Insect Biochemistry and Molecular Biology 105 (2019) 69–78

Contents lists available at ScienceDirect

Insect Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/ibmb

The chemoreceptors and odorant binding proteins of the soybean and pea aphids

Hugh M. Robertson[a,*,1], Erica C.N. Robertson[a], Kimberly K.O. Walden[a], Laramy S. Enders[b], Nicholas J. Miller[c,**]

[a] Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[b] Department of Entomology, Purdue University, West Lafayette, IN 47907, USA
[c] Department of Biology, Illinois Institute of Technology, Chicago, IL 60616, USA

ARTICLE INFO

Keywords:
Odorant receptor
Gustatory receptor
Ionotropic receptor
Sex-biased expression
Stress-related expression

ABSTRACT

We examined the genome of the soybean aphid, *Aphis glycines*, and an updated genome assembly of the pea aphid, *Acyrthosiphon pisum*, for members of the three major families of chemoreceptors, the Odorant Receptors (ORs), Gustatory Receptors (GRs) and Ionotropic Receptors (IRs), as well as the Odorant Binding Proteins (OBPs). The soybean aphid has 47 ORs, 61 GRs, 19 IRs, and 10 OBPs, compared with 87 ORs, 78 Grs, 19 IRs, and 18 OBPs in the pea aphid, with variable numbers of pseudogenes in the OR and GR families. Phylogenetic analysis reveals that while all of the IRs are simple orthologs between these two species, the OR, GR, and OBP families in the pea aphid have experienced major expansions of particular gene lineages and fewer losses of gene lineages. This imbalance in birth-and-death of chemosensory genes has led to the larger pea aphid gene repertoire, which might be related to the broader host range of pea aphids versus the specialization of soybean aphids on a single summer host plant. Examination of the expression levels of these chemosensory genes in parthenogenetic and sexual females and males of pea aphids revealed multiple genes that are differentially expressed in sexual females or males and might be involved in reproductive biology. Examination of the soybean aphid genes in parthenogenetic females under multiple stressors revealed multiple genes whose expression levels changed with heat or starvation stress, the latter potentially important in finding new food sources.

1. Introduction

Aphids are a major family of phytophagous hemipteran insects with significant impacts on host plant biology that commonly extends to pest insect status (van Emden and Harrington, 2007). They exhibit many unusual features, such as parthenogenetic reproduction during the summer alternating with sexual reproduction each autumn in temperate species. Some species also exhibit both winged and wingless adult morphs, while all are dependent on endosymbiotic bacteria, especially *Buchnera*, for their ability to live on phloem. Some aphids have become crop pests, like the soybean aphid *Aphis glycines*, while others through their specialization on diverse host plants have become a model for speciation studies, like the pea aphid *Acyrthosiphon pisum*. A genome sequence for the latter was produced a decade ago (International Aphid Genomics Consortium, 2010) and has facilitated studies of the molecular basis of many aspects of their biology, including the symbiosis with *Buchnera* (e.g. Kim et al., 2018), their facultative winged state (e.g. Grantham and Brisson, 2018), chromosomal evolution (e.g. Jaquiéry et al., 2013, 2018), and their chemosensory biology and host race formation (e.g. Smadja et al., 2009, 2012; Duvaux et al., 2014; Eyres et al., 2016; Nouhaud et al., 2018).

Wenger et al. (2017) published a draft genome sequence for the soybean aphid, however they did not analyse their chemosensory gene repertoire. Aphids use chemicals to detect and identify their host plants, locate and identify mates, and escape predators through alarm pheromones (Caillaud and Via, 2000; Del Campo et al., 2003; van Emden and Harrington, 2007), so a complete description of their chemosensory gene families is crucial to initiate studies of their roles in aphid chemical ecology (Pickett et al., 1992; Powell et al., 2006). Here we describe, and begin to examine the expression of, the three major families of membrane-bound chemoreceptors expressed in their olfactory and gustatory receptor neurons (Leal, 2013; Joseph and Carlson, 2015), as

---

[*] Corresponding author.
[**] Corresponding author.
E-mail addresses: hughrobe@uiuc.edu (H.M. Robertson), nmiller11@iit.edu (N.J. Miller).
[1] Corresponding author for the review process is Hugh Robertson – hughrobe@uiuc.edu.

https://doi.org/10.1016/j.ibmb.2019.01.005
Received 2 December 2018; Received in revised form 10 January 2019; Accepted 14 January 2019

well as the small globular odorant binding proteins expressed by support cells at the base of chemosensory sensilla and secreted into the sensillar lymph (Zhou et al., 2010; Wang et al., 2019; Bruno et al., 2018), using the new soybean aphid genome assembly of Giordano et al. (2019) and a new genome assembly v2 for the pea aphid (see Jaquiéry et al., 2018; Nouhaud et al., 2018). These two aphids differ considerably in the breadth of their host plants. The soybean aphid is a specialist on soybeans during the spring and summer, and *Rhamnus* tree species in winter (Ragsdale et al., 2004, 2011). Pea aphids are rather more complex, having evolved diverse host races of varying differentiation on multiple legumes (Eastop, 1971; Via, 2001; Peccoud et al., 2015; Nouhaud et al., 2018), which on one level indicates specialization on single plant hosts, but on the other hand suggests an ability to exploit multiple host plants much like a generalist species. These two species therefore also provide another comparison of host specialists and generalists to join many already studied (Robertson, 2019).

2. Methods

2.1. Gene modeling

The chemosensory genes in the soybean aphid genome assembly (v6) were detected using the original pea aphid proteins from Smadja et al. (2009) for the ORs and GRs, Croset et al. (2010) and Terrapon et al. (2014) for the IRs, and Zhou et al. (2010) for the OBPs as queries in TBLASTN searches of the genome assembly. Iterative searches were done to find all members of each family. We examined assembly versions 2 and 3 for any genes that appeared to be missing from v6. Gene models were built manually using the Apollo genome browser available at AphidBase (Legeai et al., 2010) at BioInformatics Platform for Agroecosystem Arthropods (BIPAA) (https://bipaa.genouest.org/is/). Existing gene models from the genome-wide automated gene modeling effort (Giordano et al., 2019) were either accepted or modified in light of both these TBLASTN results and guidance from spliced RNA-Seq reads from diverse samples available in the genome browser. Frameshifts resulting from apparent single-base insertions or deletions were encountered within exons of several genes, and each was checked against raw ILLUMINA reads in the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI), and the genome assembly repaired if that was indicated.

This manual annotation effort revealed several genes that were absent from the original pea aphid gene sets for these families (Smadja et al., 2009), as well as gene models that were incorrect or incomplete, so the pea aphid gene families were updated to v2 of the pea aphid genome assembly, also available from BIPAA, again with assistance from RNA-Seq from diverse samples. This new genome assembly, while considerably more contiguous, has misassemblies affecting 6 of these 232 genes, which were corrected, as were a few frameshifted genes. Misassemblies in the v2 assembly were also noted by Jaquiéry et al. (2018). Multiple alignments of the soybean and pea aphid proteins were generated by ClustalX v2.0 (Larkin et al., 2007) using default settings, and gene models were refined if necessary in light of these alignments. All soybean aphid proteins and the updated pea aphid proteins are available in FASTA format in the Supplement.

2.2. Phylogenetic analysis

The final protein alignments were generated using only the proteins from the two aphids for the OR, GR, and OBP families, excluding AglyGr10 and its ortholog ApisGr12 as they are so divergent as to disrupt the tree analysis. For the much smaller and more conserved IR family, the entire *Drosophila melanogaster* 60-protein set was included. The resultant alignments were trimmed using TrimAl v1.4 (Capella-Gutiérrez et al., 2009), with the “gappyout” option employed for the ORs, GRs and OBPs, and the “strictplus” option for the IRs, which removes much of their length- and sequence-divergent N-termini. Phylogenetic analysis was conducted using the maximum likelihood methods employed in PhyML v3.0 (Guindon et al., 2010) using the webserver http://www.atgc-montpellier.fr/phyml/with default settings, and in each case PhyML chose the VT model for amino acid substitutions. The resultant trees were configured using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/), and the final figures prepared using Adobe Illustrator.

2.3. Gene expression

For the pea aphid, the RNA-Seq datasets of Jaquiéry et al. (2013) for the adult morphs and Richard et al. (2017) for embryos were employed. Jaquiéry et al. (2013) generated two samples each for adult males, sexual females, and parthenogenetic females. These libraries range in size from 12 to 22 million reads. They provided the numbers of reads that mapped to genome assembly v1, and these have a narrow range of approximately 300–800 fewer reads (Table S1). Richard et al. (2017) generated many libraries from sexual and asexual embryos and we chose one from each of comparable size to examine, because some chemosensory genes appeared to be unusually lowly expressed in adults, see the Results section. Because the millions of reads from the adults almost all mapped to the genome, and we do not know the number of mapped reads for each of the embryo libraries, we used the total number of reads in each library to standardize our read counts. Reads mapping to each chemosensory cDNA were counted using the Burroughs-Wheeler Algorithm as described in Robertson et al. (2018b) and standardized as Reads Per Kilobase transcript per Million reads in each library (RPKM). Each gene was first examined in the Apollo browser for evidence of overlap of each end with highly-expressed neighboring genes, and if necessary one or more exons were removed from the cDNA to avoid misleading results from such overlap. These cDNAs are indicated with the suffix “m”, and mostly involved the last one or two exons overlapping the extended 3’ UTR of a downstream gene. Pseudogenes were not included in this analysis as they are all very similar to intact genes.

Comparable RNA-Seq datasets are not available for the soybean aphid, in part due to the difficulty of generating sexual males and females in this species in the lab (R. Giordano, personal communication). We instead examined a RNA-Seq set generated by Enders et al. (2015) who studied the responses of adult parthenogenetic female aphids to heat, starvation, and plant defense. We used the same approach as for the pea aphid to generate the results illustrating overall expression levels (employing one library of three replicates for each treatment – Table S1). Enders et al. (2015) found that on average 56% of reads from these 12 libraries mapped to their assembled transcriptome, but for standardization purposes here we again used the total number of reads per library. We also used the full RNA-Seq dataset of three replicate libraries per stress condition to test for significant effects of stress on transcript abundance. Abundance of each transcript in each library was quantified by pseudoaligning the RNA-Seq reads from each library to the predicted cDNA sequence of each chemosensory gene using kallisto (Bray et al., 2016). One hundred bootstrap replicate sets of transcript abundance estimates were generated for each library to estimate the “inferential variance” due to reads that pseudoaligned to multiple transcripts (see Bray et al., 2016 for details). The likelihood ratio test implemented in the sleuth package, which takes into account bootstrap estimates of inferential variance (Pimentel et al., 2017), was used to test for differential gene expression in response to each stress condition versus un-stressed controls. Genes were declared to be significantly differentially expressed at a false discovery rate of 0.1. Obp2, Gr4, and Ir68a and 324 completely overlap the 3’ UTRs of highly-expressed flanking genes, so could not be included in the analysis.

3. Results

3.1. The OR family

The OR family originated from within the GR family at the base of the insects (Brand et al., 2018). In the soybean aphid it consists of the single highly conserved Odorant receptor co-receptor (Orco) and 46 "specific" ORs, each of which is thought to pair with Orco to form a functional olfactory receptor tetramer (Butterwick et al., 2018). All of these genes are modeled as encoding full-length proteins, with five of them being pseudogenes, and all but five of the existing gene models in the automated annotation were improved (Table S2). Four genes are missing from the v6 assembly, but are present in the v2/3 assemblies. Updating the 79 gene models in the pea aphid to genome assembly v2 resulted in the loss of two models (ApisOr19 and Or47), which were nearly identical to ApisOr20 and Or48, respectively, and appear to have been separate assemblies of alternative haplotypes in the original genome assembly v1. Eleven new models were added to the family, two of which are pseudogenes, and these were named ApisOr80-90 to avoid changing any of the original gene names as these have now been employed in multiple studies (e.g. Smadja et al., 2009, 2012; Duvaux et al., 2014; Eyres et al., 2016, 2017; Nouhaud et al., 2018). As these new genes are phylogenetically and chromosomally near various already-named genes, they are significantly "out of order" in the numbering scheme. There is also a duplicate copy of Or5 37 kb upstream of the original copy but missing the final short exon, which was ignored as a possible assembly artifact because raw read depth for Or5 is similar to singleton genes. In addition, 35 of the original models were improved in significant ways involving changes in gene structure beyond just occasional changes in particular amino acid residues. The final count is 86 "specific" OR genes, of which 17 are pseudogenes. Most of these pseudogenes are in a recently highly expanded clade, and there are many additional gene fragments in the genome indicating considerable additional gene duplication, pseudogenization, and loss (as well as possible genome assembly artifacts). Phylogenetic analysis reveals that while these two aphids have 1:1 orthologs for 15 genes in addition to Orco, the major differences between them are in the large expansions specific to the pea aphid, as well as losses of four gene lineages from the soybean aphid (the orthologs of ApisOr16 and 41 are present as severely damaged and short pseudogenes in the soybean aphid genome, while the ApisOr35/36 and Or26-30 lineages appear to have been completely lost) (Fig. 1). Conversely, the soybean aphid has only a few small gene-lineage expansions, the largest of which are six genes each (AglyOr30-25 and Or41-46), while the pea aphid has lost only one lineage (AglyOr4). The end result is that the pea aphid has almost twice as many OR genes as the soybean aphid.

3.2. The GR family

The GR family is far older in animals (Saina et al., 2015; Robertson, 2015; Eyun et al., 2017) and consists of several major subfamilies. In the soybean aphid the family has 60 genes, again all full-length gene models, twelve of which are pseudogenes, but again only five of which were correctly modeled by the automated methods, while one is only present in the v2/3 assemblies (Table S3). One gene is modeled as encoding two isoforms in a fashion typical of these chemoreceptors in many insects, with alternative long first exons spliced into three shared short final exons, a model supported by spliced RNA-Seq reads (AglyGr23a/b, orthologous to ApisGr21a/b). Updating the pea aphid 77 GR gene set revealed that five models no longer exist and appear to have been alternative haplotype assemblies (ApisGr23, 49, 52, 61, and 76), while five new models were added to the gene set, two of which are pseudogenes. In addition, 7 existing models were improved. The resultant set of 77 ApisGr genes encoding 78 GRs includes 14 pseudogenes. Phylogenetic analysis reveals that in addition to 31 1:1 orthologous relationships (including AglyGr10/ApisGr12 excluded from the tree analysis), there are many instances of gene duplications in each species, for example, one of the four shared sugar receptor lineages was duplicated in the pea aphid into three genes (ApisGr2-4) (Fig. 2). Unlike the OR family, there are no obvious losses of gene lineages from either species. Like the pea aphid, soybean aphid does not have relatives of the carbon dioxide receptor subfamily of endopterygote insects, a gene lineage present back to basal pterygote insects (Benoit et al., 2016; Ioannidis et al., 2017; Robertson et al., 2018a). The only other aphid GR of likely known function is the fructose receptor subfamily related to DmGr43a (Miyamoto et al., 2012), represented here by AglyGr9 and ApisGr11. The amplification of GR lineages in both species and no gene lineage losses results in roughly comparable gene family sizes in these two aphids, with the slightly larger pea aphid family resulting again from some very recent gene duplications.

3.3. The IR family

The IR family is evolutionarily independent of the OR/GR families, which together form the insect chemoreceptor superfamily (Robertson et al., 2003), having arisen in basal protostomes as a variant lineage of the ancient ionotropic glutamate receptor superfamily (Benton et al., 2009; Croset et al., 2010; Rytz et al., 2013; Eyun et al., 2017). Croset et al. (2010) included 11 IRs in their compilation for the pea aphid, and Terrapon et al. (2014) added 8 to these for a total of 19. The soybean aphid has simple 1:1 orthologs for each of these, all of which are full-length gene models with no pseudogenes, only two of which were correctly modeled in the automated gene set while one is only present in the v2/3 assemblies (Table S4). The pea aphid gene set was updated for 12 of these 19 gene models. For the phylogenetic analysis the entire IR family from *D. melanogaster* (Benton et al., 2009) was included, showing why the subset of conserved proteins are named for their fly homologs, with the more divergent proteins named Ir322-327 (Fig. 3). The IR family is far more conserved in these two aphids than are the OR or GR families, with no gene losses or duplications. Likely functions can be assigned to many of these aphid IRs based on their conservation or similarity with Drosophila IRs. Ir8a, 25a, and 76b are co-receptors that function with subsets of the other IRs (Rytz et al., 2013), and are the most conserved members of the family. The next most conserved proteins are Ir21a, 40a, 68a, and 93a, which are involved in perception of temperature and humidity in *D. melanogaster* (Knecht et al., 2017). The members of the Ir75 clade with known functions detect acids in *Drosophila* flies (Prieto-Godino et al., 2017). Ir100a in the aphids was named for DmelIr100a (Croset et al., 2010), however our analysis suggests these are not really orthologs, instead there is a small clade of aphid IRs related to Ir100a/10a in Drosophila, but their ligands are unknown. The Ir7 and Ir20a clades in flies are both involved in gustation (Croset et al., 2010; Koh et al., 2014; Stewart et al., 2015; Sánchez-Alcañiz et al., 2018), so it is reasonable to suggest that the remaining divergent aphid IRs distantly related to these two clades might also be involved in gustation.

3.4. The OBP family

OBPs are short secreted proteins that can bind biomolecules and are thought to function in transport of chemicals (Pelosi et al., 2018) and quenching of the chemosensory signal (Larter et al., 2016). Thirteen full-length OBPs were described in the pea aphid (Zhou et al., 2010), for which 10 orthologs were found in the soybean aphid, albeit one is only in assemblies v2/3 (Table S5) (six are perfectly modeled in the automated gene set). These OBPs were named for their orthologs, following the approach of Wang et al. (2019) who examined them in multiple aphids. An ortholog for ApisOBP1 is not present in any of the genome assemblies, or in the independent genome sequence of Wenger et al. (2017), a finding that agrees with Wang et al. (2019). Most of the remaining OBPs had simple 1:1 orthologs in the two species, however Obp3 is of special interest. The soybean aphid has a single gene

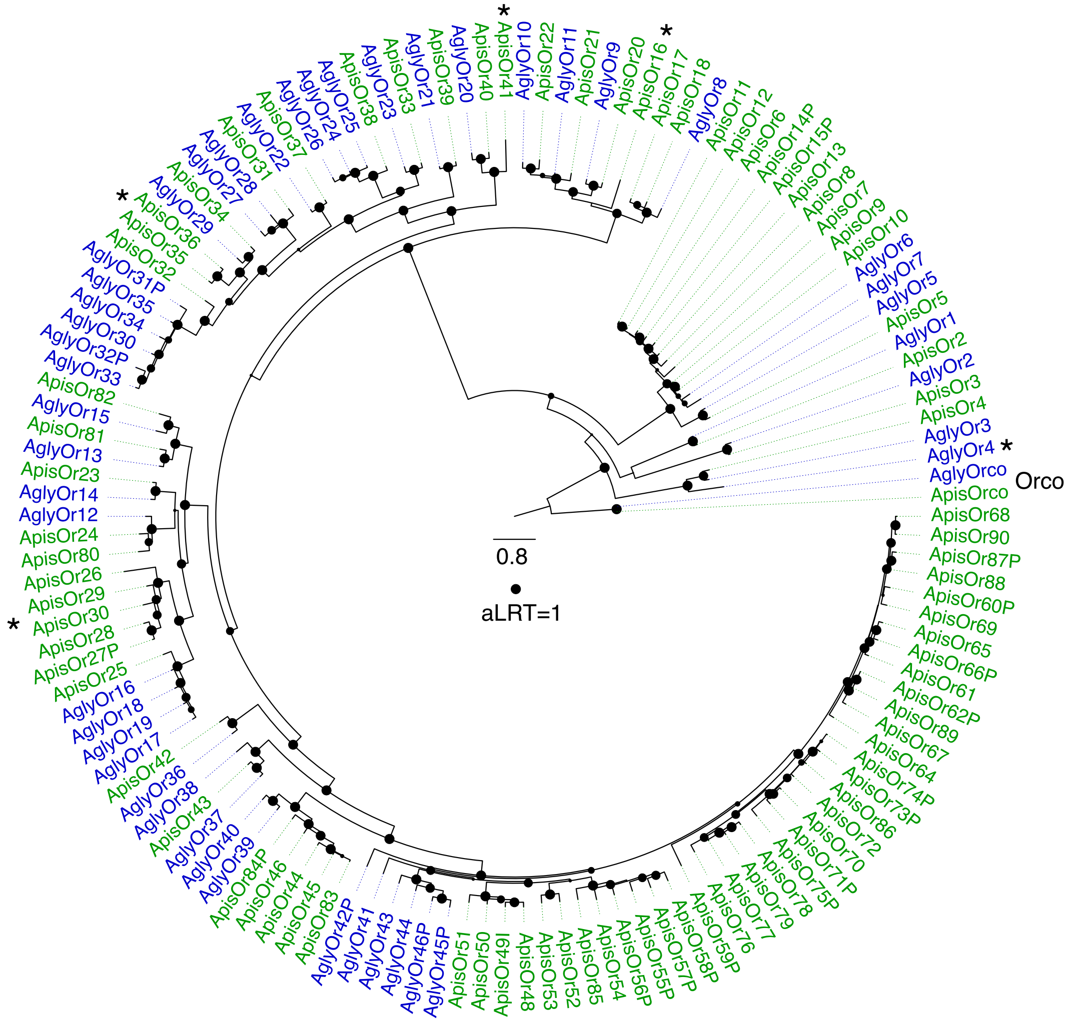

Fig. 1. Phylogenetic relationships of the soybean and pea aphid odorant receptors (ORs). This maximum likelihood tree was rooted with the Orco proteins, which are highly conserved 1:1 orthologs in most insects, and represent a basal lineage of the OR family (Robertson et al., 2003; Brand et al., 2018). The two Orco proteins are indicated outside the circle, while asterices indicate the locations of soybean aphid OR gene losses. Soybean aphid names abbreviated as Agly are in blue, while pea aphid names abbreviated as Apis are in green. The scale bar indicates amino acid substitutions per site, and the filled circles on nodes indicate support levels from approximate Likelihood Ratio Tests (aLRT) in PhyML ranging from 0 to 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

encoding this protein, as do most other aphids (Wang et al., 2019), however there are at least six paralogs of this gene in the pea aphid genome assembly v2. Zhou et al. (2010) included two of these in their compilation as Obp11 and 12, and mentioned two more possible paralogs as incomplete models Obp14/15. We find that v2 of the genome assembly does not have an exact copy of Obp12, which was apparently an alternative haplotype assembly, and it is hard to determine which of the six paralogs are their Obp14/15, so we name them Obp14-18. Obp16 is a pseudogene, and there are at least three additional shorter and more highly degraded likely-pseudogenic fragments in the genome assembly. All of these paralogs and gene fragments are inside large scaffolds, so are unlikely to be alternative haplotype assemblies or misassemblies. Furthermore, examination of raw read datasets in the SRA at NCBI using BLASTN with an internal exon that is near 100% identical across these paralogs as query shows that read depth for these genes is several fold higher than the other OBPs, consistent with multiple copies of them. Obp3/11/14–18 share at least 90% DNA and protein identity, so despite being distributed around the genome, are recent duplicates (Fig. S1). Furthermore, there is a pseudogenic duplicate of Obp10 immediately upstream of Obp10, which we name Obp12P. Finally, the version of Obp13 provided in Zhou et al. (2010) and Wang et al. (2019) has the first 13 amino acids incorrect, they should be an unusually long N-terminal 133 amino acids, something shared by the soybean aphid ortholog and well-supported by RNA-Seq reads in both species (although in the pea aphid assembly v2 the first two exons encoding these 133 amino acids are misassembled 62 kb downstream of the rest of the gene, and also duplicated in another large scaffold – the gene in assembly v1 is intact and not duplicated). Thus the OBP family is considerably larger in the pea aphid, however most of this is due to the duplications of Obp3, and only Obp1 has been lost from the soybean aphid, a loss that Wang et al. (2019) found in all examined members of the *Aphis* genus.

3.5. *Expression of the pea aphid chemosensory genes*

We examined the expression of these pea aphid chemosensory genes in published RNA-Seq sets representing the three adult forms (Jaquiéry et al., 2013) and embryos (Richard et al., 2017), specifically two samples each from males, sexual females, and parthenogenetic females, as well as single samples from sexual and asexual embryos (from a much larger dataset). The full results are in Tables S6–9 and Figs. S2–5, with representative and interesting results for the chemoreceptors shown in Fig. 4 (each result in Fig. 4 was confirmed by examining the raw RNA-Seq reads mapped against the genome in the Apollo browser

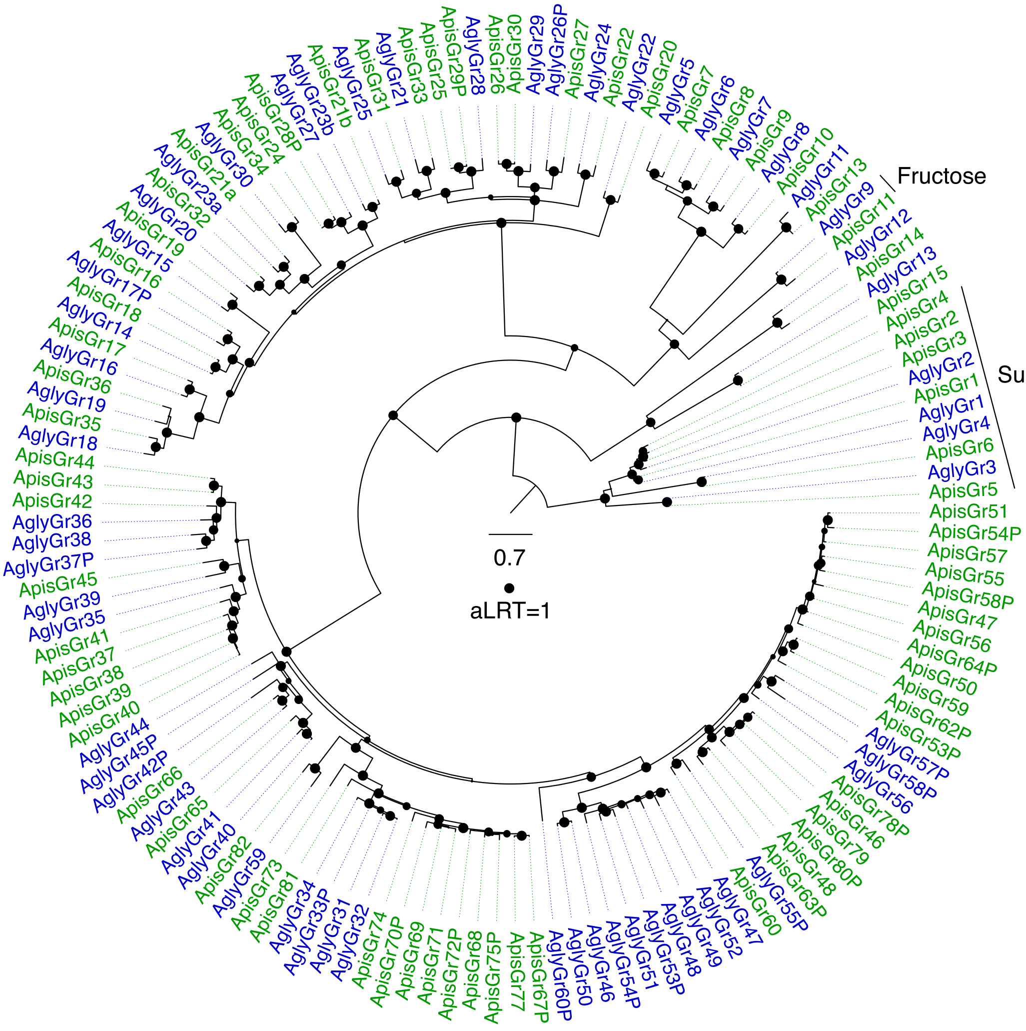

Fig. 2. Phylogenetic relationships of the soybean and pea aphid gustatory receptors (GRs). This maximum likelihood tree was rooted with the candidate sugar receptors, which are a divergent lineage at the base of the insect GR tree in larger analyses (Robertson, 2015). The candidate sugar receptor subfamily and the candidate fructose receptor are indicated outside the circle. Other details as for Fig. 1.

at BIPAA).

As expected for a gene expressed in each olfactory sensory neuron that has a specific OR (Butterwick et al., 2018), Orco is the most consistently highly expressed member of the OR family, and the RPKM values from the two samples of each sex are reasonably consistent. Asexual embryos have considerably higher expression than sexual embryos, consistent with the relatively high expression in parthenogenetic females (Fig. 4). The deep RNA-Seq for Orco also revealed that there are three possible transcription start sites for this gene, the most upstream of which yields a possible protein that is seven amino acids longer than the other two, suggesting that the shorter version might be the actual protein sequence. Most of the specific OR genes have low levels of expression, as expected for genes expressed in a small subset of olfactory sensory neurons, however some have expression biased to one sex. Thus Or17, 18, 21, 22, 26, 31, 33, 72 and 78 are relatively highly expressed in males (represented by Or31 in Fig. 4), while Or9, 29, 49, 50, and 83 are relatively highly expressed in sexual females (represented by Or9 in Fig. 4). Some of these ORs expressed primarily in the sexual lifestages might be involved in reproductive behavior, for example, some of these male-biased ORs might mediate perception of components of the female sex pheromone (Dawson et al., 1990). Despite the high levels of Orco expression in parthenogenetic females, no specific OR is unusually highly expressed in them, but most of those not exhibiting differential expression in sexuals do show expression in parthenogens (represented by Or37 in Fig. 4), so some of these ORs might be important for host plant detection. The only aphid OR with a known ligand is Or5, which mediates response to the sesquiterpene alarm pheromone (E)-**β**-farnesene (Zhang et al., 2017), however like many other ORs it is expressed at low levels (Fig. 4 – just 1 or 2 reads for three of the samples, compared with 20–379 reads per sample for Orco – Table S6).

Among the GRs (Table S7, Fig. S3), five of the six sugar receptors (Gr1-6) are expressed in most samples (represented by Gr1 in Fig. 4), however Gr4 has no reads mapping to it in any samples, suggesting that despite being intact in the coding regions, it might have lost regulatory regions, perhaps during the recent tandem duplications that led to Gr2-4. Most of the other GRs have low levels of expression, among the highest being the candidate fructose receptor Gr11, as well as Gr24, which is relatively highly expressed in parthenogens (Fig. 4). Only Gr3, 21a/b, 42, and 57 are clearly more highly expressed in males (represented by Gr21b in Fig. 4), while none are clearly differentially expressed in females, suggesting that taste is not as important in pea aphid reproductive biology. It is noteworthy that most GRs are expressed in embryos, suggesting that GR expression is well underway before they are required for function, or some might be involved in earlier aphid biology.

Among the IRs (Table S8, Fig. S4), the three co-receptors (Ir8a, 25a, and 76b) are not expressed at particularly high levels in adults, except

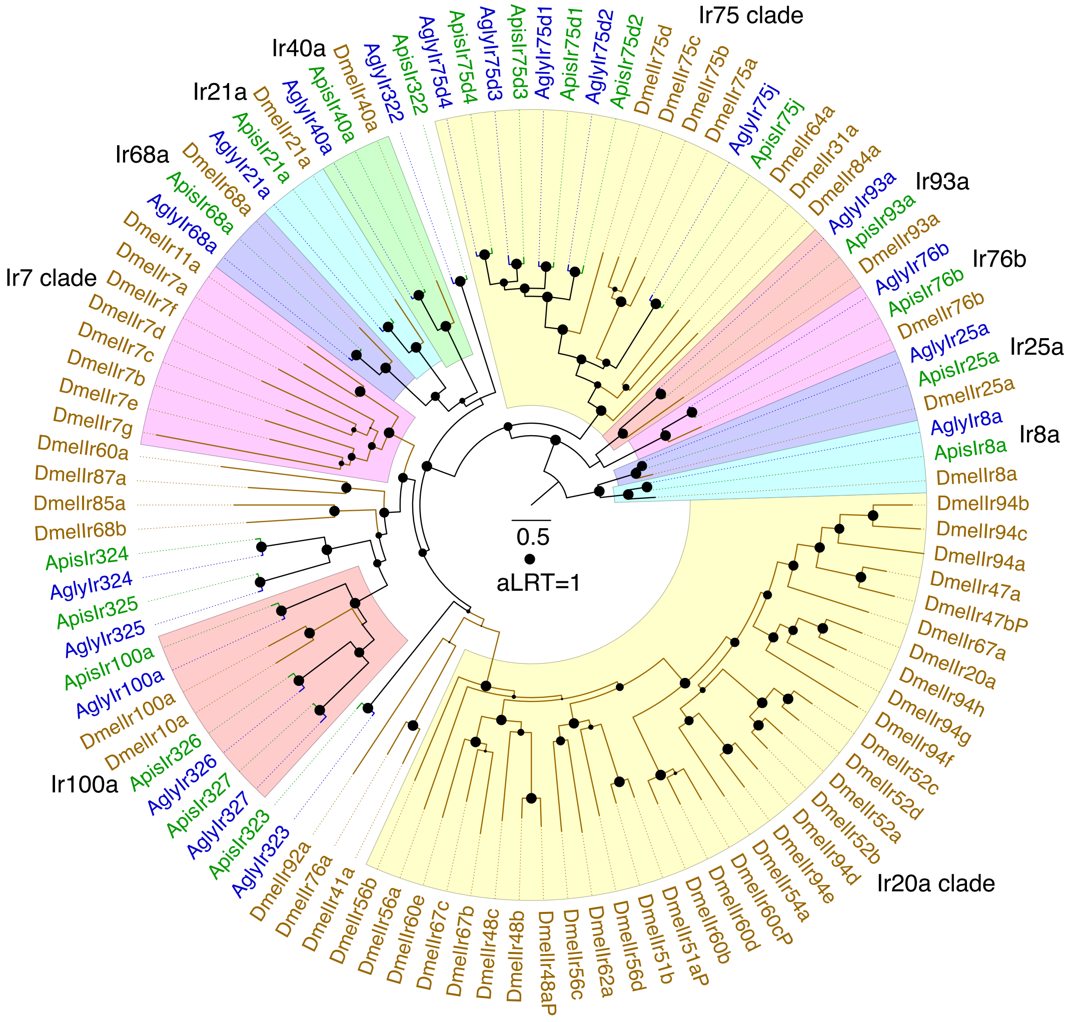

Fig. 3. Phylogenetic relationships of the soybean and pea aphid ionotropic receptors (IRs). This maximum likelihood tree was rooted with the Ir8a and Ir25a proteins, which are basal to the family (Croset et al., 2010; Terrapon et al., 2014; Eyun et al., 2017). The proteins with clear *Drosophila* relatives are indicated outside the circle, as are other major clades or subfamilies, and highlighted with colors. *D. melanogaster* IRs are colored tan. Other details as for Fig. 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

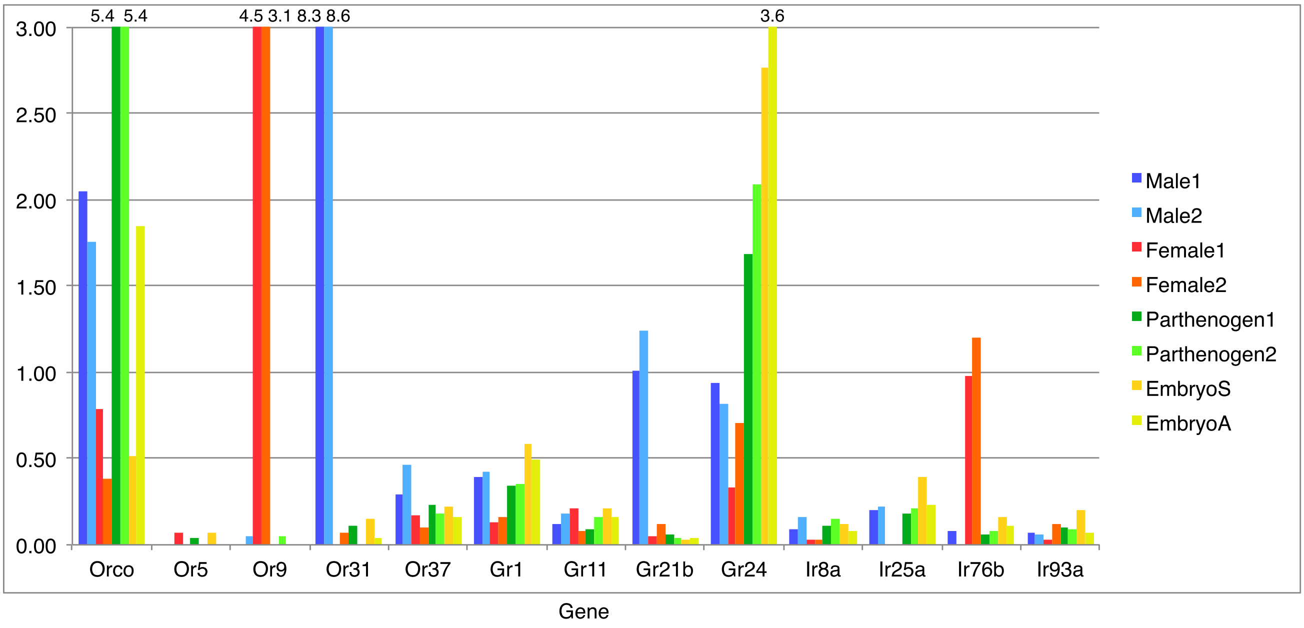

Fig. 4. Expression levels in RPKM for selected pea aphid chemoreceptors in the three adult morphs and embryos.

that Ir76b is highly expressed in females, while Ir25a is somewhat unusually not expressed in females (Fig. 4). Like most of the other IRs, all three are expressed in embryos, so perhaps this early expression suffices for their lifetime, however the high expression of Ir76b in females is enigmatic. In *Drosophila* flies, Ir76b is involved in perception of salt, amines, amino acids, and fatty acids (e.g. Zhang et al., 2013; Ganguly et al., 2017; Ahn et al., 2017), but this likely role does not readily explain why it should be highly expressed in sexual females. The four IRs involved in sensing temperature and humidity all have similar low expression profiles, exemplified by Ir93a in Fig. 4. The five members of the Ir75 clade, members of which in *Drosophila* flies are involved in perception of various acids (Prieto-Godino et al., 2017), also have generally low expression, as do the divergent IRs. Overall the levels of IR expression are comparable to those of the ORs and GRs.

Finally, the OBPs are expected to show far higher levels of expression, and indeed the highest are about 100 times higher than the highest chemoreceptor expression levels (Table S9 and Fig. S5). Obp1 is the most consistently highly expressed, and Obp6-10 are more highly expressed in males than either female, making them candidates for binding the female pheromone components (Dawson et al., 1990), although Sun et al. (2012) reported that Obp7 can bind the alarm pheromone. Obp3 and 4 are higher in females, Obp3 being known for binding the alarm pheromone (Qiao et al., 2009; Sun et al., 2012), while the remaining Obps have relatively low expression levels. The paralogs of Obp3 all have low expression, suggesting that they might not play much of a role in pea aphid biology.

3.6. *Expression of the soybean aphid chemosensory genes*

Comparable RNA-Seq datasets for soybean aphid by morph are not available, so instead we analysed a dataset generated to examine responses of these aphids to three stressors, specifically starvation, heat, and plant defense (Enders et al., 2015). These four libraries are approximately 10-fold larger than those for the pea aphid (Table S1). Unfortunately the Gr4, Ir68a and 324, and Obp2 genes all completely overlap neighboring highly-expressed genes, so they had to be excluded from the analysis. The complete data are shown in Tables S10–13 and Figs. S6–9, with selected genes shown in Fig. 5. The genes showing statistically significant up or down expression are shown in Table 1, and the statistical analyses presented in Figs. S10–14.

As expected, Orco is the most consistently highly expressed member of the OR family, most genes having relatively low expression such as Or1. Several ORs showed responses to the various stressors, e.g. Or11 to heat and starvation, Or29 and 40 to heat only, and Or37 to starvation only (Fig. 5, others listed in Table 1). Among the GRs, the sugar receptors (represented by Gr1 in Fig. 5) are up-regulated under starvation, as are several other genes like the candidate fructose receptor Gr9 and most impressively Gr39, while Gr40 and 41 led the way in being down-regulated with starvation. Several GRs had elevated expression under heat stress, especially the tandemly-arrayed set of close relatives, Gr5-8, while Gr41 and 51 were down-regulated. Among the IRs, the co-receptors Ir8a, 25a, and 76b have relatively uniform expression in these adults, as do the temperature- and humidity-sensing Ir21a, 40a, and 93a (Ir68a could not be evaluated). Most of the other IRs have lower expression levels (exemplified by Ir75d2 in Fig. 5), and only Ir323 and 326 have elevated expression with heat and starvation, respectively. Finally, the OBPs are again expressed at approximately 100-fold higher levels, however none exhibited particularly striking expression changes with these stressors. As was the case in the transcriptome-wide analysis of Enders et al. (2015), plant stress caused relatively little expression level changes for these chemosensory genes.

4. Discussion

Our annotation of these four major families of chemosensory genes in these two aphids reveals some major differences between them. The most prominent is that the OR family is almost twice as large in the pea aphid, resulting from recent increased birth of genes in the pea aphid and a few losses from the soybean aphid. Similar but less extreme patterns are present for the GR and OBP families, while the IRs are conserved in number. These differences for the OR and GR families are most likely related to the breadth of host plants each aphid lives on. Soybean aphids are specialists on just one legume, the soybean, albeit utilizing a second woody host in the winter (Ragsdale et al., 2004, 2011), while the pea aphid is a complex of multiple host races of different levels of genetic divergence living on a diversity of legume hosts (Eastop, 1971; Via, 2001; Peccoud et al., 2015; Nouhaud et al., 2018). In addition to the sequence differentiation of these host races at several of these chemosensory genes (discussed below), they differ in copy number for over 50% of ORs and GRs (Duvaux et al., 2014), indicating an ongoing process of gene birth and death. On-going gene flow might explain why individual host races, like the sequenced reference strain examined here, retain large numbers of duplicates including pseudogenes. This pattern of larger chemosensory gene repertoires in

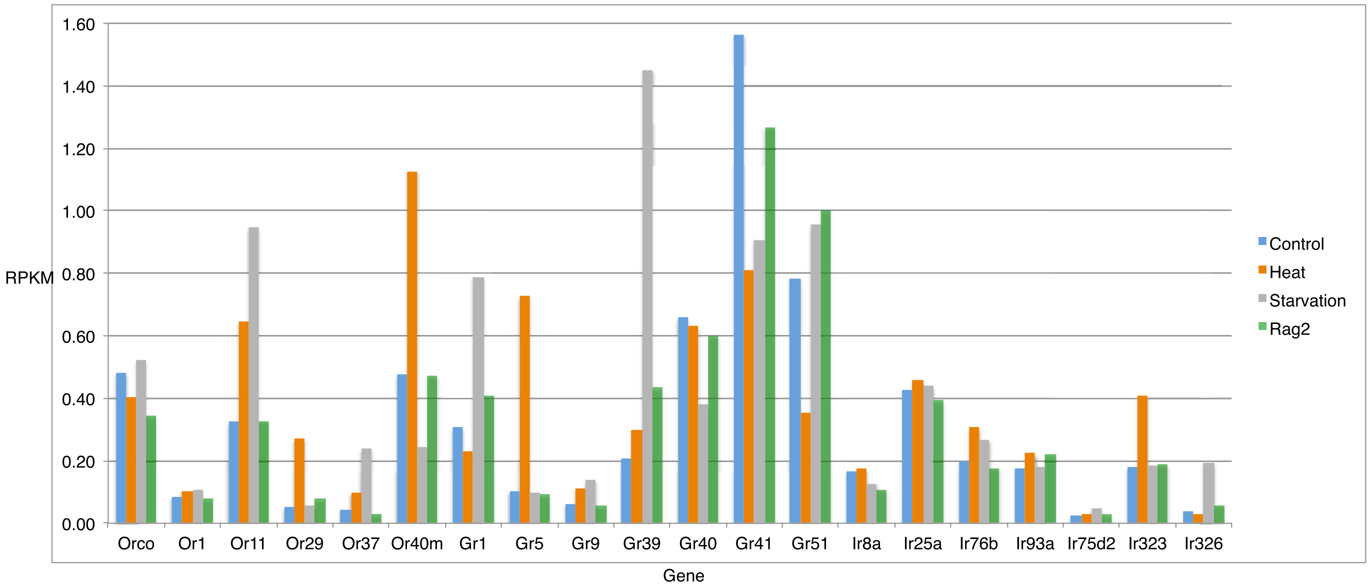

Fig. 5. Expression levels in RPKM for selected soybean aphid chemoreceptors under starvation, heat, and plant defense stress.

Table 1
Genes significantly up- or down-regulated in soybean aphids exposed to stress conditions versus unstressed controls.

Condition	Change	Gene family			
		OR	GR	IR	OBP
Starvation	up	Or37, Or11, Or6, Or16, Or35, Or19	Gr39, Gr23a, Gr60P, Gr22, Gr23b, Gr30, Gr17P, Gr3, Gr14	Ir326, Ir75j, Ir75d2, Ir40a, Ir100a	Obp9, Obp10
Starvation	down	Or21, Or7, Orco	Gr40, Gr41, Gr6, Gr12, Gr59, Gr51	Ir75d4, Ir25a, Ir93a, Ir8a, Ir323, Ir76b	Obp13, Obp8
Heat	up	Or40, Or29, Or16, Or25, Or11, Or18, Or27, Or41, Or17, Or12	Gr5, Gr7, Gr55P, Gr6, Gr22, Gr17P, Gr23a, Gr8, Gr23b, Gr18, Gr60P, Gr15	Ir323, Ir325	Obp3
Heat	down	Or22, Or9, Orco, Or21, Or2, Or39, Or3	Gr41, Gr51, Gr49, Gr3, Gr56, Gr26P, Gr53P, Gr40, Gr11	Ir100a, Ir75d3, Ir75d1, Ir25a, Ir40a, Ir93a	Obp6, Obp8, Obp4, Obp10, Obp5
Resistant plant	up				Obp9
Resistant plant	down		Gr41		

generalists versus specialists has now been found in many other studies (Robertson, 2019). The different sizes of the OBP family, however, are probably not related to host plant breadth, because the loss of Obp1 from the soybean aphid is true of the *Aphis* genus (Wang et al., 2019), while the duplications of Obp3 in the pea aphid are unlikely to be related to host breadth as it is apparently a binding protein for the alarm pheromone (Qiao et al., 2009; Sun et al., 2012), and these duplicate genes have extremely low expression levels for OBPs.

The pea aphid has become a prominent study system for understanding the differentiation of phytophagous insects on different host plants, potentially leading to sympatric or ecological speciation (Via, 2001; Peccoud et al., 2009, 2015), a process that is expected to involve differentiation of host plant recognition, especially mediated by chemosensation (Pickett et al., 1992; Powell et al., 2006). Nouhaud et al. (2018) reported the first genome-wide re-squencing effort to identify genomic hotspots of differentiation between different host races, identifying 392 such hotspots, which overlap 10 OR and 14 GR genes. Of course, not all these genes are necessarily involved in host race differentiation, however they noted that several (ORs 3, 14, 20, 21, and 22, and GRs 15, 37, 45, 60, 65, and 66) were also identified by Smadja et al. (2012) and Eyres et al. (2017) as exhibiting high levels of sequence differentiation between host races. Our observations on these genes and their expression levels might be relevant. The Or14 gene was initially described as a pseudogene with a stop codon within the first coding exon (Smadja et al., 2009) and the stop codon is also present in v2 of the genome assembly, however the raw ILLUMINA reads from Nouhaud et al. (2018) reveal that this stop codon is not present in the three host races they examined or in nine more races with data available in the SRA at NCBI, so the model was fixed herein. This observation is more consistent with a possible role in host plant choice, however we find no expression for this gene in any adult morph (Table S6, Fig. S2), although low expression of chemoreceptors in whole body RNA-Seq studies does not preclude expression in a few olfactory sensory neurons and a role in host plant specificity. Or3 and 20 also have almost no expression in our analysis, while Or21 and 22 are highly differentially expressed in males, which seems at odds with involvement in host plant selectivity (Table S6, Fig. S2). Similarly, all of these six GR genes have low expression levels, with Gr65, which was considered a pseudogene in Smadja et al. (2009) due to a ten-amino-acid insertion relative to its closest relatives but here is considered to be intact, being the most highly expressed and in all morphs (Table S7, Fig. S3). Again, low expression in whole animal RNA-Seq does not preclude a role in host plant specificity.

Our expression analyses in pea aphid, which must be considered preliminary because they are from entire animals, rather than from separated chemosensory organs like antennae, mouthparts, legs, or genitalia, nevertheless reveal some interesting patterns that suggest receptors for future investigation. In particular we identified several ORs and a few GRs that are differentially expressed in males and sexual females, which are candidates for involvement in reproductive biology. These putatively differentially-expressed chemoreceptor genes were not previously noted by the authors of this RNA-Seq dataset because they focused on questions of chromosomal gene evolution (Jaquiéry et al., 2013). In other insects some chemoreceptors have been found to be expressed in testes (e.g. Pitts et al., 2014), which might explain some differential expression in males. The spatial expression patterns of a subset of the OBP genes have been studied in pea aphid using RT/PCR and most are expressed in antennae and heads, and immunostaining shows presence of the proteins in chemosensory sensilla on both structures (De Biasio et al., 2015; Bruno et al., 2018), so most are clearly involved in chemosensation. Differential expression in the sexes has apparently not previously been examined, so our finding that OBPs 8–10 are differentially highly expressed in males warrants further investigation of their possible roles in reproductive biology.

Our examination of the soybean aphid revealed that several genes are differentially expressed with the three stressors, most impressively with heat and starvation, in agreement with the overall results of Enders et al. (2015). The changes with heat are not easily interpreted, however some of the changes with starvation do make sense, especially the up-regulation of the sugar receptors, which might improve ability to find new food sources, as chemical stimulants are particularly important to aphids (Del Campo et al., 2003). Similar up-regulation with starvation might suggest the importance of some other GRs like Gr39 in detection of food (ortholog of ApisGr45, one of the host-race-differentiated GRs), as well as ORs like Or11 (ortholog of ApisOr21, a host-race-differentiated OR) and Or37 as well as Ir326. Differential expression of chemoreceptors with changing physiological needs have been noted before in other insects, most prominently in mosquitoes after a blood meal (e.g. Rinker et al., 2013). While difficult to do, it would be interesting to look for different chemoreceptors up-regulated in the sexual soybean aphid morphs because in addition to possibly being involved in mating behaviors, some might be involved in detection of their *Rhamnus* tree hosts in autumn (Ragsdale et al., 2004, 2011).

The most limiting aspect of this kind of study is our very limited knowledge of the ligands of almost all the ORs and most of the GRs, with functions for half of the IRs only imputed from *Drosophila* flies. This study nevertheless indicates particular chemoreceptors worthy of follow-up study of their ligands that would shed additional light on the chemical ecology of these important players in insect-plant interactions.

Declarations of interest

None.

Acknowledgements

We thank Anthony Bretaudeau for the Apollo browser for v2 of the pea aphid genome assembly at BIPAA. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibmb.2019.01.005.

References

Ahn, J.E., Chen, Y., Amrein, H., 2017. Molecular basis of fatty acid taste in Drosophila. Elife 6, e30115.

Benoit, J.B., Adelman, Z.N., Reinhardt, K., Dolan, A., Poelchau, M., Jennings, E.C., Szuter, E.M., Hagan, R.W., Gujar, H., Shukla, J.N., Zhu, F., 2016. Unique features of a global human ectoparasite identified through sequencing of the bed bug genome. Nat. Commun. 7, ncomms10165.

Benton, R., Vannice, K.S., Gomez-Diaz, C., Vosshall, L.B., 2009. Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila. Cell 136 (1), 149–162.

Brand, P., Robertson, H.M., Lin, W., Pothula, R., Klingeman, W.E., Jurat-Fuentes, J.L., Johnson, B.R., 2018. The origin of the odorant receptor gene family in insects. Elife 7, e38340.

Bray, N.L., Pimentel, H., Melsted, P., Pachter, L., 2016. Near-optimal RNA-Seq quantification with kallisto. Nat Biotechnol 34 (5), 525–527.

Bruno, D., Grossi, G., Scala, A., Farina, D., Grimaldi, A., Salvia, R., Zhou, J.J., Bufo, S.A., Vogel, H., Grosse-Wilde, E., Hansson, B.S., 2018. Sensilla morphology and complex expression pattern of Odorant Binding Proteins in the vetch aphid Megoura viciae (Hemiptera: Aphididae). Front. Physiol. 9, 777.

Butterwick, J.A., del Mármol, J., Kim, K.H., Kahlson, M.A., Rogow, J.A., Walz, T., Ruta, V., 2018. Cryo-EM structure of the insect olfactory receptor Orco. Nature 560 (7719), 447.

Caillaud, M.C., Via, S., 2000. Specialized feeding behavior influences both ecological specialization and assortative mating in sympatric host races of pea aphids. Am. Nat. 156 (6), 606–621.

Capella-Gutiérrez, S., Silla-Martínez, J.M., Gabaldón, T., 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25 (15), 1972–1973.

Croset, V., Rytz, R., Cummins, S.F., Budd, A., Brawand, D., Kaessmann, H., Gibson, T.J., Benton, R., 2010. Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. PLoS Genet. 6 (8), e1001064.

Dawson, G.W., Griffiths, D.C., Merritt, L.A., Mudd, A., Pickett, J.A., Wadhams, L.J., Woodcock, C.M., 1990. Aphid semiochemicals—a review, and recent advances on the sex pheromone. J. Chem. Ecol. 16 (11), 3019–3030.

De Biasio, F., Riviello, L., Bruno, D., Grimaldi, A., Congiu, T., Sun, Y.F., Falabella, P., 2015. Expression pattern analysis of odorant-binding proteins in the pea aphid Acyrthosiphon pisum. Insect Sci. 22 (2), 220–234.

Del Campo, M.L., Via, S., Caillaud, M.C., 2003. Recognition of host-specific chemical stimulants in two sympatric host races of the pea aphid Acyrthosiphon pisum. Ecol. Entomol. 28 (4), 405–412.

Duvaux, L., Geissmann, Q., Gharbi, K., Zhou, J.J., Ferrari, J., Smadja, C.M., Butlin, R.K., 2014. Dynamics of copy number variation in host races of the pea aphid. Mol. Biol. Evol. 32 (1), 63–80.

Eastop, V.F., 1971. Keys for the identification of Acyrthosiphon (Hemiptera: Aphididae). Bulletin of the British Museum (Natural History), B. Entomology 26 (1), 1–115.

Enders, L.S., Bickel, R.D., Brisson, J.A., Heng-Moss, T.M., Siegfried, B.D., Zera, A.J., Miller, N.J., 2015. Abiotic and biotic stressors causing equivalent mortality induce highly variable transcriptional responses in the soybean aphid. G3: Genes, Genomes, Genetics 5 (2), 261–270.

Eyres, I., Jaquiéry, J., Sugio, A., Duvaux, L., Gharbi, K., Zhou, J.J., Legeai, F., Nelson, M., Simon, J.C., Smadja, C.M., Butlin, R., 2016. Differential gene expression according to race and host plant in the pea aphid. Mol. Ecol. 25 (17), 4197–4215.

Eyres, I., Duvaux, L., Gharbi, K., Tucker, R., Hopkins, D., Simon, J.C., Ferrari, J., Smadja, C.M., Butlin, R.K., 2017. Targeted re-sequencing confirms the importance of chemosensory genes in aphid host race differentiation. Mol. Ecol. 26 (1), 43–58.

Eyun, S.I., Soh, H.Y., Posavi, M., Munro, J.B., Hughes, D.S., Murali, S.C., Qu, J., Dugan, S., Lee, S.L., Chao, H., Dinh, H., 2017. Evolutionary history of chemosensory-related gene families across the Arthropoda. Mol. Biol. Evol. 34 (8), 1838–1862.

Ganguly, A., Pang, L., Duong, V.K., Lee, A., Schoniger, H., Varady, E., Dahanukar, A., 2017. A molecular and cellular context-dependent role for Ir76b in detection of amino acid taste. Cell Rep. 18 (3), 737–750.

Giordano, R., et al., 2019. Genome of the Soybean Aphid. IBMB in press.

Grantham, M.E., Brisson, J.A., 2018. Extensive differential splicing underlies phenotypically plastic aphid morphs. Mol. Biol. Evol. 35 (8), 1934–1946.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59 (3), 307–321.

International Aphid Genomics Consortium, 2010. Genome sequence of the pea aphid Acyrthosiphon pisum. PLoS Biol. 8 (2), e1000313.

Ioannidis, P., Simao, F.A., Waterhouse, R.M., Manni, M., Seppey, M., Robertson, H.M., Misof, B., Niehuis, O., Zdobnov, E.M., 2017. Genomic features of the damselfly Calopteryx splendens representing a sister clade to most insect orders. Genome Biol. Evol. 9 (2), 415–430.

Jaquiéry, J., Rispe, C., Roze, D., Legeai, F., Le Trionnaire, G., Stoeckel, S., Mieuzet, L., Da Silva, C., Poulain, J., Prunier-Leterme, N., Ségurens, B., 2013. Masculinization of the X chromosome in the pea aphid. PLoS Genet. 9 (8), e1003690.

Jaquiéry, J., Peccoud, J., Ouisse, T., Legeai, F., Prunier-Leterme, N., Gouin, A., Nouhaud, P., Brisson, J.A., Bickel, R., Purandare, S., Poulain, J., 2018. Disentangling the causes for faster-X evolution in aphids. Genome Biol. Evol. 10 (2), 507–520.

Joseph, R.M., Carlson, J.R., 2015. Drosophila chemoreceptors: a molecular interface between the chemical world and the brain. Trends Genet. 31 (12), 683–695.

Kim, D., Minhas, B.F., Li-Byarlay, H., Hansen, A.K., 2018. Key transport and Ammonia recycling genes involved in aphid symbiosis respond to host-plant specialization. G3: Genes, Genomes, Genetics 8 (7), 2433–2443.

Knecht, Z.A., Silbering, A.F., Cruz, J., Yang, L., Croset, V., Benton, R., Garrity, P.A., 2017. Ionotropic Receptor-dependent moist and dry cells control hygrosensation in Drosophila. Elife 6, e26654.

Koh, T.W., He, Z., Gorur-Shandilya, S., Menuz, K., Larter, N.K., Stewart, S., Carlson, J.R., 2014. The Drosophila IR20a clade of ionotropic receptors are candidate taste and pheromone receptors. Neuron 83 (4), 850–865.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23 (21), 2947–2948.

Larter, N.K., Sun, J.S., Carlson, J.R., 2016. Organization and function of Drosophila odorant binding proteins. Elife 5, e20242.

Leal, W.S., 2013. Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. Annu. Rev. Entomol. 58, 373–391.

Legeai, F., Shigenobu, S., Gauthier, J.P., Colbourne, J., Rispe, C., Collin, O., Richards, S., Wilson, A.C., Murphy, T., Tagu, D., 2010. AphidBase: a centralized bioinformatic resource for annotation of the pea aphid genome. Insect Mol. Biol. 19, 5–12.

Miyamoto, T., Slone, J., Song, X., Amrein, H., 2012. A fructose receptor functions as a nutrient sensor in the Drosophila brain. Cell 151 (5), 1113–1125.

Nouhaud, P., Gautier, M., Gouin, A., Jaquiéry, J., Peccoud, J., Legeai, F., Mieuzet, L., Smadja, C.M., Lemaitre, C., Vitalis, R., Simon, J.C., 2018. Identifying genomic hotspots of differentiation and candidate genes involved in the adaptive divergence of pea aphid host races. Mol. Ecol. 27 (16), 3287–3300.

Peccoud, J., Ollivier, A., Plantegenest, M., Simon, J.C., 2009. A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. Proc. Natl. Acad. Sci. Unit. States Am. 109 (18), 1495–1500.

Peccoud, J., Mahéo, F., De La Huerta, M., Laurence, C., Simon, J.C., 2015. Genetic characterisation of new host-specialised biotypes and novel associations with bacterial symbionts in the pea aphid complex. Insect Conservation and Diversity 8 (5), 484–492.

Pelosi, P., Iovinella, I., Zhu, J., Wang, G., Dani, F.R., 2018. Beyond chemoreception: diverse tasks of soluble olfactory proteins in insects. Biol. Rev. 93 (1), 184–200.

Pickett, J.A., Wadhams, L.J., Woodcock, C.M., Hardie, J., 1992. The chemical ecology of aphids. Annu. Rev. Entomol. 37 (1), 67–90.

Pimentel, H., Bray, N.L., Puente, S., Melsted, P., Pachter, L., 2017. Differential analysis of

RNA-seq incorporating quantification uncertainty. Nat. Methods 14 (7), 687.

Pitts, R.J., Liu, C., Zhou, X., Malpartida, J.C., Zwiebel, L.J., 2014. Odorant receptor-mediated sperm activation in disease vector mosquitoes. Proc. Natl. Acad. Sci. Unit. States Am. 111 (7), 2566–2571.

Powell, G., Tosh, C.R., Hardie, J., 2006. Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. Annu. Rev. Entomol. 51, 309–330.

Prieto-Godino, L.L., Rytz, R., Cruchet, S., Bargeton, B., Abuin, L., Silbering, A.F., Ruta, V., Dal Peraro, M., Benton, R., 2017. Evolution of acid-sensing olfactory circuits in drosophilids. Neuron 93 (3), 661–676.

Qiao, H., Tuccori, E., He, X., Gazzano, A., Field, L., Zhou, J.J., Pelosi, P., 2009. Discrimination of alarm pheromone (E)-β-farnesene by aphid odorant-binding proteins. Insect Biochem. Mol. Biol. 39 (5–6), 414–419.

Ragsdale, D.W., Voegtlin, D.J., O'neil, R.J., 2004. Soybean aphid biology in North America. Ann. Entomol. Soc. Am. 97 (2), 204–208.

Ragsdale, D.W., Landis, D.A., Brodeur, J., Heimpel, G.E., Desneux, N., 2011. Ecology and management of the soybean aphid in North America. Annu. Rev. Entomol. 56, 375–399.

Richard, G., Legeai, F., Prunier-Leterme, N., Bretaudeau, A., Tagu, D., Jaquiéry, J., Le Trionnaire, G., 2017. Dosage compensation and sex-specific epigenetic landscape of the X chromosome in the pea aphid. Epigenet. Chromatin 10 (1), 30.

Rinker, D.C., Pitts, R.J., Zhou, X., Suh, E., Rokas, A., Zwiebel, L.J., 2013. Blood meal-induced changes to antennal transcriptome profiles reveal shifts in odor sensitivities in Anopheles gambiae. Proc. Natl. Acad. Sci. Unit. States Am. 110 (20), 8260–8265.

Robertson, H.M., 2015. The insect chemoreceptor superfamily is ancient in animals. Chem. Senses 40 (9), 609–614.

Robertson, H.M., 2019. Molecular evolution of the major arthropod chemoreceptor gene families. Annu. Rev. Entomol 64, 227–242.

Robertson, H.M., Warr, C.G., Carlson, J.R., 2003. Molecular evolution of the insect chemoreceptor gene superfamily in Drosophila melanogaster. Proc. Natl. Acad. Sci. Unit. States Am. 100 (Suppl. 2), 14537–14542.

Robertson, H.M., Baits, R.L., Walden, K.K., Wada-Katsumata, A., Schal, C., 2018a. Enormous expansion of the chemosensory gene repertoire in the omnivorous German cockroach Blattella germanica. J. Exp. Zool. B Mol. Dev. Evol. 330 (5), 265–278.

Robertson, H.M., Waterhouse, R.M., Walden, K.K., Ruzzante, L., Reijnders, M.J., Coates, B.S., Legeai, F., Gress, J.C., Biyiklioglu, S., Weaver, D.K., Wanner, K.W., 2018b. Genome sequence of the wheat stem sawfly, Cephus cinctus, representing an early-branching lineage of the Hymenoptera, illuminates evolution of hymenopteran chemoreceptors. Genome Biol. Evol. 10 (11), 2997–3011.

Rytz, R., Croset, V., Benton, R., 2013. Ionotropic receptors (IRs): chemosensory ionotropic glutamate receptors in Drosophila and beyond. Insect Biochem. Mol. Biol. 43 (9), 888–897.

Saina, M., Busengdal, H., Sinigaglia, C., Petrone, L., Oliveri, P., Rentzsch, F., Benton, R., 2015. A cnidarian homologue of an insect gustatory receptor functions in developmental body patterning. Nat. Commun. 6, 6243.

Sánchez-Alcañiz, J.A., Silbering, A.F., Croset, V., Zappia, G., Sivasubramaniam, A.K., Abuin, L., Sahai, S.Y., Münch, D., Steck, K., Auer, T.O., Cruchet, S., 2018. An expression atlas of variant ionotropic glutamate receptors identifies a molecular basis of carbonation sensing. Nat. Commun. 9 (1), 4252.

Smadja, C., Shi, P., Butlin, R.K., Robertson, H.M., 2009. Large gene family expansions and adaptive evolution for odorant and gustatory receptors in the pea aphid, Acyrthosiphon pisum. Mol. Biol. Evol. 26 (9), 2073–2086.

Smadja, C.M., Canbäck, B., Vitalis, R., Gautier, M., Ferrari, J., Zhou, J.J., Butlin, R.K., 2012. Large-scale candidate gene scan reveals the role of chemoreceptor genes in host plant specialization and speciation in the pea aphid. Evolution: Int. J. Organ. Evol. 66 (9), 2723–2738.

Stewart, S., Koh, T.W., Ghosh, A.C., Carlson, J.R., 2015. Candidate ionotropic taste receptors in the Drosophila larva. Proc. Natl. Acad. Sci. 112 (14), 4195–4201.

Sun, Y.F., De Biasio, F., Qiao, H.L., Iovinella, I., Yang, S.X., Ling, Y., Riviello, L., Battaglia, D., Falabella, P., Yang, X.L., Pelosi, P., 2012. Two odorant-binding proteins mediate the behavioural response of aphids to the alarm pheromone (E)-ß-farnesene and structural analogues. PLoS One 7 (3), e32759.

Terrapon, N., Li, C., Robertson, H.M., Ji, L., Meng, X., Booth, W., Chen, Z., Childers, C.P., Glastad, K.M., Gokhale, K., Gowin, J., 2014. Molecular traces of alternative social organization in a termite genome. Nat. Commun. 5, 3636.

van Emden, H.F., Harrington, R., 2007. Aphids as Crop Pests. CAB International, Wallingford. https://doi.org/10.1079/9780851998190.0000.

Via, S., 2001. Sympatric speciation in animals: the ugly duckling grows up. Trends Ecol. Evol. 16 (7), 381–390.

Wang, Q., Zhou, J.J., Liu, J.T., Huang, G.Z., Xu, W.Y., Zhang, Q., Chen, J.L., Zhang, Y.J., Li, X.C., Gu, S.H., 2019. Integrative transcriptomal and genomic analysis of odorant binding proteins and chemosensory proteins in aphids. Insect Mol. Biol. 28 (1), 1–22.

Wenger, J.A., Cassone, B.J., Legeai, F., Johnston, J.S., Bansal, R., Yates, A.D., Coates, B.S., Pavinato, V.A., Michel, A., 2017. Whole genome sequence of the soybean aphid, Aphis glycines. Insect Biochem. Mol. Biol. https://doi.org/10.1016/j.ibmb.2017.01.005.

Zhang, Y.V., Ni, J., Montell, C., 2013. The molecular basis for attractive salt-taste coding in Drosophila. Science 340 (6138), 1334–1338.

Zhang, R., Wang, B., Grossi, G., Falabella, P., Liu, Y., Yan, S., Lu, J., Xi, J., Wang, G., 2017. Molecular basis of alarm pheromone detection in aphids. Curr. Biol. 27 (1), 55–61.

Zhou, J.J., Vieira, F.G., He, X.L., Smadja, C., Liu, R., Rozas, J., Field, L.M., 2010. Genome annotation and comparative analyses of the odorant-binding proteins and chemosensory proteins in the pea aphid Acyrthosiphon pisum. Insect Mol. Biol. 19, 113–122.