

RESOURCE ARTICLE

Chromosomal-level assembly of *Bactericera cockerelli* reveals rampant gene family expansions impacting genome structure, function and insect-microbe-plant-interactions

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Handling Editor: Kin-Ming (Clement) Tsui**Abstract**

Lineage specific expansions and gene duplications are some of the most important sources of evolutionary novelty in eukaryotes. Although not as prevalent in eukaryotes compared to bacteria, horizontal gene transfer events can also result in key adaptations for insects, especially for those involved in insect-microbe interactions. In this study we assemble the first chromosomal assembly of the psyllid *Bactericera cockerelli* and reveal that the *B. cockerelli* genome has experienced significantly more gene expansion events compared to other Hemipteran representatives with fully sequenced genomes. We also reveal that *B. cockerelli*'s genome is the largest psyllid genome (567 Mb) sequenced to date and is ~15% larger than the other two psyllid species genomes sequenced (*Pachypsylla venusta* and *Diaphorina citri*). Structurally, *B. cockerelli* appears to have an additional chromosome compared to the distantly related psyllid species *P. venusta* due to a previous chromosomal fission or fusion event. The increase in genome size and dynamic nature of the *B. cockerelli* genome may largely be contributed to the widespread expansion of type I and II repeat elements that are rampant across all of *B. cockerelli*'s chromosomes. These repeat elements are distributed near equally in both euchromatic and heterochromatic regions. Furthermore, significant gene family expansions and gene duplications were uncovered for genes that are expected to be important in its adaptation to insect-plant and microbe interactions, which include transcription factors, proteases, odorant receptors, and horizontally transferred genes that are involved in the nutritional symbioses with their long-term nutritional endosymbiont *Carsonella*.

KEYWORDS*Bactericera cockerelli*, *Candidatus Carsonella ruddii*, carboxypeptidases, Psyllid genome, symbiosis-genes, transposable elements

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1 | INTRODUCTION

Lineage specific gene expansions are one of the most important means of adaptation in eukaryotes. These lineage specific expansions are primarily in response to microbes, environmental stress, and species specific signalling factors, often enabling both functional and expression divergence among gene copies (Lespinet et al., 2002). Although less common in animals compared to bacteria, horizontal gene transfer events have also been recognized as important sources of new genes and evolutionary novelty in eukaryotic genomes (Keeling & Palmer, 2008; Van Etten & Bhattacharya, 2020). For example, it has been observed in some insect genomes that horizontal gene transfer has played a major role in helping orchestrate symbiotic integration between the insect host and its nutritional endosymbionts (Husnik et al., 2013; Sloan et al., 2014). Genome sequences of both insects and their obligate nutritional endosymbionts have been crucial in shedding light onto the evolution of nutritional endosymbioses (Moran et al., 2008; The International Aphid Genomics Consortium, 2010). Nevertheless, far more genome sequences have been acquired from bacterial endosymbionts compared to insect hosts (NCBI, 2022). Therefore, by sequencing of additional genomes of insects with obligate symbionts we can gain a deeper understanding of how insect host genomes evolve with their long-term endosymbionts (Blaxter et al., 2022).

The evolution of endosymbiont genomes in sap-feeding insects has been extensively documented revealing extreme cases of genome reduction leading to the loss of essential genes for basic cellular processes (Bennett & Moran, 2013; McCutcheon & Moran, 2012). One of the most extreme examples of genome reduction in an endosymbiont was first observed in *Candidatus Carsonella ruddii* (hereafter referred to as *Carsonella*; Nakabachi et al., 2006), a nutritional endosymbiont that is universally present in psyllids (Spaulding & von Dohlen, 2001; Thao et al., 2000). Psyllids feed on plant sap and belong to the insect superfamily Psylloidea (psyllids) within the sub-order Sternorrhyncha, which includes aphids, whiteflies, and scale insects (Gullan & Martin, 2009). *Carsonella* supplements the psyllid's diet with essential amino acids that are deficient in the psyllid's sap diet (Baumann, 2005; McCutcheon & Moran, 2012; Moran & Wernegreen, 2000; Nakabachi et al., 2006).

At this time genome assemblies are only available for two psyllid species, *Diaphorina citri* (Saha et al., 2017) and *Pachypsylla venusta* (Li et al., 2020) which belong to two of seven psyllid families, the Psyllidae and Carsidaridae, respectively (Burckhardt et al., 2021). Therefore, in an effort to increase our understanding of psyllid genome evolution and nutritional symbioses we sequenced the potato (tomato) psyllid, *Bactericera cockerelli* (Šulc) genome, which belongs to a third psyllid family the Triozidae. The psyllid family Triozidae shares a more recent common ancestor to the family Psyllidae compared to the more divergent psyllid family Carsidaridae (Burckhardt et al., 2021). Like other psyllid species *B. cockerelli* feeds on plant sap and possesses the obligate nutritional endosymbiont *Carsonella*-BC (Riley et al., 2017). Similar to

P. venusta, *B. cockerelli* possesses one long-term obligate endosymbiont, *Carsonella*-BC, and has not been identified with an additional cosymbiont such as *Candidatus Proffttella armatura* in *D. citri* (hereafter referred to as *Proffttella*-DC; Nakabachi et al., 2013). The psyllid, *B. cockerelli* is native to North America (Crawford, 1914; Šulc, 1909) and is recognized as a major pest of solanaceous crops in its native range and as an invasive pest in New Zealand (Crosslin et al., 2010; Hansen et al., 2008; Liefting et al., 2008; Munyaneza et al., 2007). This is largely because *B. cockerelli* is a vector of the bacterium "*Candidatus Liberibacter psyllaourous*," which is associated with psyllid yellows disease (Hansen et al., 2008). Interestingly *Liberibacter* species are only known to infect the insect superfamily Psylloidea (Kwak et al., 2021; Raddadi et al., 2011; Wang et al., 2017), and may influence insect-plant interactions by playing a dual role as a plant pathogen and insect endosymbiont (Casteel et al., 2012). At this time *P. venusta* is not known to harbour a *Liberibacter* species; however, *D. citri* is a notorious vector of *Ca. L. asiaticus*, which is associated with Huanglongbing, a highly destructive disease of citrus (Bové, 2006). Sequencing additional genomes of psyllids that harbour and vector *Liberibacter* will increase our understanding of this unique and specialized relationship between *Liberibacter* taxa and Psylloidea.

In this study we sequence and annotate the first genome of *B. cockerelli* using short and long read sequencing technologies combined with proximity ligation to obtain a high quality chromosomal-level genome assembly for the research community. First, to gain insight into gene expansion and contraction events and natural selection on *B. cockerelli*'s genome we conducted evolutionary genomic analyses. Moreover, to further understand how the psyllid host and endosymbiont metabolisms integrate and evolve together we conducted comparative genomics analyses on the psyllid and endosymbiont genomes of *B. cockerelli*, *D. citri*, and *P. venusta*.

2 | MATERIALS AND METHODS

2.1 | Insect rearing

The *Bactericera cockerelli* culture sequenced in this study was derived ~2 years ago from a wild population in Temecula, California, USA. To prepare genomic material for genome sequencing a single mated female of *B. cockerelli* was used to establish an inbred line to reduce heterozygosity. The established isofemale line was maintained for over 6 generations on 4–8-week-old *Capsicum annuum* (California Wonder pepper) at 25°C under a 16L:8D light/dark. Both DNA and RNA material was obtained from this line at the same time for genome sequencing and annotation (see below). To increase coverage of the insect genome this insect line was verified to not be infected with *Ca. Liberibacter psyllaourous*, an insect symbiont/plant pathogen that commonly infects this psyllid species (Hansen et al., 2008), using the primers Y DRAG Q-PCR-IGS-7F/R and the qPCR conditions detailed in Casteel et al. (2012).

2.2 | Genome sequencing and assembly

In order to obtain a high-quality genome of *B. cockerelli*, a chromosome-level genome assembly of *B. cockerelli* was generated from long-read sequencing by Pacific Biosciences (PacBio) and short-read sequencing by Illumina with the Omni-C proximity ligation technique developed by Dovetail Genomics. Briefly, over 200 mg of both female and male adults from the *B. cockerelli* inbred line were collected and were starved overnight for 8–9 h. Psyllid adults were then flash frozen in liquid nitrogen, stored at -80°C , and shipped to Dovetail Genomics for genomic DNA extraction, library preparations, sequencing, and genome assemblies.

Briefly, genomic DNA was extracted using the Qiagen DNA extraction kit following the manufacturer's instructions (Qiagen) and quantified using the Qubit 2.0 Fluorometer (Life Technologies). The PacBio SMRTbell library was constructed using the SMRTbell Express Template Prep Kit 2.0 (PacBio) following the manufacturer's protocol. The SMRTbell library was then bound to sequencing polymerase with the Sequel II Binding Kit 2.0 (PacBio). DNA sequencing was performed on PacBio Sequel II 8M SMRT cells generating 137 Gb of raw data. A de novo assembly of PacBio reads was then generated using the WTDBG2 version 2.5 pipeline (Ruan & Li, 2020). Nontarget sequences of the output assembly were filtered using BLASTN and blobtools version 1.1.1 (Laetsch & Blaxter, 2017), and contig overlaps and/or potential haplotypic duplications were also removed using purge_dups version 1.2.3 (Guan et al., 2020). The final PacBio de novo assembly was then used with the Omni-C data to create the final chromosome-level genome assembly (below).

2.3 | Chromosome-length sequencing and genome assembly

For the Omni-C library, chromatin was fixed in place with formaldehyde in the nucleus to create a three-dimensional scaffold. The fixed chromatin was then extracted and randomly fragmented with DNase I. The resulting chromatin fragments were end-repaired, ligated to a biotinylated bridge adapter, and were followed by proximity ligation. After the proximity ligation, crosslinks were reversed for DNA purification, and the purified DNA was treated to remove biotin that was not internal to ligated fragments. The sequencing library was generated using NEBNext Ultra enzymes and Illumina-compatible adapters (NEB). Biotinylated fragments were isolated using streptavidin beads before PCR enrichment of library, and the library was sequenced on an Illumina HiSeqX.

To obtain chromosome-level scaffolds, the Omni-C library reads were aligned to the PacBio draft assembly using BWA-0.7.17 (Li, 2013). Read pairs mapped onto draft scaffolds were then used to generate a likelihood model using HiRise, a software pipeline designed specifically for using proximity ligation data to scaffold genome assemblies (Putnam et al., 2016). The generated likelihood model was used to identify and break putative misjoins, to score prospective joins, and make joins above a threshold. To evaluate the completeness of the

HiRise assembly, Benchmarking universal single-copy orthologues (BUSCOs) version 4.0.5 (Simão et al., 2015) was performed against the eukaryote_odb10 database (Table S1).

2.4 | TAD analyses

The three-dimensional structure of the *B. cockerelli* genome was analysed through the analysis of topologically associated domains (TADs) from Omni-C data (Table S2). TADs are a fundamental unit of chromatin topology where DNA sequences within a TAD region are in proximity and physically interact more frequently with one another compared to DNA sequences that occur between TAD regions (Dixon et al., 2012). For the analysis of TADs first Omni-C contact matrices were generated in two formats, namely cool and hic. Both contact matrices were generated from the same BAM file by using read pairs where both ends were aligned with a mapping quality of 60. TADs were identified using the Arrowhead (Rao et al., 2014) implemented with Juicer (Durand et al., 2016). TADs were called at three different resolutions: 10, 25, and 50 kbp. The parameters used were $-k$ KR $-m$ 2000 $-r$ 10,000, $-k$ KR $-m$ 2000 $-r$ 25,000, and $-k$ KR $-m$ 2000 $-r$ 50,000. A/B compartments were identified at 1 Mbp using the eigenvector program implemented in Juicer (Durand et al., 2016). The parameters used were KR BP 1,000,000. Isochores were predicted using the IsoFinder (Oliver et al., 2004). The parameters used were 0.90 p2 3000. The output was post-processed to convert into a bedpe format. CTCF sites were predicted using CREAM (Smith et al., 2006). The position weight matrix was downloaded from CTCFBSDB 2.0 website (Ziebarth et al., 2013). The output was then post-processed to convert it to a bed file. Multires files were generated for visualization in HiGlass using the clodius package (Kerpedjiev et al., 2018).

2.5 | RNA-seq and annotation

To annotate the final assembly of the *B. cockerelli* genome, repeat sequences in the final HiRise genome assembly were identified de novo and masked using RepeatModeler version 2.01 (Flynn et al., 2020) and RepeatMasker version 4.1.0 (Smit et al., 2013). We then used coding sequences (CDS) from *Acrythosiphon pisum*, AL4f downloaded from NCBI, GCA_005508785.1 (Li et al., 2019), *D. citri*, Dcitri_OGSv2.0 downloaded from https://citrusgreening.org/organism/Diaphorina_citri/genome (Saha et al., 2017) and *P. venusta*, AUS-FW-20181119 downloaded from NCBI (GCA_012654025.1) and github (lly005; Li et al., 2020) to train the initial ab initio models for *B. cockerelli* using AUGUSTUS version 2.5.5 (Stanke et al., 2006) and SNAP version 2006-07-28 (Korf, 2004).

We also performed RNA-seq from the same colony DNA was collected from for genome sequencing (above) to improve the *B. cockerelli* genome annotation. Five pooled psyllid samples, each containing >8 mg of both female and male adults per sample, were collected and flash frozen using liquid nitrogen. Samples were then shipped to

GENEWIZ for total RNA extraction and sequencing. Total RNA was extracted using Qiagen RNeasy Plus kit (Qiagen) following the manufacturer's instructions. Purified RNA integrity and quantity were measured using 4200 TapeStation (Agilent Technologies) and Qubit 2.0 Fluorometer (Life Technologies), respectively. After the rRNA depletion using Qiagen FastSelect rRNA HMR kit (Qiagen), the sequencing library was generated using NEBNext Ultra II RNA library preparation kit for Illumina by following the manufacturer's instructions (NEB). Briefly, enriched RNAs were fragmented for 15 min at 94°C. First strand and second strand cDNA were subsequently synthesized. cDNA fragments were end repaired and adenylated at 3'ends, and universal adapters were ligated to cDNA fragments, followed by index addition and library enrichment with limited cycle PCR. The sequencing libraries were then quality-checked using 4200 TapeStation (Agilent Technologies), and quantified with Qubit 2.0 fluorometer (Invitrogen) and quantitative PCR (Applied Biosystems). The sequencing was performed on Illumina HiSeq4000 150PE platform.

RNA-seq reads were analysed, prior to trimming, using FASTQC version 0.11.7 (Andrews, 2010). Adapter sequences were then trimmed using Trimmomatic version 0.39 (Bolger et al., 2014). For the annotation of genes RNA-seq reads were mapped onto the genome using STAR version 2.7 (Dobin et al., 2013), and a hint file was generated to predict intron-exon boundaries using AUGUSTUS version 2.5.5 (Stanke et al., 2006). MAKER (Cantarel et al., 2008), SNAP (Korf, 2004), and the hint file generated from AUGUSTUS (Stanke et al., 2006) were then used to predict genes in the repeat-masked reference genome. To help guide the prediction process, Swiss-Prot peptide sequences of *D. citri* and *P. venusta* were used from UniProt to generate peptide evidence in the MAKER pipeline (Cantarel et al., 2008). The database was downloaded and used in conjunction with the protein sequences from *A. pisum*. Only genes that were predicted by both SNAP (Korf, 2004) and AUGUSTUS version 2.5.5 (Stanke et al., 2006) were retained in the final gene sets. To help assess the quality of the gene prediction, annotation edit distance (AED) scores were generated for each of the predicted genes as part of the MAKER pipeline (Cantarel et al., 2008). Functional annotation was further performed using BLASTP against the UniProt database.

To determine the relative read count for annotated genes, a custom index was created for the *B. cockerelli* genome using the HISAT2 version 2.2.1 build command, and all trimmed reads were aligned to the genome using HISAT2 version 2.2.1 (Kim et al., 2015). The outputs of the HISAT2 alignment were compressed into BAM files and then sorted using SAMTOOLS version 1.14 (Li et al., 2009). Gene abundance tables were created using StringTie version 2.0.4 (Pertea et al., 2015) after inputting the sorted BAM files.

2.6 | Detecting contaminant proteins from the official Diaci_version 2.0 protein data set prior to orthologue analysis

To determine if the official Dcitri_OGSv2.0 (Diaci_version 2.0 protein) data set contained contaminated proteins from the

common microbial contaminant *Walleimia mellicola* a BLASTP database was constructed using proteins from *W. mellicola* from NCBI (GCF_000263375.1_Walleimia_sebi_v1). A separate BLASTP database was also made with *B. cockerelli* proteins from our study. BLASTP was conducted separately on both databases using the Diaci_version 2.0 proteins as the query with an E value of 10e-10 as a significance cutoff.

2.7 | Comparative genomics and orthologue analyses

Orthologous clusters of CDSs from *B. cockerelli*, *D. citri* and *P. venusta* were determined using OrthoVenn2 (Xu et al., 2019) using default settings. Similar to Li et al. (2020) synteny of one-to-one orthologues in the chromosomes of *B. cockerelli* and *P. venusta* was determined using MCSanX_h (Wang et al., 2012), and SynVisio (Bandi & Gutwin, 2020) was used to visualize results. All *B. cockerelli* CDSs not assigned to clusters were screened for matches to the NCBI nr database (downloaded on 04/2021) using DIAMOND version 2.0.13 (Buchfink et al., 2015) with an E value cutoff of 10e-10. GO enrichment analyses were conducted within OrthoVenn2 (Xu et al., 2019). For KEGG pathway analysis of psyllid proteins, KO numbers were retrieved using KofamKoala (Aramaki et al., 2020) and analyses were conducted using KEGG-Decoder (Graham et al., 2018) and KEGG Mapper (Kanehisa et al., 2022; Kanehisa & Sato, 2020).

To estimate rates of molecular evolution among *B. cockerelli*, *D. citri* and *P. venusta* we calculated nonsynonymous substitutions per nonsynonymous site (dN) and synonymous substitutions per synonymous site (dS) for one-to-one CDS orthologues from *B. cockerelli*, *D. citri*, and *P. venusta*. Similar to Degnan et al. (2010) the CDS were aligned based on the MAFFT (version 7.427) alignment of the amino acid sequences (Kato & Standley, 2013), then all gaps and stop codons were removed, and pairwise estimates of (dN) and (dS) were calculated in Paml (Yang, 1997) based on the method of Goldman and Yang (1994). Gene pairs were excluded when dS was saturated (dS ≥ 3.0). In addition, this method of estimating rates of molecular evolution was used to evaluate SNP differences detected in our previously published *Carsonella*-BC genome (Riley et al., 2017) and the short-read data generated above (see below). Given the paucity of mutations in these *Carsonella*-BC gene comparisons, we also excluded gene pairs when the number of synonymous or nonsynonymous changes was 0.

2.8 | Gene expansion and contraction analyses

For analyses of gene contractions and expansions in psyllids, proteomes of *B. cockerelli*, 10 other hemipterans and 1 outgroup (*Frankliniella occidentalis*; Table S3) were first analysed with the BUSCO (version 5.2.2; Manni et al., 2021) hemiptera_odb10 database to identify single-copy orthologous genes (Table S4). Genes identified in all 12 genomes were individually aligned with MAFFT (version 7.427;

Katoh & Standley, 2013), trimmed of gap containing positions and merged into a single alignment. This alignment was used for phylogenetic reconstruction of a species tree with RAxML-HPC BlackBox (version 8.2.12; Stamatakis, 2014) on the CIPRES (Miller et al., 2010) webserver using the PROTCATJTT model of evolution and recommended default bootstrap parameter of "Let RAxML halt bootstrapping automatically". Following the method outlined in the CAFÉ tutorial by Mendes et al. (2020) the species tree was converted to an ultrametric tree using r8s (version 1.7; Sanderson, 2003). This was based on an estimated divergence between the Psylloidea and Aphidoidea of 338 million years ago (Mya; Johnson et al., 2018).

The proteomes were then grouped into orthologous gene clusters using OrthoVenn2 with the default settings (Xu et al., 2019). Note that prior to clustering proteomes predicted gene isoforms were reduced to the single longest isoform. These clusters were then analysed to determine patterns of expansion and contraction over evolutionary time with CAFÉ (version 5; Mendes et al., 2020). As recommended by Mendes et al. (2020), all clusters with ≥ 100 gene copies in a single species were initially filtered out, and a final birth-death (λ) parameter was estimated based on an error model that accounted for the possibility of genome assembly errors. Large gene clusters were then analysed and final estimates of gene families significantly expanding or contracting across the phylogenetic tree were determined. Annotations, locations, organizations and sequences of genes belonging to expanding and contracting families within the Psylloidea were assessed. In addition to previous annotations discussed, cluster genes were also subjected to HMMER (2022) (hmmmer.org) searches with the Pfam database (version 35.0, --cut_tc) (Mistry et al., 2021) and PANNZER2 (Törönen et al., 2018) rapid functional annotation tool using default parameters. Cluster functions were assigned based on a majority rule consensus of predicted functional annotations. Further, gene locations for members of rapidly expanding families predicted to be transposable elements were retrieved and identified as being euchromatic or heterochromatic based on the TAD analysis (above). The frequency of gene localizations within these distinct chromosomal regions were compared both within families and chromosomes using a two-sample test for equality of proportions with continuity correction in R (version 4.1.2; R Core Team, 2020).

2.9 | Identifying sequences from insect mitochondria and its obligate symbiont *Carsonella*

The mitochondrion genome sequence was acquired by mapping the short reads to the existing *B. cockerelli* mitochondrion reference sequence (NC_030055.1; Wu et al., 2016) from NCBI GenBank. Specifically, the OmniC Illumina paired-end 150nt reads were trimmed using Trimmomatic 0.39 (Bolger et al., 2014) using the following settings (LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36). A total of 167,060,472 read pairs were trimmed with 92.86% surviving. Trimmed paired-end reads were mapped to the *Bactericera cockerelli* mitochondrion (NC_030055.1) using

BWA-0.7.17 (Li, 2013) using default settings. A total of 132,773 reads mapped onto the reference genome and had a total of 1309 \times coverage. Consensus calling was performed with SAMTOOLS 1.14 (Li et al., 2009) and bcftools 1.14 (Li, 2011) and the final consensus fasta file was created within bcftools 1.14 (Li, 2011) using the vcf2fq program and EMBOSS 6.6.0 (Rice et al., 2000) using the seqret program. The *Carsonella-BC* genome sequence was acquired as above for the mitochondrion by mapping the short reads to the existing *Carsonella-B. cockerelli*-reference sequence (CP019943.1; Riley et al., 2017). A total of 395,485 short reads mapped onto the reference genome and had a total of 341 \times coverage. Similar to Thairu et al. (2021) a custom Perl script was used to count the number of SNPs between the previously published *Carsonella-BC* genome (Riley et al., 2017) and the consensus generated by short read mapping.

2.10 | Identifying nutritional symbiosis-related genes

To identify putative nutritional symbiosis-related genes, KofamKOALA-KEGG and NCBI-BLAST were used to annotate amino acid metabolism pathways for all three psyllid species, *B. cockerelli*, *D. citri* and *P. venusta*. Specifically, we conducted both BLASTP and TBLASTN against the *B. cockerelli* protein data set and the initial PacBio assembly, respectively, using protein sequences from *P. venusta* and *D. citri* as the query with an E value of $10e-10$ as a significance cutoff. In addition, *B. cockerelli*'s de novo mRNA transcripts were also investigated for putative symbiosis-related genes in amino acid pathways based on previous symbiosis studies (Sloan et al., 2014). Specifically, the trimmed pair-ended RNA-seq reads from each of the five libraries (see above) were concatenated into a single de novo transcriptome assembly using Trinity version 2.13.2 (Grabherr et al., 2011). Any putative symbiosis transcripts identified using TBLASTN against *P. venusta/D. citri* query sequences were further screened against the final *B. cockerelli* scaffolds using nucleotide BLASTN to confirm that the transcripts were encoded in the psyllid's genome. If putative symbiosis genes were identified on unassembled PacBio contigs, contigs were further screened using BLASTX against the NCBI nr database (downloaded on 04/2021) using DIAMOND version 2.0.13 (Buckfink et al., 2015) with an E value cutoff of $10e-10$ to determine if the contigs contained other psyllid/insect related genes, to further confirm that the transcripts were encoded in the psyllid's genome. Genes acquired through horizontal gene transfer that were initially identified in *P. venusta* (Sloan et al., 2014) were also searched against *B. cockerelli*'s genome using the same BLAST protocol that was used for the symbiosis genes.

Amino acid biosynthetic pathways were reconstructed manually with HGT and symbiosis related genes using KEGG Mapper (Kanehisa et al., 2022; Kanehisa & Sato, 2020) for psyllids, the reference genomes for the psyllid symbionts *Carsonella* and *Proffttella* (NC008512.1; CP003467.1; CP019943.1; CP003468.1), and the EcoCyc database (Keseler et al., 2013). The HGT genes were then aligned with other bacterial representative sequences using MAFFT

(version 7.427) (Kato & Standley, 2013). Phylogenetic analysis was performed on the alignment with RAxML-HPC BlackBox version 8.2.12 on the CIPRES webserver (Miller et al., 2010) using the GTR model and 100 bootstrap replicates. The bipartition trees generated from RAxML were visualized and exported using FIGTREE version 1.4.4 (Rambaut, 2018).

3 | RESULTS

3.1 | Whole chromosome assembly and annotation of *Bactericera cockerelli*

PacBio sequencing of *Bactericera cockerelli* DNA resulted in 5,869,245 reads with the initial filtered assembly resulting in 4411 scaffolds with 4409 scaffolds over 1 kbp resulting in a total assembly size of 567 Mb (Table 1). Using both the PacBio assembly and the Omni-C library reads Dovetail HiRise scaffolding resulted in 51,238,984 read-pairs with 3000 joins and 0 breaks to the initial assembly resulting in a total final assembly size of 567 Mb (Table 1). This final Dovetail HiRise Assembly was a significant improvement from the initial PacBio assembly in contiguity resulting in a 32% reduction in scaffolds (1411 total) with 1409 scaffolds >1 kbp and ~a 98X increase in N_{50} value compared to the initial filtered PacBio assembly (Table 1). The %GC of this final alignment was ~35% (± 4.6 SD). To further evaluate the completeness of the genome Benchmarking universal single-copy orthologues (BUSCOs; Simão et al., 2015) analysis was conducted on the final assembly and we found 93.7% of the complete BUSCOs were present based on the eukaryota database (Table S1).

The TAD analysis pipeline identified 4998 CTCF Binding Sites, which are located at the boundaries of the TAD regions and are generally highly enriched in the regions of genome that are transcriptionally active (Bonev & Cavalli, 2016). TAD analysis further identified ~48% of the whole genome as active/euchromatin and ~51% as inactive/heterochromatin (Table S2).

TABLE 1 Assembly statistics for the initial PacBio and the final Dovetail assembly of *Bactericera cockerelli*

	PacBio filtered assembly	Dovetail HiRise assembly
Total length (bp)	567,134,386	567,434,386
Largest scaffold	4,017,258	65,445,692
Number of scaffolds	4411	1411
Number of scaffolds >1 kbp	4409	1409
Number of gaps	16	3016
Number of N's per 100 kbp	0.06	52.93
N_{50}	423,673	41,634,607
L_{50}	331	6
N_{90}	53,963	21,995,751
L_{90}	1830	13

The TAD analysis further revealed a total of 13 putative chromosomes that correspond to the 13 largest scaffolds from the final Dovetail HiRise assembly representing 92% of the final assembly length (~522 Mb; Table 2; Figure S1). We analysed the synteny of one-to-one orthologues from the 13 largest chromosomes/scaffolds of both *B. cockerelli* and *P. venusta* and found syntenic blocks of one-to-one orthologues among 11 autosomes and 1 sex chromosome (X) (Figure 1). However, all of the one-to-one orthologue blocks were highly rearranged within each scaffold/chromosome pair. In addition, we detected evidence of a chromosomal fission/fusion event accounting for two *B. cockerelli* chromosomes (bc9 and bc12) and one *P. venusta* chromosome (pv3; Figure 1). The 13th largest scaffold in *P. venusta* (pv12) was not indicated as a chromosome in Li et al. (2020); however, this scaffold shares 24 one-to-one orthologues with chromosome bc4 and one orthologue in both bc1 and bc3. These latter orthologues are not arranged in syntenic blocks (≥ 2 genes), however, and therefore are not indicated in Figure 1.

Using our annotation pipeline (see Materials and Methods) we annotated 22,280 genes with an average length of 1726 bp within a total coding region of 38,474,975 bp. A total of 15.4% of genes were annotated as single-exon genes. Using RepeatModeler version 2.01 (Flynn et al., 2020) and RepeatMasker version 4.1.0 (Smit et al., 2013) we found that 43.3% of the total genome was masked for repeats and 10.9%, 4.63%, 0.71%, and 2.07% of the genome is composed of Class I transposable element (TE) repeats, Class II TEs, low complexity repeats, and simple repeats, respectively. Annotation edit distance (AED) scores, were calculated for each gene (Table S5) to measure how well a predicted gene is supported by external evidence (UniProt protein and mRNA sequences). The AED score ranges from 0–1 where a lower score represents more evidence support for a gene. We found that the majority of annotated genes (80%) possess an AED score under 0.5 (Figure S2).

The 15.2 kbp mitochondrion genome sequence obtained here had 100% coverage and 100% identity to the *B. cockerelli* mitochondrion reference sequence (NC_030055.1) using BLASTN. In contrast, in the *Carsonella*-BC symbiont genome we identified a total of 2143 SNPs in the 174 kbp genome. These differences were distributed among 182 of the 195 predicted CDSs. None of the differences resulted in premature stop codons, but 155 CDSs did have ≥ 1 nonsynonymous mutation that would alter the amino acid position. However, estimates of dN/dS indicated that all of these *Carsonella*-BC CDSs are under strong purifying selection (negative selection) ($dN/dS = 0.065 \pm 0.0743$). This is consistent with what has been previously observed among other *Carsonella* genomes (Sloan & Moran, 2012).

3.2 | Comparative genomics of psyllid species with complete genomes

We compared *B. cockerelli* coding sequences annotated in this study (Table S5) to two other psyllid species with fully sequenced genomes, *P. venusta* (Li et al., 2020) and *D. citri* (Saha et al., 2017).

During the preliminary analysis of proteins from the official *D. citri* (Diaci version 2.0) protein data set (Saha et al., 2017) that is available publicly at <https://citrusgreening.org>, we found hundreds of putative fungal proteins, many that are only conserved in fungi and bacteria in the psyllid protein data set. We conducted BLASTp on these suspect proteins and they had 100% amino acid identity to the fungus *Walleimia mellicola* (previously *sebi*) and did not hit any of the *D. citri* v. 1.0 proteins that are currently available in the NCBI database. On

further inspection we also found conserved housekeeping proteins (which are present in animals as well) that had 100% amino acid identity to *W. mellicola*. A total of 717 Diaci_version 2.0 proteins were identified as likely fungal contaminants, with 389 having 100% identity to *W. mellicola* proteins. While many ($n = 421$) had some homology with *B. cockerelli*, these proteins had an average of 47% greater amino acid identity to *W. mellicola*. The remainder had no *B. cockerelli* homologue ($n = 296$) and $\geq 90\%$ amino acid identity to *W. mellicola*. *Walleimia mellicola* is a xerophilic food- and airborne fungus that commonly contaminates foodstuffs, especially highly sugared materials (Zalar et al., 2005). We removed these putative contaminant *W. mellicola* fungal proteins ($n = 717$) from the official Diaci_version 2.0 protein data set for all analyses conducted in this study.

We found that all three psyllid species share 6139 orthologous clusters and genes within these clusters are significantly enriched for three GO term categories (DNA integration, GO:0015074; transposition, DNA-mediated, GO:0006313; RNA-directed DNA polymerase activity, GO:0003964; Table S6). Orthologous clusters that are only present in *B. cockerelli* (557 clusters) compared to *P. venusta* and *D. citri* are primarily related to transposition and protein processing based on GO enrichment analyses (Table S6).

Among the shared orthologous clusters found in all three psyllid species 2016 genes were identified as single-copy 1:1:1 gene clusters (Table S7). We analysed these single-copy genes for signatures of natural selection by calculating dN and dS values among each species pair. Overall, for all single copy orthologue pairs, where dS was not saturated the dN/dS ratio was < 1 , indicating

TABLE 2 Chromosome scaffolds of *Bactericera cockerelli* identified using the TAD analysis pipeline

Chromosomes	Scaffolds	Sizes
Chr. 1	Scaffold_1	65,445,692
Chr. 2	Scaffold_2	55,905,524
Chr. 3	Scaffold_3	52,961,393
Chr. 4	Scaffold_4	50,895,300
Chr. 5	Scaffold_5	46,310,608
Chr. 6	Scaffold_6	41,634,607
Chr. 7	Scaffold_7	38,204,804
Chr. 8	Scaffold_8	33,343,378
Chr. 9	Scaffold_9	32,048,629
Chr. 10	Scaffold_10	30,660,657
Chr. 11	Scaffold_11	29,265,850
Chr. 12	Scaffold_12	23,608,477
Chr. X (sex chr.)	Scaffold_13	21,995,751

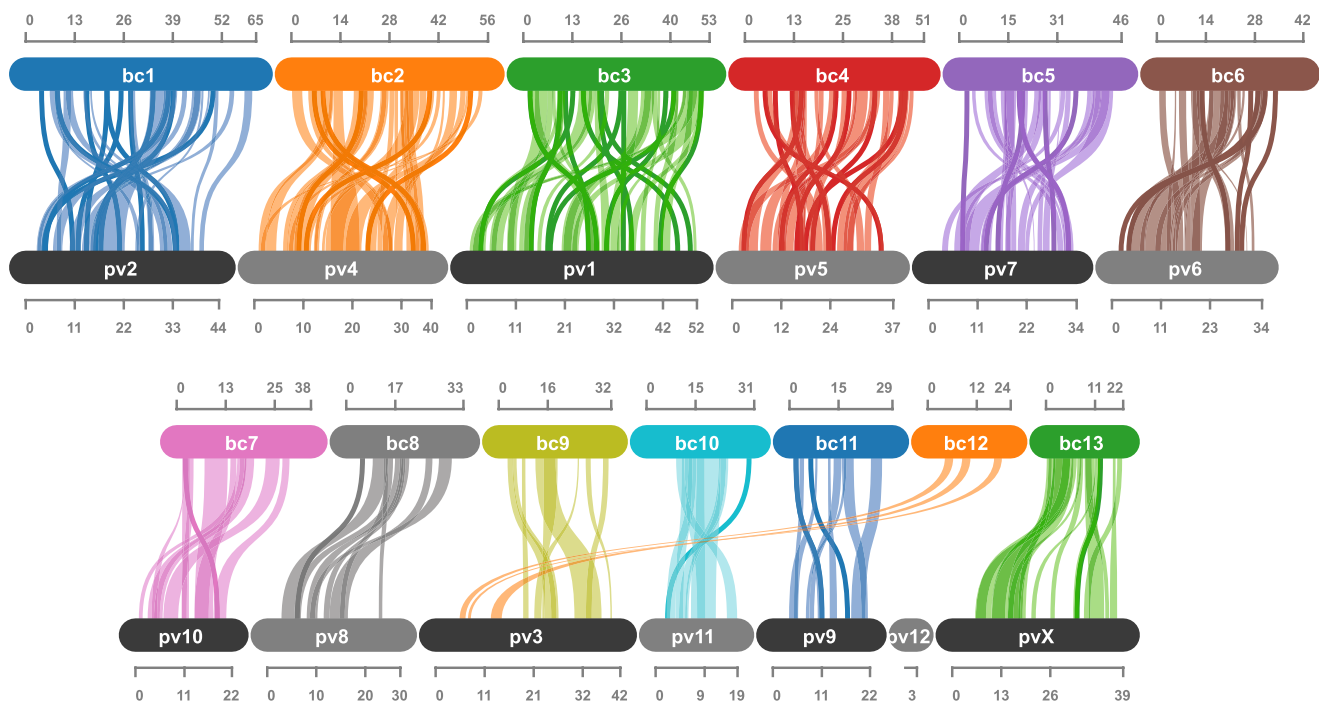


FIGURE 1 Comparison of syntenic orthologue blocks (≥ 2) between the 13 largest chromosomes/scaffolds identified in *B. cockerelli* (bc) and *P. venusta* (pv). Chromosomes/scaffold numbers correspond to the rank size of the scaffold within each species (1 is the largest). pvX represents the sex chromosome identified in Li et al. (2020). Rulers represent chromosome/scaffold length in Mb

that purifying selection is widespread among single-copy 1:1:1 orthologues. Specifically, we found 1323 gene pairs between *B. cockerelli* and *D. citri* that had an average dN/dS ratio of 0.06 (± 0.04 SD). A total of 127 gene pairs between *B. cockerelli* and *P. venusta* had an average dN/dS ratio of 0.05 (± 0.05 SD), and a total of 285 gene pairs between *P. venusta* and *D. citri* had an average dN/dS ratio of 0.05 (± 0.05 SD). None of these gene pairs were significantly enriched for GO terms; however, 33, 2, and 10 gene pairs under purifying selection in *B. cockerelli* and *D. citri*, *B. cockerelli* and *P. venusta*, and *P. venusta* and *D. citri*, respectively, were related to nutrition and 19, 10, and 16 gene pairs in *B. cockerelli* and *D. citri*, *B. cockerelli* and *P. venusta*, and *P. venusta* and *D. citri*, respectively, were related to environmental response genes and immunity (Table S8).

A total of 3892 proteins in *B. cockerelli* were not found in any orthologous clusters and they were characterized further with DIAMOND (Buchfink et al., 2015). A total of 64.3% (1973) of these query sequences had a significant best hit to *D. citri* (mean amino acid identity $\sim 69.9\%$ [± 17.1 SD]). While the remaining 35.7% (1,094) of query sequences with significant hits primarily hit species within Insecta with a minority hitting species in Arachnida (mean amino acid identity $\sim 58.2\%$ [± 17.0 SD]). Most of these putative proteins were classified as "hypothetical", "unnamed", or "uncharacterized". A minority of query sequences (4.7% [825]) had no significant hits to the NCBI nr database and appear to be orphan genes.

3.3 | Large number of gene family expansions in *Bactericera cockerelli*

A fully resolved hemipteran phylogeny with strong maximum likelihood bootstrap support was estimated based on 632 complete, single-copy genes shared by *B. cockerelli* and 11 additional genomes (of 2510 Hemipteran BUSCOs; Tables S3 and S4; Figure 2). After converting the tree branch lengths to ages (Mya), the expansions and contractions of 22,478 predicted gene families were estimated (Table S9; Figure 2). This analysis suggested that *B. cockerelli* and *D. citri* diverged from one another ~ 86 Mya and their most recent common ancestor diverged from *P. venusta* ~ 130 Mya. Note that the proteomes used were not masked for repeats prior to clustering (Table S3). Across the entire phylogeny *B. cockerelli* had the greatest number of significantly expanding protein families ($n = 157$) and zero contracting families. Nearly a third of these families (52/157) are unique to psyllids and nearly a fifth (30/157) are unique to *B. cockerelli*. Perhaps unsurprisingly, over three quarters of the families have annotations consistent with either type I (RNA intermediate) or type II (DNA intermediate) repetitive elements (120/157; Figure 3).

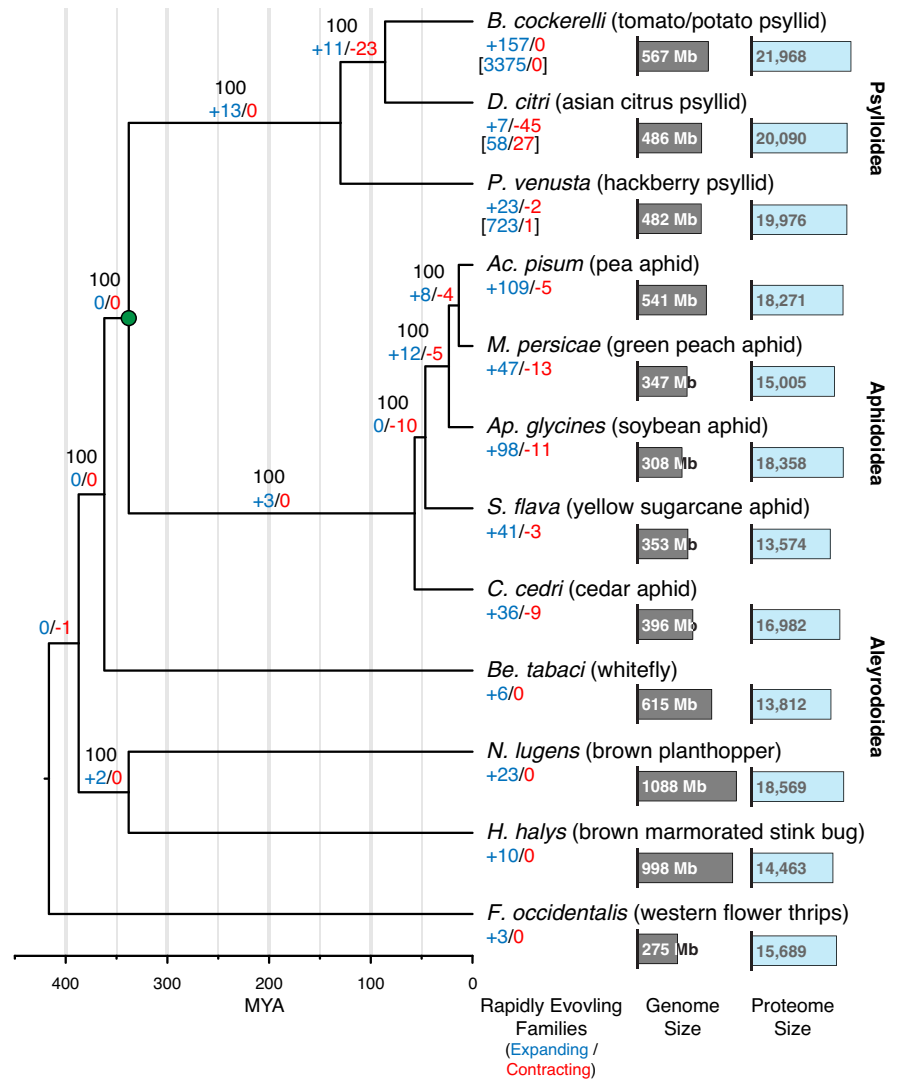
To evaluate the effect of chromosomal confirmation and activity on recent transposition, we determined the proportion of recently expanded transposon gene families within euchromatic (A) or heterochromatic (B) regions. We compared the relative

proportions on each chromosome to the proportion of each chromosome comprised of euchromatin and heterochromatin. Throughout the majority of the genome, there is no significant bias in recent transposition events within euchromatic or heterochromatic regions (Figure S3). However, on chromosome/scaffold_1 we did detect a significantly greater proportion of recent transpositions within the heterochromatic regions of this chromosome/scaffold. By analysing the euchromatic or heterochromatic localization within individual gene families with more than 10 gene copies ($n = 94$) we identified 14 families significantly biased towards heterochromatin and 17 significantly biased towards the euchromatic regions (Proportion test, d.f. = 1, $p < .05$). This revealed that at least part of the trend detected on chromosome/scaffold_1 was associated with three, type II (DNA) repeat families where the vast majority of copies were in the heterochromatic regions of chromosome/scaffold_1.

While 16 families have no identifiable functions, there were several expanded families we detected in *B. cockerelli* with putative functional annotations that were not type I or II repeats. For instance, expansions of putative regulatory factors such as zinc finger genes, including C2H2-ZNF domain containing homologues (PF00096) which constitutes one of the largest classes of transcription factors in eukaryotes (119 proteins in eight families) and helix-turn-helix motif regulators (12 proteins in 1 family) were detected. In addition, we detected expansions in several families of proteases including metalloproteases and zinc carboxypeptidases. Genes from one of these protease families consistently had a significant best BLASTP hit to NCBI nr for carboxypeptidase D in other insects however identity in the top 10 hits was low and ranged 36%–42% with a query coverage $>90\%$. Finally, a family of 12 *B. cockerelli* specific putative seven transmembrane odorant receptors (7tm) were identified. Together these regulatory, proteolytic and odorant receptor genes may be key factors in *B. cockerelli* for species specific regulation and host plant interactions.

In contrast to *B. cockerelli*, substantially fewer gene families were identified as expanding or contracting in *D. citri* and *P. venusta* despite the general similarity in their proteome sizes (Figure 2). In fact, the entirety of contracting gene families in *D. citri* ($n = 45$) were identified as expanding families in *B. cockerelli* (Figure 3). Further, unlike *B. cockerelli* only one of the expanding families in *D. citri* is a type I repetitive element. The six remaining expanding families are involved in chromosome structure ($n = 3$; histone like proteins, kinetochore component), detoxification ($n = 1$; cytochrome P450), cell-cell interactions ($n = 1$; fibronectin III binding domain proteins) and organic solute transport ($n = 1$). In *P. venusta*, the overwhelming majority of expanding gene families have no predicted functions (19/23). Of the remainder, three are likely type I or type II repetitive elements and one is a cysteine protease. Finally, we examined the rapidly evolving families along the internal branches of the Psylloidea clade only to find that all but one of the expanding and contracting families are represented in the terminal gene families. The only unaccounted for gene family is yet another type I repetitive element.

FIGURE 2 Evolutionary relationships of *B. cockerelli* and representative hemipterans. Maximum likelihood phylogeny of 632 conserved, single copy orthologues (a 234,854 amino acid alignment) was used as the basis to identify trends in gene family expansions and contractions (Lambda [birth-death] = 0.0023307 - probability any gene will be gained or lost). The node marked with a green circle represents the estimated divergence between the Psylloidea and Aphidoidea of 338Mya (Johnson et al., 2018) used to calibrate the ultrametric tree. Numbers in black at each node represent the bootstrap values, and the numbers of significantly expanding (blue) and contracting (red) gene families are also labelled. Among the Psylloidea the number of genes represented by the expanding or contracting families are listed within brackets. Below each species name the assembled genome and proteome sizes are indicated and shown as proportionally sized horizontal bars, grey and blue, respectively



3.4 | Identification of putative symbiosis genes in *Bactericera cockerelli*

We investigated the presence and absence of amino acid biosynthesis genes among the three psyllid species, *B. cockerelli*, *D. citri* and *P. venusta*, as well as their obligate endosymbionts (Figure 4). We identified a total of 54 *B. cockerelli* genes that may collaborate with *Carsonella* based on previous bacteriocyte gene expression studies in psyllids, aphids and other sap-feeding insects (Hansen & Moran, 2011; Husnik et al., 2013; Sloan et al., 2014; Tables 3 and Table S10). Overall, these genes are largely conserved across all three psyllid species including those that were horizontally transferred from bacteria (Figure 4, Table 4).

3.4.1 | Nonessential amino acid biosynthesis pathways

Carsonella does not encode nonessential amino acid biosynthesis pathways except for *glyA* in the glycine pathway (Nakabachi

et al., 2006). In turn, these metabolites need to be provided by either their psyllid host and/or their psyllid host's diet. All three psyllid species encode asparagine, aspartate and tyrosine biosynthesis pathways via asparagine synthase (ASNS, EC 6.3.5.4), asparaginase (ASPG, EC 3.5.1.1) and phenylalanine-4-hydroxylase (PAH, EC 1.14.16.1), respectively (Table 3, Figure 4). In addition, psyllid genes involved in the biosynthesis of other nonessential amino acids, such as proline, tyrosine, serine and alanine, have also been identified (Table S10, Figure 4). Moreover, all three psyllid genomes contain glutamine synthase (GS, EC 6.3.1.2) and glutamate synthase (GOGAT, EC 1.4.1.13). These latter genes are potentially crucial for recycling ammonia in bacteriocytes via the GOGAT cycle to serve as the building blocks and amino donors in EAA biosynthesis (Table 3, Figure 4; Hansen & Moran, 2011; Sloan et al., 2014). Interestingly, four intact copies of GS are encoded in *B. cockerelli* whereas only one copy of this ammonia recycling enzyme is present in the other two psyllid genomes (Table 3). All four gene copies are unique in identity and have an average amino acid identity of 82.8% (± 7.1 SD) based on BLASTP. Gene copies are located in tandem in pairs (e.g., ANN10145-RA & ANN10146-RA; ANN10148-RA & ANN10149-RA) with one gene

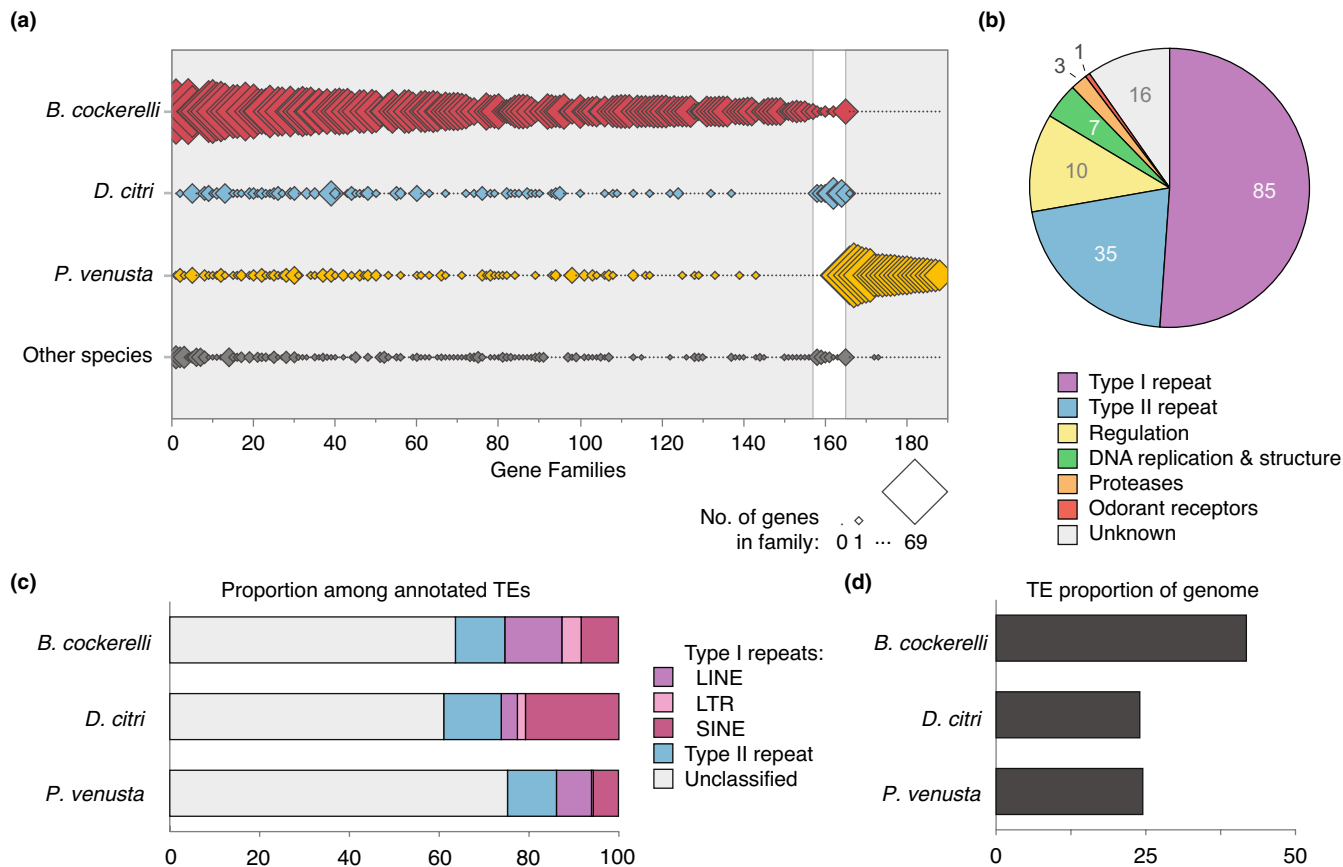


FIGURE 3 Characteristics of the significantly expanding and contracting gene families among the Psylloidea. (a) Diamond plot of the 188 total significantly expanding and/or contracting gene families in the Psylloidea. The size of the diamond represents the number of genes in each gene family and range from 0 to 69 genes. The category “other species” represent the average gene copy number among the nine other species analysed in Table S3. The horizontal axis represents rank order of homologous gene families that are present or absent in each lineage. Gene families 1–157 are expanding in *B. cockerelli*, families 158–164 are expanding in *D. citri*, and family 165 is contracting and families 166–188 are expanding in *P. venusta*. (b) Distribution of predicted functions of expanding gene families in *B. cockerelli*. (c) Proportion of different transposable element (TE) classes ≥ 100 nt in length and (d) total TE percent content in the genomes of *B. cockerelli*, *D. citri*, *P. venusta*. Data from *D. citri* was estimated from Gilbert et al. (2021), and data from *P. venusta* was retrieved from Petersen et al. (2019)

(COPA; Coatomer subunit alpha, ANN10147-RA) located between the gene pairs on Scaffold 5. Gene expression of one gene copy in each tandem gene pair (ANN10146-RA and ANN10148-RA) was highly expressed in *B. cockerelli* (>the 96th percentile) whereas the other two gene copies were expressed moderately (42nd and 52nd percentile) relative to other genes in *B. cockerelli*'s whole body (Table 3).

3.4.2 | Psyllid genes that collaborate with *Carsonella* for essential amino acid biosynthesis

Similar to the nutritional endosymbionts of other plant phloem-feeding insects, *Carsonella* has lost the genes *argABCDE*, which synthesize ornithine (Hansen & Moran, 2014). All three psyllid genomes encode the genes delta-1-pyrroline-5-carboxylate synthetase (P5CS, EC 2.7.2.11/1.2.1.41) and ornithine transaminase (OAT, EC 2.6.1.13) that synthesize ornithine from glutamate in the arginine biosynthesis pathway, and they are highly expressed (above the

80th percentile) in the whole body relative to other genes in *B. cockerelli* (Table 3, Figure 4). *Carsonella* has also lost the terminal step for phenylalanine biosynthesis (*aspC*), nevertheless all three psyllid species genomes encode aspartate aminotransferase (AAT, EC 2.6.1.1), which may complement *Carsonella*'s phenylalanine biosynthesis pathway (Table 3, Figure 4). There is a total of one cytoplasmic and four mitochondrial gene copies of AAT that are expressed at a moderate to high level in *B. cockerelli* relative to other genes in *B. cockerelli* (Table 3).

In a few cases psyllid host genes are similar in enzymatic function, depending on the direction of the reaction, when compared to genes in *Carsonella*. For example, *metE*, which encodes homocysteine S-methyltransferase is retained in all three *Carsonella* genomes and homocysteine S-methyltransferase (BHMT, EC 2.1.1.10) is encoded in all three psyllid genomes as well (Figure 4). We found two BHMT gene copies in *B. cockerelli* and their expression levels were both above the 60th percentile relative to the expression of all other *B. cockerelli* genes for the whole body (Tables S10 and S11). The gene *ilvE*, which encodes branched-chain aminotransferase (BCAT,

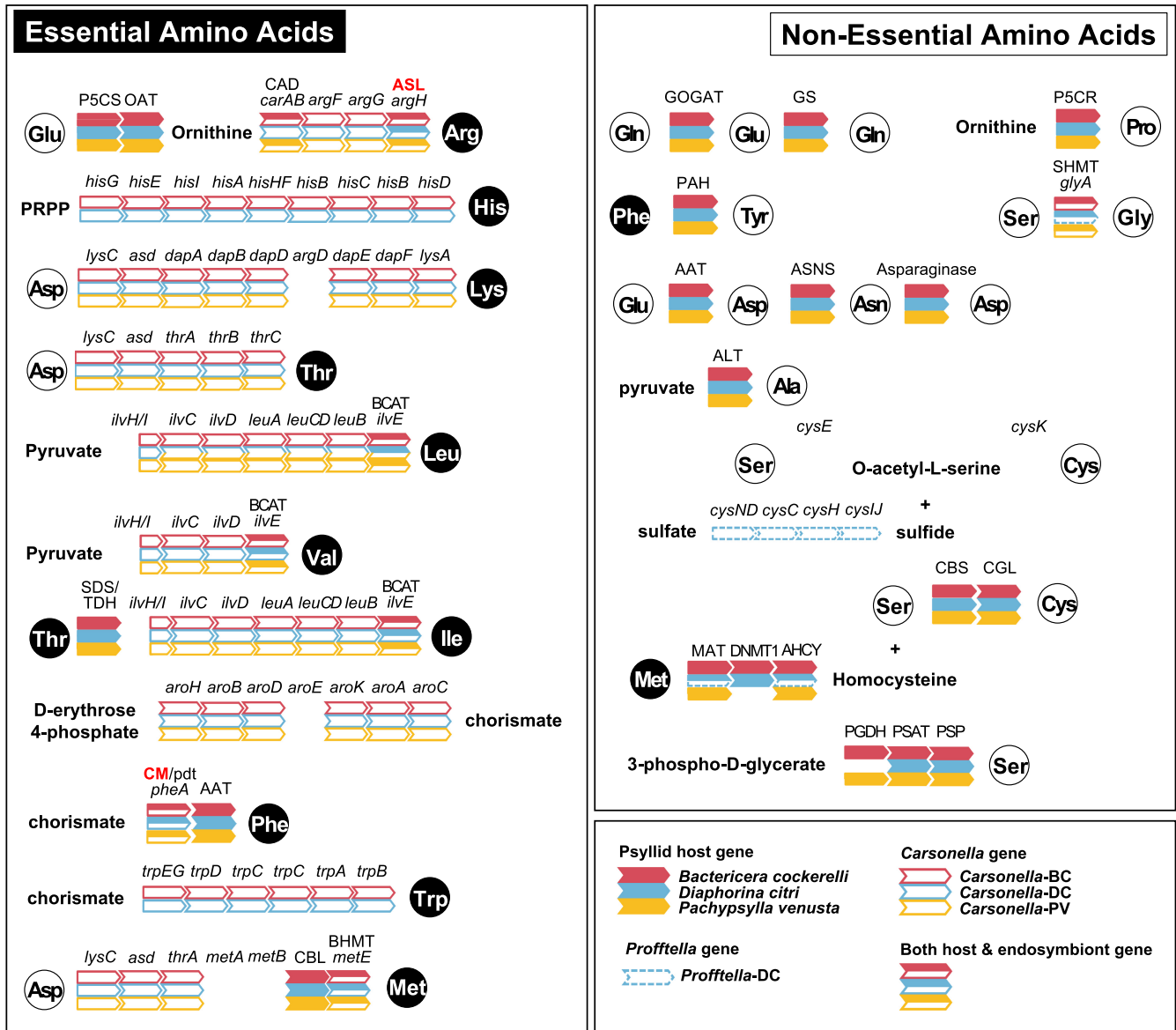


FIGURE 4 Amino acid biosynthesis pathways present in *B. cockerelli*, *D. citri*, *P. venusta*, and their endosymbionts. Arrows are coloured/ lined accordingly for each host/endosymbiont-encoded gene (see legend). Gene names in bold (red) indicate horizontally transferred genes of bacterial origin into the psyllids' genomes

EC 2.6.1.42) and catalyses the terminal step in the biosynthesis of isoleucine, leucine, and valine, is retained in all three *Carsonella* genomes as well (Figure 4). The BCAT gene in *B. cockerelli* was identified (ANN10973-RA) and was expressed above the 85th percentile relative to the expression of all other *B. cockerelli* genes for the whole body (Table 3).

3.4.3 | HGT in the *B. cockerelli* genome

We identified seven *B. cockerelli* genes that were horizontally transferred from bacteria into psyllids (Table 4). These results are largely consistent with the horizontally transferred genes identified initially in Sloan et al. (2014) in *P. venusta*, except the *mutY* gene

(comp106533_c0_seq1) was not identified in the *B. cockerelli* genome; however, it is still retained in *D. citri*. However, we identified that multiple gene duplications occurred among four of these horizontally transferred genes in the *B. cockerelli* and *D. citri* genomes but not *P. venusta* (Table 4). For example, we found that both *B. cockerelli* and *D. citri* encode three copies of the arginosuccinate lyase (ASL, EC 4.3.2.1) gene while *P. venusta* only encodes two (Sloan et al., 2014) (Figure 5a). ASL is hypothesized to be involved in the end step of the arginine pathway with *Carsonella* (Figure 4; Sloan et al., 2014). Our phylogenetic reconstruction of the ASL genes reveals that all three psyllid species encode two divergent copies of ASL that share a most recent common ancestor and are most similar to ASL gene copies found within the Gammaproteobacteria (Figures 5a, S4 and S5). The most parsimonious explanation for this observation is that the ASL

TABLE 3 Amino acid/symbiosis-related genes involved in the amino acid metabolism of *Bactericera cockerelli* based on KEGG and BLAST

Name	KO	Enzyme	<i>B. cockerelli</i> Protein ID ^a	Expression percentile ^b
P5CS	K12657	Delta-1-pyrroline-5-carboxylate synthetase [EC:2.7.2.11/1.2.1.41]	ANN07147(8)-RA	98% (90%)
OAT	K00819	Ornithine--oxo-acid transaminase [EC:2.6.1.13]	ANN07990(1)-RA	91% (84%)
BCAT	K00826	Branched-chain amino acid aminotransferase [EC:2.6.1.42]	ANN10973-RA	85%
AAT	K14455	Aspartate aminotransferase [EC:2.6.1.1]	ANN05170-RA	62%
			ANN05183-RA	69%
			ANN05186-RA	65%
			ANN05257-RA	99%
			ANN17803(4)-RA	98% (99%)
BHMT	K00547	Homocysteine S-methyltransferase [EC:2.1.1.10]	ANN06058-RA	80%
			ANN06059-RA	60%
GOGAT	K00264/K00265/K00266	Glutamate synthase (NADH) [EC:1.4.1.13/1.4.1.14]	ANN19375-RA	94%
GS	K01915	Glutamine synthetase [EC:6.3.1.2]	ANN10145-RA	52%
			ANN10146-RA	97%
			ANN10148-RA	96%
			ANN10149-RA	42%
PAH	K00500	Phenylalanine-4-hydroxylase [EC:1.14.16.1]	ANN09753-RA	95%
ASNS	K01953	Asparagine synthase [EC:6.3.5.4]	ANN02335-RA	81%
			ANN09881-RA	71%
			ANN21723-RA	84%
ASPG	K01424/K13051	L-asparaginase [EC:3.5.1.1]	ANN07324-RA	68%
			ANN21497-RA	17%

^aPsyllid genes in tandem that were reannotated to be a single protein after manual annotation are indicated with parentheses.

^bThe average gene expression percentile was determined based on relative gene expression (transcript per million, TPM) of the target gene relative to all genes annotated in the final assembly. RNAseq data were derived from five pooled psyllid whole body samples ($n = 5$; see Table S11).

gene was acquired by horizontal transfer and was duplicated early in the ancestor of psyllids as suggested by Sloan et al. (2014). Curiously, *B. cockerelli* and *D. citri* both appear to have had a very recent independent duplications of one of these gene copies (Figures 5a, S4 and S5). The amino acid percent identity between these new paralogs in *B. cockerelli* are 100% and both paralogues have two introns. For *D. citri*, the two paralogs have 99% nucleotide similarity with one intron. In *B. cockerelli* all ASL gene copies are located on different scaffolds (Table 4) and are surrounded by different genes suggesting that the paralogs did not arise from a tandem duplication. Relative gene expression of all three gene copies are very similar to one another and range between 48th–56th percentile relative to other genes in *B. cockerelli* (Table 4). Sloan et al. (2014) previously suggested that the ASL gene was derived from *Carsonella*; however, here we find inconsistencies in our results in regard to whether this gene is derived from *Carsonella* or another related endosymbiont due to long branch attraction (Bergsten, 2005) of psyllid/endosymbiont sequences and lower branch support for some nodes (Figures S4 and S5).

A second horizontally transferred gene that may collaborate with *Carsonella* for essential amino acid biosynthesis is chorismite mutase (CM, EC 5.4.99.5), which is involved in the phenylalanine

pathway (Sloan et al., 2014; Table 4, Figure 4). We identified three CM genes in the *B. cockerelli* genome, two genes in *P. venusta* (Sloan et al., 2014), and six genes in *D. citri* (Figure 5b). The three CM gene copies in *B. cockerelli* were all expressed at variable levels (35th–81st percentile) in the *B. cockerelli* body (Table 4). A phylogenetic analysis indicates that there were two early duplications of CM in psyllids and a likely loss of one of those copies in *P. venusta* (Figure 5b). Subsequently, *D. citri* experienced further duplications for each of these paralogues resulting in the six copies present in the proteome. These *D. citri* copies consist of three nearly identical sets of gene copies with 100% nucleotide sequence similarity except for the gene pair, P019075.1 and P094730.1.1 (Figure 5b). Precise origins of the transfer event are unclear; however, it is clear that a member of the Proteobacteria was the donor (Figures 5b and S6).

4 | DISCUSSION

In this study we reveal that the *B. cockerelli* genome has experienced significantly more gene family expansion events compared to other Hemipteran representatives with fully sequenced genomes (Figure 2).

TABLE 4 *Bactericera cockerelli* genes of bacterial origin acquired by horizontal gene transfer

Gene	Protein ID	Scaffold	Expression ^a	<i>P. venusta</i> unigene ^b	Amino acid similarity ^b
<i>Argininosuccinate lyase</i>					
ASL-1	ANN12874-RA	Scaffold_7	56%	116240_c1	68%
ASL-2a	ANN10361-RA	Scaffold_5	58%	113612_c8	91%
ASL-2b	ANN20354-RA	Scaffold_15	48%	113612_c8	91%
<i>Chorismate mutase</i>					
CM-1	ANN05927-RA	Scaffold_2	35%	113157_c6	30%
CM-2	ANN06705-RA	Scaffold_2	93%	113157_c6	33%
CM-3	ANN17115-RA	Scaffold_9	81%	113157_c6	62%
<i>A/G-specific adenine glycosylase</i>					
MUTY	ANN05978-RA	Scaffold_2	N/A	106533_c0	64%
<i>AAA-ATPase-like</i>					
ORF-1a	ANN01458-RA	Scaffold_1	61%	111207_c2	44%
ORF-1b	ANN01460-RA	Scaffold_1	68%	111207_c2	68%
ORF-2	ANN17656-RA	Scaffold_10	30%	115390_c0	25%
ORF-3a	ANN18155-RA	Scaffold_10	18%	115390_c0	57%
ORF-3b	ANN18147-RA	Scaffold_10	0%	115390_c0	53%
<i>Riboflavin synthase</i>					
ribC	ANN14810-RA	Scaffold_11	73%	97442_c1	60%
<i>16S rRNA methyltransferase</i>					
RSMJ	ANN13802-RA	Scaffold_8	10%	107791_c0	41%
<i>VOC family protein</i>					
YDCJ	ANN12599-RA	Scaffold_7	93%	111380_c3	70%

^aThe average gene expression percentile was determined based on relative gene expression (transcript per million, TPM) of the target gene relative to all genes annotated in the final assembly. RNAseq data were derived from five pooled psyllid whole body samples ($n = 5$; see Table S11).

^bAmino acid similarity of *B. cockerelli* HGT genes using BLASTX against *P. venusta*'s Trinity unigene assemblies (Sloan et al., 2014).

Nearly 75% of these expanding gene families in *B. cockerelli* are transposable elements that are rampant across all of *B. cockerelli*'s chromosomes. These transposable elements are primarily composed of type I and II elements which occur in equal proportions throughout both the euchromatin and the heterochromatin regions of all of *B. cockerelli*'s chromosomes except for chromosome 1, where we detected a bias of transposable elements in heterochromatic regions. Various drivers of transposable element expansions in animals including insects have been determined including the mode of reproduction, social complexity, ecology, and host mechanisms for repeat containment (Gilbert et al., 2021; Lynch, 2007). At this time, it is unclear what is driving mobile element gene family expansions in *B. cockerelli*. For example, *B. cockerelli* has a sexual mode of reproduction with a 50/50 sex ratio (Abernathy, 1991; Yang & Liu, 2009) therefore a reduced level of recombination, which can result in transposable element accumulation, is not expected for this insect species. It is also unlikely that these expansions are exclusively an artifact of laboratory rearing as 154/157 of the expanded gene families (representing ~99% of the genes) have average amino acid identities $\leq 90\%$. In insects the likeliest candidate for host mechanisms for transposon containment is the Piwi-interacting RNA (piRNAs) pathway (Brennecke et al., 2007). Although *B. cockerelli*

encodes the proteins Piwi and Argonaute 3 (BC, ANN11580-RA and ANN07423-RA, respectively) which are involved in this pathway (Dowling et al., 2016), the functional role of piRNAs in silencing transposons in *B. cockerelli* has not yet been described.

The expansion of mobile elements in *B. cockerelli*'s genome has implications for impacting the evolution of insect genome architecture and adaptation to its ecological niche. For example, Petersen et al. (2019) revealed a positive correlation between transposable element content and insect genome size. We found that *B. cockerelli*'s genome is the largest psyllid genome (567 Mb) sequenced to date and is ~15% larger than the other two psyllid species genomes (*P. venusta* and *D. citri*). The fraction of transposable element content in *B. cockerelli*'s genome is ~2× larger than the content measured in *P. venusta* (Petersen et al., 2019) and *D. citri* (Gilbert et al., 2021; Peccoud et al., 2017), in addition to the median size of transposable element content (24.4% [$\pm 12.5\%$ SD]) that is found in insects belonging to five orders (Petersen et al., 2019). Consequently *B. cockerelli*'s genome size may partly be driven by the expansion of transposable elements in its genome. Transposable elements can also significantly impact genome architecture by inducing chromosomal rearrangements via non-homologous recombination (Burns & Boeke, 2012). Structurally,

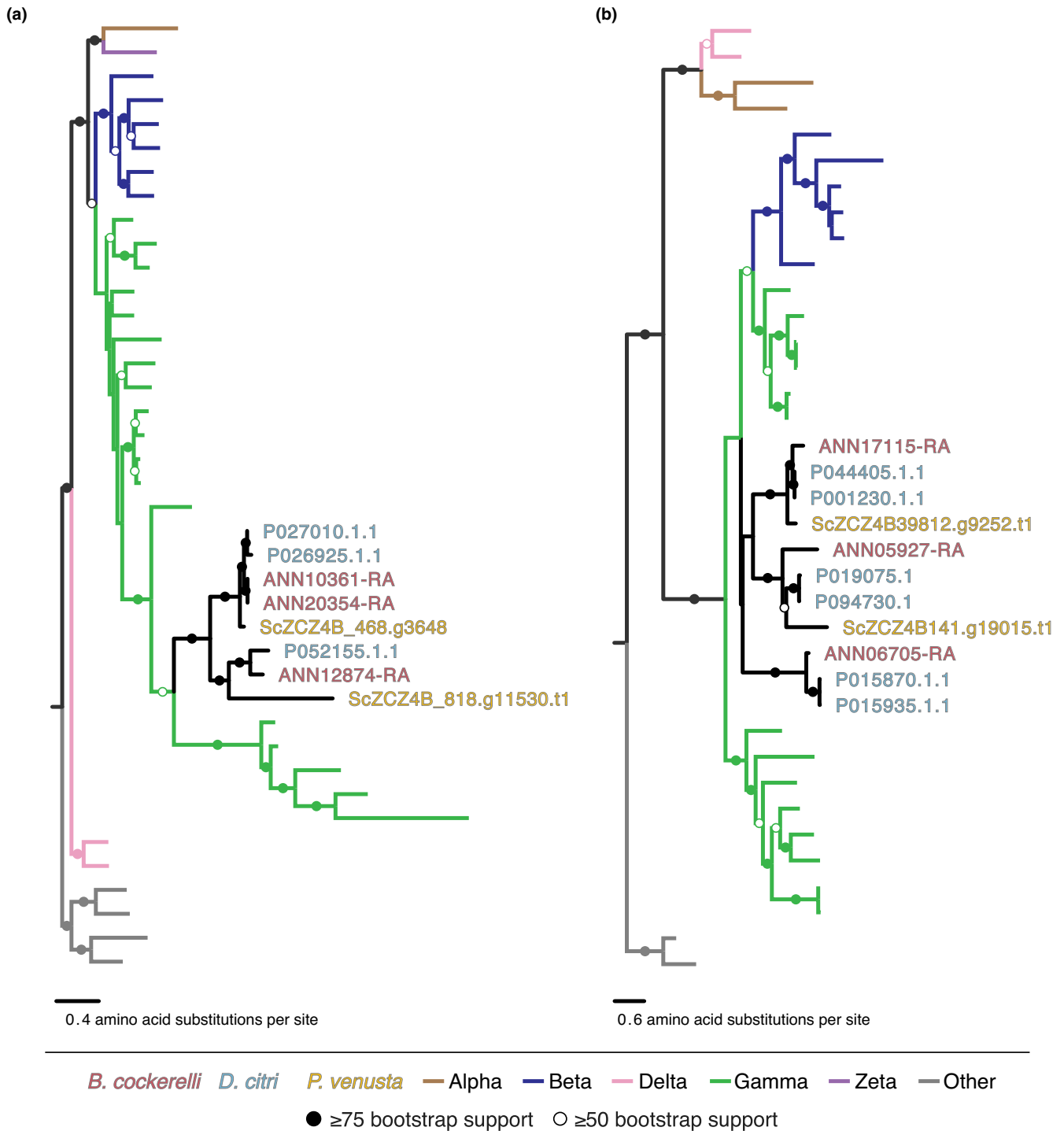


FIGURE 5 Phylogenetic analyses of gene duplications for horizontally transferred genes in the psyllid genomes of *P. venusta*, *D. citri*, and *B. cockerelli* for (a) argininosuccinate lysase (ASL) genes and (b) chorismite mutase (CM) genes. Branches are coloured according to their bacterial classes within the Proteobacteria and ranges of bootstrap values are indicated by filled or open circles as indicated in the key. See Figures S4, S5, and S6 for trees with detailed taxa names and accession numbers

B. cockerelli appears to have an additional chromosome compared to the distantly related psyllid species *P. venusta* due to a previous chromosomal fission or fusion event (Figure 1). When more psyllid species genome assemblies are resolved at a chromosomal-level we will have a better understanding of the dynamics of chromosomal loss and/or gain across Psylloidea. Although we detected

extensive conservation of syntenic gene blocks among the *B. cockerelli* and *P. venusta* shared chromosomes that we estimated diverged ~130 Ma, we detected a substantial amount of intrachromosomal gene block rearrangements between the homologous chromosomes (Figure 1). The dynamic nature of the *B. cockerelli* genome may largely be contributed to the widespread expansion

of type I and II repeat elements that are omnipresent throughout all of *B. cockerelli*'s chromosomes.

Transposable elements can also impact insect adaptation either negatively or positively depending on the environmental context (Gilbert et al., 2021). Given that transposable elements are equally expanding in the euchromatin regions of *B. cockerelli*'s genome the disruption of coding sequences and/or the rewiring of regulatory circuits is plausible. It is hypothesized that transposable element insertions are primarily deleterious or neutral however transposable element mediated mutations can occasionally be adaptive. For example, transposable element mediated insecticide resistance, adaptation to temperate climates and oxidative stress, and faster developmental times have all been documented in insects (Gilbert et al., 2021; Petersen et al., 2019). Classic examples of natural selection in insects have also been contributed to transposable element insertions such as the industrial melanism colour polymorphism of the peppered moth *Biston betularia*, which helped populations evade bird predation in coal polluted environments (van't Hof et al., 2016). Interestingly, transposable elements have recently been found to be important, and sometimes essential components of antiviral immunity in insects (Gilbert et al., 2021). The psyllid *B. cockerelli* is known to be associated with insect viruses in field populations (Dahan et al., 2022), however it is unclear at this time what role its mobilome plays in facilitating antiviral interactions.

In addition to mobile element gene family expansions, we also found significant expansions of gene families in *B. cockerelli* that are associated with transcription factors, proteases, and odorant receptors. The expansion of transcription factors in *B. cockerelli*'s genome may increase the plasticity of gene expression among environments or conversely provide more stability for the transcription of conserved genes depending on how many gene targets the transcription factors bind to (Yang & Wittkopp, 2017). The expansion of protease gene families in *B. cockerelli* may also be very important in the evolution of *B. cockerelli* by facilitating species specific responses to host plant defenses. For example, the host plants of *B. cockerelli*, such as tomato and potato are widely known to produce carboxypeptidase inhibitors during insect feeding and wounding (Díez Díaz et al., 2004; Graham & Ryan, 1981). These inhibitors can produce an antifeedant effect for insects (Zhu-Salzman & Zeng, 2015), and therefore *B. cockerelli* may be involved in an arms race with its solanaceous host plant defences and its rapidly evolving carboxypeptidase families. Another expanding gene family in *B. cockerelli* that may help this psyllid species adapt to its environment especially in regard to host-plant volatiles, mating and intraspecific communication are odor receptors. Compared to the other two psyllids species examined in this study only *B. cockerelli* was observed to have significant gene family expansions in odorant receptors. It will be of interest for further studies to determine how and if these expanding seven transmembrane odorant receptors influence *B. cockerelli* host plant recognition, as *B. cockerelli* is a polyphagous herbivore that can feed and reproduce on a wide variety of host plant species (Pletsch, 1947; Wallis, 1955).

The analysis of gene duplication events is largely dependent on the quality of genome assemblies. Taking advantage of our chromosomal-level assembly we were able to detect gene duplications for several genes that are important for insect-microbe interactions. Three of these genes that duplicated in *B. cockerelli*, argininosuccinate lyase (ASL), chorismate mutase (CM), and the AAA-ATPase-like protein, were horizontally transmitted from bacteria into psyllids before the divergence of the psyllid lineages examined (Sloan et al., 2014). The genes ASL and CM may be important in the regulation and collaboration with *Carsonella* for the biosynthesis of the essential amino acids arginine and phenylalanine, respectively (Figure 4; Sloan et al., 2014). Our analyses uncovered additional copies of the genes ASL and CM in *B. cockerelli* and *D. citri* compared to *P. venusta* (Figure 5). It will be of interest in future studies to determine if these additional copies have different functions and differential expression patterns in the symbiotic cells of psyllids compared to other body tissues. Additionally validating that these copies in *D. citri* are actual gene copies will be important when a chromosomal assembly for *D. citri* is publicly available, as several copies shared 100% amino acid identity. Another important gene duplication in *B. cockerelli* that is involved in the nutritional symbiosis is glutamine synthase (GS), which is hypothesized to recycle ammonia in symbiotic cells via the GOGAT cycle to provide amino donors for EAA biosynthesis (Table 3, Figure 4; Hansen & Moran, 2011; Sloan et al., 2014). Four gene copies of GS were identified in *B. cockerelli* and we expect future studies will be able to characterize the expression patterns and functional role of these multiple copies.

Our analysis of core single copy orthologues shared by *B. cockerelli* and the other psyllids identified an array of genes under strong purifying selection that are related to insect-microbe interactions and immunity (Table S8). Genes that are maintained under purifying selection infer that these genes have an important conserved function and are candidates for future investigations. Interestingly two of these genes have homologues in pea aphids that are hypothesized to be involved in the nutritional amino acid symbiosis with its endosymbiont *Buchnera* (Feng et al., 2019; Pers & Hansen, 2021). This includes the amino acid transporter SLC36-like AAAP, which is hypothesized to be important in transmembrane transport of the small nonessential amino acids such as proline, alanine, and glycine in symbiotic cells of aphids (bacteriocytes; Feng et al., 2019). This transporter is expressed the highest in first instar bacteriocytes of *A. pisum* and is significantly downregulated in bacteriocytes during the first to second instar transition and it is downregulated further in the second to third instar transition (Pers & Hansen, 2021). The other protein that is under purifying selection in *B. cockerelli* is in the kynurenine pathway (kynurenine formamidase/cyclase-like) and is involved in the degradation of tryptophan. The homologue of this protein in *A. pisum* is expressed the highest in *A. pisum* bacteriocytes at first and second instar and is significantly downregulated from second to third instar and is further downregulated during its third to fourth instar transition (Pers & Hansen, 2021). The functional role of kynurenine intermediates in insect bacteriocytes is not well understood; however, ommochromes from this pathway

are primarily found in insect ommatidia but are also known to be important in reducing oxidative stress (Figon & Casas, 2019; Lugo-Huitrón et al., 2011), which may be crucial for allowing this symbiosis to function in metabolically active bacteriocytes.

In summary, taking advantage of our high-quality chromosomal genome assembly we uncovered a large expansion of gene families and gene duplications events that play a large role in shaping the genome architecture and function of *B. cockerelli*. The significant increase of transposable elements in *B. cockerelli*'s genome probably has significant consequences for *B. cockerelli*'s genome architecture and adaptation through the restructuring of regulatory circuits and insertion mutations of important genes. Moreover, recent gene duplications of genes that were horizontally transferred into *B. cockerelli*'s genome from bacteria may have species specific consequences for its nutritional symbioses with *Carsonella*. The genomic resources and candidate genes uncovered here will provide ample resources for future studies on this system.

AUTHOR CONTRIBUTIONS

Younghwan Kwak performed the research, analysed data, and wrote the manuscript. Jacob A. Argandona analysed data. Patrick H. Degnan analysed data, and wrote the manuscript. Allison K. Hansen designed research, analysed data, and wrote the manuscript.

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CONFLICT OF INTEREST

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

GENOMIC DATA

Bioproject/biosample for the genomic data were created in NCBI under accession number PRJNA822651/SAMN27256069. Raw PacBio data have been submitted to NCBI SRA under accession number SRR18584432. Raw Illumina data have been submitted to NCBI SRA under accession number SRR18584429. Final DoveTail chromosomal assembly have been submitted to NCBI Genome under accession number JAMCCL000000000.

ANNOTATION DATA

Protein sequences annotated in this study have been submitted to Github at https://github.com/AllisonHansenLab/BC_psyllid_Genome. The GFF file created in this study for the assembly has been submitted to Github at https://github.com/AllisonHansenLab/BC_psyllid_Genome.

GENE EXPRESSION DATA

Biosamples for gene expression data were created in NCBI under accession number SAMN27256084 to SAMN27256088. Raw RNAseq Data were submitted to NCBI SRA under accession number SRR18584596 to SRR18584600. Trinity assembled unigenes NCBI TSA under accession number SRR11276723

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