *Oocerea biroi* ants (McKenzie *et al*., 2014) but its chemosensory functions are debated (Kulmuni and Havukainen, 2013). The specific functions of *CSP11precursor* and *probable CSP* are not known, but since *CSP11precursor* expression was not consistent across samples from the stimulation treatment, we cannot be sure that it plays a role in larval chemosensory perception. We also found overexpression of genes belonging to the OR and GR gene families, as well as two *protein takeout-like* genes that were overexpressed under stimulation. In adult blowflies and silkworm larvae, a *takeout-like* gene is expressed in chemosensory organs (Fujikawa *et al*., 2006; Yoshizawa *et al*., 2011). Similarly, *protein artichoke*, a gene which is required for normal morphology and function of sensory organs in *Drosophila* embryos (Andrés *et al*., 2014) was overexpressed in stimulated larvae.

Several other sensory genes were overexpressed in stimulated larvae, including *apolipoprotein d*, *apolipoprotein d-like*, *probable cytochrome P450 303a1* and *Myosin XV*. *Apolipoprotein d* and *apolipoprotein d-like* were among the most overexpressed genes in stimulated larvae, and act in lipid binding and transport, both of which are crucial steps in the chemosensory process. The related apolipoprotein *apolipophorin III* is a major antennal protein in the fire ant (Guntur *et al*., 2004), and *apolipophorin I* and *apolipophorin II* carry CHCs in termites (Sevala *et al*., 2000). *Probable cytochrome P450 303a1* codes for a cytochrome P450, a protein that is expressed in sensory bristles in *Drosophila melanogaster* (Willingham and Keil, 2004). We found differential expression in eight other cytochrome P450 genes, which are known to be expressed in insect sensory organs and are related to odorant detection (Wang *et al*., 1999; Maïbèche-Coisne *et al*., 2002; 2005) and maintenance of olfactory sensitivity (Wang *et al*., 2008). Finally, *Myosin XV* is associated with sound perception in mammals (Libby and Steel, 2000), and a homologue in *D. melanogaster*, *Sisyphus*, transports key sensory proteins (Liu *et al*., 2008). Albeit speculative, these results suggest a possible role in acoustic communication, a widespread phenomenon in ants (Hunt and Richard, 2013; Schönrogge *et al*., 2017). Overall, the increase in expression of genes with putative sensory functions following manipulation of social environment appear to validate their role in sensory perception in ants, but whether these genes also act in other biological processes in larvae remains to be studied.

Compared with stimulated larvae, fewer genes were overexpressed in isolated larvae, which may be indicative of a transcriptional shutdown due to the stress of isolation. Indeed, isolation is not a natural state for ant larvae, and stress of isolation has been shown to cause changes in gene expression in adult fruit flies (Zhou *et al*., 2009). At the same time, overexpressed genes in isolated larvae

were enriched for GO terms related to RNA processing, suggesting increased transcriptional activity. In addition, isolated larvae expressed roughly the same number of genes as stimulated larvae, and the most highly expressed genes showed significant overlap between treatments, confirming that isolation did not have significant negative effects on larval gene expression. Finally, the chemosensory genes *CSP6* and *OBP7* were overexpressed in isolated larvae. *CSP6* is also expressed in adult ant antennae (McKenzie *et al*., 2014), and is likely to bind CHCs. Thus, while our data do not allow us to discern whether changes in gene expression reflect a transcriptomic response to stimulation, or rather a response to lack of stimulation, they clearly show that social environment influences larval gene expression.

F. fusca ant larvae expressed some of the same sensory-related genes as adult ants and other insect larvae. For example, larvae expressed eight CSPs, whereas 12 CSPs (six of which overlapped with our data) are expressed in adults and pupae of the closely related ant *Formica exsecta* (Dhaygude *et al*., 2017). Similarly, six ORs were expressed in larvae; orthologs of five of these ORs have been shown to respond to odorants in antennae of *Harpegnathos saltator* worker ants (Pask *et al*., 2017; Slone *et al*., 2017). The sixth gene, *ORCO*, is a conserved, insect-specific odorant co-receptor subunit necessary for the function of the OR complex (Brand *et al*., 2018), which plays a critical role in social behaviour in ants (Trible *et al*., 2017; Yan *et al*., 2017). We also detected expression of 11 OBPs, whereas only three OBPs were expressed in *F. exsecta* pupae (Dhaygude *et al*., 2017). This indicates development-stage specificity in sensory gene expression, as has been found in *D. melanogaster* (Fishilevich and Vosshall, 2005; Zhou *et al*., 2009). We furthermore detected five GRs, which encode gustatory receptors in *Drosophila* larvae (Scott *et al*., 2001), as well as two pickpocket genes (PPKs): *PPK19* and *PPK28*. The *D. melanogaster* homologues *PPK23* and *PPK29* modulate responses to larval aggregation pheromones, and knockdown prevents larvae from detecting social cues (Mast *et al*., 2014). PPKs have not been studied in ants, but our results suggest that they may play a role in larval chemosensory perception.

Only few of the chemosensory genes identified in our study were commonly expressed across ant, honeybee and fruit fly larvae. This is probably due to sequence divergence between lineages, as most of the sequences were identified from studies on ant gene expression, and insects are known to vary widely in the number and sequence divergence of genes from gene families involved in sensory perception (Robertson and Wanner, 2006; Engsontia *et al*., 2008; Brand and Ramírez, 2017; Slone *et al*., 2017; McKenzie and Kronauer, 2018). This study is therefore only a first step in understanding larval perception in social insects, with three potential avenues for future research. First, the whole-body transcriptomes generated for this study do not allow inference about the localization of chemosensory gene expression. Ant larvae have two antennae, each of which possesses 1–5 olfactory sensillae (Wheeler and Wheeler, 1976). Whether sensory gene expression in ant larvae is restricted to these sensillae, or also occurs in sensory-specific organs similar to those found in *Drosophila* larvae (Joseph and Carlson, 2015), remains to be shown. Second, individual larval traits such as development stage, caste and sex may influence perception abilities. In *Formica* ants, male larvae are more likely to cannibalize related eggs than female larvae (Schultner *et al*., 2013; 2014), but it is unknown whether this is linked to differences in egg recognition abilities. While we could not confirm the sex of individual larvae from our data as this requires information about sex-specific gene expression, sex ratio estimates from previous studies were female-biased (Schultner *et al*., 2014; Pulliainen *et al*., 2019). We thus assume that the samples used in this study included no or few males, and that the results are not influenced by sex-specific differences in larval perception. Understanding how perception varies with larval sex will require separate testing of male and female larvae. Finally, species-level traits are likely to be associated with larval perception abilities. For instance, plasticity of gene expression can be expected to differ between ants, bees and wasps because bee and wasp larvae are reared in individual cells, while ants rear brood in piles. As a result, ant larvae are in close contact with nestmates, providing ample opportunities for social interactions (Schultner *et al*., 2017), which likely shape larval sensory biology. Comparative studies across a range of social life histories will reveal how social environment influences the sensory biology of larvae. Together, this will shed new light on the evolution of communication in social insects.

Experimental procedures

Collection and experimental setup

We collected colonies of *F. fusca* ants (n = 6) containing one queen and workers (>200) in southwestern Finland (59°540 46.30 N, 23°150 55.90 E) in April 2016, coinciding with the end of hibernation. Colonies were transferred to nest boxes and kept in the dark at +4 °C for ∼10 days, a well-established procedure used to synchronize the onset of egg laying [Ozan *et al*., 2013]. Nests were then brought to room temperature, and fed with a diet based on honey and eggs adapted from (Bhatkar and Whitcomb, 1970) and watered daily.

We monitored egg laying and hatching, removed young larvae (1–3 days post hatching) from nests, and size-matched them visually. The number of larval instars in *F. fusca* is unknown, but related species exhibit 3–4 instars (Solis *et al*., 2010). Based on visual inspection of larvae, only young, ie, first or second instar larvae, were included in the experiments. Each larva was placed on a Petri dish lined with sponge cloth to maintain moisture. Larvae

were either isolated without contact to others ('isolation') or kept in a pile with four size-matched nestmate larvae and five nestmate eggs ('stimulation'). To avoid sampling effects, treatments were set up in parallel with size-matched larvae over 3 days. To avoid potential effects of starvation on larval gene expression, larvae were isolated for 24 h in accordance with a previous study showing that *Formica* larvae isolated with five eggs survived for ~2.5 days when starved (Schultner *et al*., 2013). After 24 h, six larvae from each treatment were placed in individual Eppendorf tubes with 200 μl Trisure (Bioline) and stored at −80 °C until RNA extraction. As *F. fusca* larvae are known to cannibalize eggs (Schultner *et al*., 2014; Pulliainen *et al*., 2019), we only sampled larvae for the stimulation treatment if all nestmate larvae and eggs could be accounted for after the 24 h period. To minimize effects caused by inter-colony variation, we aimed to collect colony-matched larvae, ie, larvae from the same colonies, for the two treatments. In addition, we attempted to avoid pseudo-replication by sampling larvae from independent colonies. As this was not always possible, the final sampling included larvae from five colonies in the isolation treatment and larvae from four colonies in the stimulation treatment, with three colonies overlapping between treatments (Table S1). We checked whether pseudo-replication affected the results but found no obvious effect of colony origin (Figure S1) and the logFC values of all colonies were highly correlated, meaning that they had the same effects overall.

Transcriptome assembly

The samples were sequenced on five lanes of an Illumina HiSeqTM 2500 2 × 100 bp (~400 M paired-end reads for each lane). Detailed descriptions of the RNA extraction and library construction protocols, and the *de novo* transcriptome assembly and annotation pipelines can be found in (Morandin *et al*., 2018). In brief, after reads were checked for quality, we used Trinity (Grabherr *et al*., 2013) with a combination of *de novo* assembly and genome-guided assembly to construct the *de novo* transcriptome. Contigs were filtered to keep only coding sequences, to obtain a set of nonredundant contigs and to remove low-quality contigs and probable exogenous RNAs known to be abundant in social insect *de novo* transcriptomes (Johansson *et al*., 2013). The final assembly included 24 765 unigenes. The paired-end reads were mapped to the final assembly using RSEM (Li and Dewey, 2011), and Bowtie2 (Langmead and Salzberg, 2012).

Differential gene expression and GO term enrichment analysis

Read counts were used in differential gene expression analysis with EdgeR (Robinson *et al*., 2009). For comparison of the two treatments, we filtered out transcripts with very low read counts by removing loci with reads <1 per kilobase of exons per million fragments (FPKM) mapped in at least half of the sequenced libraries. We found 23 149 unigenes expressed in stimulated larvae, 13 440 (58%) of which had a FPKM count >1 in at least half of the samples. In isolated larvae, 21 919 unigenes were expressed, 14 493 (66%) of which had a FPKM count >1 in at least half of the samples. TMM normalization was applied to account for compositional differences between libraries, and expression differences were considered significant at a FDR < 0.05. GOstat (Beißbarth and Speed, 2004) was used for gene ontology (GO) term enrichment analysis on differentially expressed gene sets, using all genes with GO terms as the universe. GO terms were plotted in a scatterplot with semantic space, clustering semantically similar GO terms after reducing redundant terms with REVIGO (Supek *et al*., 2011) (displaying terms with dispensability of <0.15).

Candidate sensory genes

From the literature on insect chemosensory biology (Scott *et al*., 2001; Ishida *et al*., 2002; Fishilevich *et al*., 2005; Kreher *et al*., 2005; Ozaki *et al*., 2005; Kulmuni and Havukainen, 2013; Mast *et al*., 2014; McKenzie *et al*., 2014; Hojo *et al*., 2015; De Carvalho *et al*., 2017; Pask *et al*., 2017; Slone *et al*., 2017), we chose candidate sensory genes, ie, genes we suspected *a priori* to be expressed in larvae, because they have been identified in ants or other insect larvae (File S1). We identified 527 sequences representing 371 genes (several isoforms were included and/or sequences from several species were used), which belong to gene families associated with sensory perception: chemosensory proteins (CSPs), odorant binding proteins (OBPs), odorant receptors (ORs), gustatory receptors (GRs), ionotropic receptors (IRs) and pickpocket proteins (PPKs). We searched for these genes in the larval transcriptomes using BLAST and visualized their expression in the two treatments.

Larval sensory gene expression across species

We compared the transcriptomes of *F. fusca* larvae with larval transcriptomes from two ants [*C. obscurior*, (Schrader *et al*., 2015); *M. pharaonis* (Warner *et al*., 2017)], the honey bee *Apis mellifera* (Cameron *et al*., 2013; Ashby *et al*., 2016), and the fruit fly *D. melanogaster* (extracted from the GEO Profiles database (Barrett *et al*., 2013), accession GSM3285207). We focused on the candidate sensory genes identified from the literature that were expressed in our data (8.6%, 32 of 371 genes), as well as on a subset of eight unigenes identified from the differential expression analysis (see Table S2 for all genes used in species comparison) and searched for these 40 genes in all transcriptomes using BLAST. To estimate how conserved larval sensory gene expression is across insects, we calculated the proportion of commonly expressed genes in Formicidae (*F. fusca*, *C. obscurior*, *M. pharaonis*), Hymenoptera (Formicidae + *A. mellifera*) and Holometabola (Hymenoptera + *D. melanogaster*).

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Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

References

Andrés, M., Turiégano, E., Göpfert, M.C., Canal, I. and Torroja, L. (2014) The extracellular matrix protein artichoke is required for integrity of ciliated mechanosensory and chemosensory organs in *Drosophila* embryos. *Genetics*, **196**, 1091–1102.

Ashby, R., Forêt, S., Searle, I. and Maleszka, R. (2016) MicroRNAs in Honey Bee Caste Determination. *Scientific Reports*, **6**, 18794.

Barrett, T., Wilhite, S.E., Ledoux, P., Evangelista, C., Kim, I.F., Tomashevsky, M. *et al*. (2013) NCBI GEO: archive for functional genomics data sets - update. *Nucleic Acids Research*, **41**, 991–995.

Beißbarth, T. and Speed, T.P. (2004) GOstat: find statistically overrepresented Gene Ontologies with a group of genes. *Bioinformatics*, **20**, 1464–1465.

Bhatkar, A. and Whitcomb, W. (1970) Artificial diet for rearing various species of ants. *The Florida Entomologist*, **53**, 229–232.

Brand, P. and Ramírez, S.R. (2017) The evolutionary dynamics of the odorant receptor gene family in corbiculate bees. *Genome Biology and Evolution*, **9**, 2023–2036.

Brand, P., Robertson, H., Lin, W., Pothula, R., Klingeman, W., Jurat-Fuentes, J.L. *et al*. (2018) The origin of the odorant receptor gene family in insects. *eLife*, **7**, e38340.

Cameron, R.C., Duncan, E.J. and Dearden, P.K. (2013) Biased gene expression in early honeybee larval development. *BMC Genomics*, **14**, 903.

Chernenko, A., Helanterä, H. and Sundström, L. (2011) Egg recognition and social parasitism in *Formica* ants. *Ethology*, **117**, 1081–1092.

Czaczkes, T.J., Grüter, C. and Ratnieks, F.L.W. (2014) Trail pheromones: an integrative view of their role in social insect colony organization. *Annual Review of Entomology*, **60**, 581–599.

De Carvalho, W.J., Fujimura, P.T., Bonetti, A.M., Goulart, L.R., Cloonan, K., Da Silva, N.M. *et al*. (2017) Characterization of antennal sensilla, larvae morphology and olfactory genes of *Melipona scutellaris* stingless bee. *PLoS One*, **12**, 1–22.

Dhaygude, K., Trontti, K., Paviala, J., Morandin, C., Wheat, C., Sundström, L. *et al*. (2017) Transcriptome sequencing reveals high isoform diversity in the ant *Formica exsecta*. *PeerJ*, **5**, e3998.

Engsontia, P., Sanderson, A.P., Cobb, M., Walden, K.K.O., Robertson, H.M. and Brown, S. (2008) The red flour beetle's large nose: an expanded odorant receptor gene family in *Tribolium castaneum*. *Insect Biochemistry and Molecular Biology*, **38**, 387–397.

Engsontia, P., Sangket, U., Robertson, H.M. and Satasook, C. (2015) Diversification of the ant odorant receptor gene family and positive selection on candidate cuticular hydrocarbon receptors. *BMC Research Notes*, **8**, 1–13.

Ferguson, S.T., Park, K.Y., Ruff, A.A., Bakis, I. and Zwiebel, L.J. (2020) Odor coding of nestmate recognition in the eusocial ant *Camponotus floridanus*. *The Journal of Experimental Biology*, **223**(2), jeb215400.

Fishilevich, E. and Vosshall, L.B. (2005) Genetic and functional subdivision of the *Drosophila* antennal lobe. *Current Biology*, **15**, 1548–1553.

Fishilevich, E., Domingos, A.I., Asahina, K., Naef, F., Vosshall, L. B. and Louis, M. (2005) Chemotaxis behavior mediated by single larval olfactory neurons in *Drosophila*. *Current Biology*, **15**, 2086–2096.

Fleischer, J., Pregitzer, P., Breer, H. and Krieger, J. (2018) Access to the odor world: olfactory receptors and their role for signal transduction in insects. *Cellular and Molecular Life Sciences*, **75**, 485–508.

Fujikawa, K., Seno, K. and Ozaki, M. (2006) A novel takeout-like protein expressed in the taste and olfactory organs of the blowfly, *Phormia regina*. *FEBS Journal*, **273**, 4311–4321.

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I. *et al*. (2013) Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nature Biotechnology*, **29**, 644–652.

Guntur, K.V.P., Velasquez, D., Chadwell, L., Carroll, C., Weintraub, S., Cassill, J.A. *et al*. (2004) Apolipophorin-III-like protein expressed in the antenna of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Archives of Insect Biochemistry and Physiology*, **57**, 101–110.

Gupta, S.K., Kupper, M., Ratzka, C., Feldhaar, H., Vilcinskas, A., Gross, R. *et al*. (2015) Scrutinizing the immune defence inventory of *Camponotus floridanus* applying total transcriptome sequencing. *BMC Genomics*, **16**, 540.

Harrison, M.C., Hammond, R.L. and Mallon, E.B. (2015) Reproductive workers show queenlike gene expression in an intermediately eusocial insect, the buff-tailed bumble bee *Bombus terrestris*. *Molecular Ecology*, **24**, 3043–3063.

Helanterä, H. and D'Ettorre, P. (2014) A comparative study of egg recognition signature mixtures in *Formica* ants. *Evolution*, **69**, 520–529.

Helanterä, H. and Ratnieks, F.L.W. (2009) Two independent mechanisms of egg recognition in worker *Formica fusca* ants. *Behavioral Ecology and Sociobiology*, **63**, 573–580.

Helanterä, H. and Sundström, L. (2007) Worker policing and nest mate recognition in the ant *Formica fusca*. *Behavioral Ecology and Sociobiology*, **61**, 1143–1149.

Helanterä, H., Lee, Y.R., Drijfhout, F.P. and Martin, S.J. (2011) Genetic diversity, colony chemical phenotype, and nest mate recognition in the ant *Formica fusca*. *Behavioral Ecology*, **22**, 710–716.

Hojo, M.K., Ishii, K., Sakura, M., Yamaguchi, K., Shigenobu, S. and Ozaki, M. (2015) Antennal RNA-sequencing analysis reveals evolutionary aspects of chemosensory proteins in the carpenter ant, *Camponotus japonicus*. *Scientific Reports*, **5**, 13541.

Hunt, J.H. and Richard, F.J. (2013) Intracolony vibroacoustic communication in social insects. *Insectes Sociaux*, **60**, 403–417.

Ishida, Y., Cornel, A.J. and Leal, W.S. (2002) Identification and cloning of a female antenna-specific odorant-binding protein in the mosquito *Culex quinquefasciatus*. *Journal of Chemical Ecology*, **28**, 867–871.

Isingrini, M., Lenoir, A. and Jaisson, P. (1985) Preimaginal learning as a basis of colony-brood recognition in the ant *Cataglyphis cursor*. *Proceedings of the National Academy of Sciences*, **82**, 8545–8547.

Johansson, H., Dhaygude, K., Lindström, S., Helanterä, H., Sundström, L. and Trontti, K. (2013) A metatranscriptomic approach to the identification of microbiota associated with the ant *Formica exsecta*. *PLoS One*, **8**, e79777.

Joseph, R.M. and Carlson, J.R. (2015) *Drosophila* chemoreceptors: a molecular interface between the chemical world and the brain. *Trends in Genetics*, **31**, 683–695.

Kreher, S.A., Kwon, J.Y. and Carlson, J.R. (2005) The molecular basis of odor coding in the *Drosophila* larva. *Neuron*, **46**, 445–456.

Kulmuni, J. and Havukainen, H. (2013) Insights into the Evolution of the CSP Gene Family through the Integration of Evolutionary Analysis and Comparative Protein Modeling. *PLoS One*, **8**, e6368.

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

Le Conte, Y., Arnold, G., Trouiller, J. and Masson, C. (1990) Identification of a brood pheromone in honeybees. *Naturwissenschaften*, **77**, 334–336.

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

Libby, R.T. and Steel, K.P. (2000) The roles of unconventional myosins in hearing and deafness. *Essays in Biochemistry*, **35**, 159–174.

Liu, R., Woolner, S., Johndrow, J.E., Metzger, D., Flores, A. and Parkhurst, S.M. (2008) Sisyphus, the *Drosophila* myosin XV homolog, traffics within filopodia transporting key sensory and adhesion cargos. *Development*, **135**, 53–63.

Maïbèche-Coisne, M., Jacquin-Joly, E., François, M.C. and Nagnan-Le Meillour, P. (2002) cDNA cloning of biotransformation enzymes belonging to the cytochrome P450 family in the antennae of the noctuid moth *Mamestra brassicae*. *Insect Molecular Biology*, **11**, 273–281.

Maïbèche-Coisne, M., Merlin, C., François, M.C., Porcheron, P. and Jacquin-Joly, E. (2005) P450 and P450 reductase cDNAs from the moth *Mamestra brassicae*: cloning and expression patterns in male antennae. *Gene*, **346**, 195–203.

Martin, S.J., Helanterä, H. and Drijfhout, F.P. (2011) Is parasite pressure a driver of chemical cue diversity in ants? *Proceedings of the Royal Society B: Biological Sciences*, **278**, 496–503.

Mast, J.D., De Moraes, C.M., Alborn, H.T., Lavis, L.D. and Stern, D.L. (2014) Evolved differences in larval social behavior mediated by novel pheromones. *eLife*, **3**, e04205.

McKenzie, S.K. and Kronauer, D.J.C. (2018) The genomic architecture and molecular evolution of ant odorant receptors. *Genome Research*, **28**, 1757–1765.

McKenzie, S.K., Oxley, P.R. and Kronauer, D.J.C. (2014) Comparative genomics and transcriptomics in ants provide new insights into the evolution and function of odorant binding and chemosensory proteins. *BMC Genomics*, **15**, 718.

McKenzie, S.K., Fetter-Pruneda, I., Ruta, V. and Kronauer, D.J.C. (2016) Transcriptomics and neuroanatomy of the clonal raider ant implicate an expanded clade of odorant receptors in chemical communication. *Proceedings of the National Academy of Sciences*, **113**, 14091–14096.

Missbach, C., Dweck, H.K.M., Vogel, H., Vilcinskas, A., Stensmyr, M.C., Hansson, B.S. *et al*. (2014) Evolution of insect olfactory receptors. *eLife*, **3**, 02115.

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

Morandin, C., Pulliainen, U., Bos, N. and Schultner, E. (2018) Data descriptor: De novo transcriptome assembly and its annotation for the black ant *Formica fusca* at the larval stage. *Scientific Data*, **5**, 180282.

Neupert, S., Hornung, M., Grenwille Millar, J. and Kleineidam, C.J. (2018) Learning Distinct Chemical Labels of Nestmates in Ants. *Frontiers in Behavioral Neuroscience*, **12**, 1–12.

Ozaki, M., Ayako, W.-K., Kazuyo, F., Masayuki, I., Fumio, Y., Yuji, S. *et al*. (2005) Ant Nestmate and Non-Nestmate Discrimination by a Chemosensory Sensillum. *Science*, **309**, 311–314.

Ozan, M., Helanterä, H. and Sundström, L. (2013) The value of oviposition timing, queen presence and kinship in a social insect. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 20131231.

Pask, G.M., Slone, J.D., Millar, J.G., Das, P., Moreira, J.A., Zhou, X. *et al*. (2017) Specialized odorant receptors in social insects that detect cuticular hydrocarbon cues and candidate pheromones. *Nature Communications*, **8**, 297.

Pelosi, P., Calvello, M. and Ban, L. (2005) Diversity of odorant-binding proteins and chemosensory proteins in insects. *Chemical Senses*, **30**, i291–i292.

Pulliainen, U., Helanterä, H., Sundström, L. and Schultner, E. (2019) The possible role of ant larvae in the defence against social parasites. *Proceedings of the Royal Society B: Biological Sciences*, **286**, 20182867.

Robertson, H.M. and Wanner, K.W. (2006) The chemoreceptor superfamily in the honey bee, *Apis mellifera*: expansion of the odorant, but not gustatory, receptor family. *Genome Research*, **16**, 1395–1403.

Robinson, M.D., McCarthy, D.J. and Smyth, G.K. (2009) edgeR: a bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, **26**, 139–140.

Sánchez-Gracia, A., Vieira, F.G. and Rozas, J. (2009) Molecular evolution of the major chemosensory gene families in insects. *Heredity*, **103**, 208–216.

Schönrogge, K., Barbero, F., Casacci, L.P., Settele, J. and Thomas, J.A. (2017) Acoustic communication within ant societies and its mimicry by mutualistic and socially parasitic myrmecophiles. *Animal Behaviour*, **134**, 249–256.

Schrader, L., Simola, D., Heinze, J. and Oettler, J. (2015) Sphingolipids, transcription factors and conserved tool kit genes: developmental plasticity in the ant *Cardiocondyla obscurior*. *Molecular Biology and Evolution*, **32**, 1474–1486.

Schultner, E., D'Ettorre, P. and Helanterä, H. (2013) Social conflict in ant larvae: egg cannibalism occurs mainly in males and larvae prefer alien eggs. *Behavioral Ecology*, **24**, 1306–1311.

Schultner, E., Gardner, A., Karhunen, M. and Helanterä, H. (2014) Ant larvae as players in social conflict: relatedness and

individual identity mediate cannibalism intensity. *The American Naturalist*, **184**, E161–E174.

Schultner, E., Oettler, J. and Helanterä, H. (2017) The role of brood in eusocial Hymenoptera. *The Quarterly Review of Biology*, **92**, 39–78.

Scott, K., Brady, R., Cravchik, A., Morozov, P., Rzhetsky, A., Zuker, C. *et al*. (2001) A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell*, **104**, 661–673.

Sevala, V., Bagnères, A.-G., Kuenzli, M., Blomquist, G.J. and Schal, C. (2000) Cuticular hydrocarbons of the dampwood termite, *Zootermopsis nevadensis*: caste differences and the role of lipophorin in the transport of hydrocarbons and hydrocarbon metabolites. *Journal of Chemical Ecology*, **26**, 765–789.

Sharma, K.R., Enzmann, B.L., Schmidt, Y., Moore, D., Jones, G. R., Parker, J. *et al*. (2015) Cuticular Hydrocarbon Pheromones for Social Behavior and Their Coding in the Ant Antenna. *Cell Reports*, **12**, 1261–1271.

Signorotti, L., Jaisson, P. and D'Ettorre, P. (2014) Larval memory affects adult nest-mate recognition in the ant *Aphaenogaster senilis*. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20132579.

Slone, J.D., Pask, G.M., Ferguson, S.T., Millar, J.G., Berger, S.L., Reinberg, D. *et al*. (2017) Functional characterization of odorant receptors in the ponerine ant, *Harpegnathos saltator*. *Proceedings of the National Academy of Sciences*, **114**, 8586–8591.

Solis, D.R., Fox, E.G.P., Kato, L.M., de Jesus, C.M., Yabuki, A.T., Campos, A.E.D.C. *et al*. (2010) Morphological description of the immatures of the ant, *Monomorium floricola*. *Journal of Insect Science*, **10**, 15.

Steinbrecht, R. (1998) Odorant-binding proteins: expression and function. *Annals of the New York Academy of Sciences*, **855**, 323–332.

Supek, F., Bošnjak, M., Škunca, N. and Šmuc, T. (2011) Revigo summarizes and visualizes long lists of gene ontology terms. *PLoS One*, **6**(7), e21800.

Trible, W., Olivos-Cisneros, L., McKenzie, S.K., Saragosti, J., Chang, N.C., Matthews, B.J. *et al*. (2017) orco mutagenesis causes loss of antennal lobe glomeruli and impaired social behavior in ants. *Cell*, **170**, 727–735.e10.

Wang, Q., Hasan, G. and Pikielny, C.W. (1999) Preferential expression of biotransformation enzymes in the olfactory organs of *Drosophila melanogaster*, the antennae. *Journal of Biological Chemistry*, **274**, 10309–10315.

Wang, L., Dankert, H., Perona, P. and Anderson, D.J. (2008) A common genetic target for environmental and heritable influences on aggressiveness in *Drosophila*. *Proceedings of the National Academy of Sciences*, **105**, 5657–5663.

Warner, M.R., Mikheyev, A.S. and Linksvayer, T.A. (2017) Genomic signature of kin selection in an ant with obligately sterile workers. *Molecular Biology and Evolution*, **34**, 1780–1787.

Warner, M.R., Mikheyev, A. and Linksvayer, T.A. (2019) Transcriptomic basis and evolution of the ant nurse-larval social interactome. *PLoS Genetics*, **15**, e1008156.

Wheeler, G.C. and Wheeler, J. (1976) Ant larvae: review and synthesis. *Memoirs of the Entomological Society of Washington*, **7**, 1–108.

Willingham, A.T. and Keil, T. (2004) A tissue specific cytochrome P450 required for the structure and function of *Drosophila* sensory organs. *Mechanisms of Development*, **121**, 1289–1297.

Yan, H., Opachaloemphan, C., Mancini, G., Yang, H., Gallitto, M., Mlejnek, J. *et al*. (2017) An engineered *orco* mutation produces aberrant social behavior and defective neural development in ants. *Cell*, **170**, 736–747.e9.

Yoshizawa, Y., Sato, R., Tsuchihara, K., Ozaki, K., Mita, K., Asaoka, K. *et al*. (2011) Ligand carrier protein genes expressed in larval chemosensory organs of *Bombyx mori*. *Insect Biochemistry and Molecular Biology*, **41**, 545–562.

Zhou, S., Stone, E.A., Mackay, T.F.C. and Anholt, R.R.H. (2009) Plasticity of the chemoreceptor repertoire in *Drosophila melanogaster*. *PLoS Genetics*, **5**(10), e1000681.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Sample information.

Table S2. Sensory gene expression across species.

Table S3. Top ten most highly expressed genes in stimulated larvae.

Table S4. Top ten most highly expressed genes in isolated larvae.

Table S5. Candidate sensory genes expressed in *Formica fusca* ant larvae.

Table S6. Differentially expressed genes in *Formica fusca* ant larvae following manipulation of social environment.

Table S7. GO terms of differentially expressed genes in *Formica fusca* ant larvae following manipulation of social environment.

Figure S1. Multi-dimensional scaling plot depicting samples grouped by treatment.

File S1. Candidate sensory genes identified from the literature

