“The green peach aphid, *Myzus persicae*, transcriptome in response to a circulative,
nonpropagative polerovirus, *Potato leafroll virus*“.

MacKenzie F. Patton¹, Allison K. Hansen³, and Clare L. Casteel^{1,2*}

¹ Department of Plant Pathology, University of California, Davis, CA, 95616, USA.

² Plant Pathology and Plant-Microbe Biology Section, School of Integrated Plant Science,
Cornell University, Ithaca, NY, 14850, USA.

³ Department of Entomology, University of California, Riverside, CA, 92521, USA.

Corresponding author:

Clare L. Casteel^{1,2}

Email Address: ccasteel@cornell.edu (CLC)

Abstract:

Viruses in the *Luteoviridae* family, such as *Potato leafroll virus* (PLRV), are transmitted by aphids in a circulative and nonpropagative mode. This means the virions enter the aphid body through the gut when they feed from infected plants and then the virions circulate through the hemolymph to enter the salivary glands before being released into the saliva. Although these viruses do not replicate in their insect vectors, previous studies have demonstrated viruliferous aphid behavior is altered and the obligate symbiont of aphids, *Buchnera aphidicola*, may be involved in transmission. Here we provide the transcriptome of green peach aphids (*Myzus persicae*) carrying *PLRV* and virus-free control aphids using Illumina sequencing. Over 150 million paired-end reads were obtained through Illumina sequencing, with an average of 19 million reads per library. The comparative analysis identified 134 differentially expressed genes (DEGs) between the *M. persicae* transcriptomes, including 64 and 70 genes that were down- and up-regulated in aphids carrying *PLRV*, respectively. Using functional classification in the GO databases, 80 of the DEGs were assigned to 391 functional subcategories at category level 2. The most highly up-regulated genes in aphids carrying *PLRV* were cytochrome p450s, genes related to cuticle production, and genes related to development, while genes related to histone and histone modification were the most down-regulated. *PLRV* aphids had reduced *Buchnera* titer and lower abundance of several *Buchnera* transcripts related to stress responses and metabolism. These results suggest carrying *PLRV* may reduce both aphid and *Buchnera* genes in response to stress. This work provides valuable basis for further investigation into the complicated mechanisms of circulative and nonpropogative transmission.

Introduction:

Aphids are a member of the superfamily Aphidoidea, are distributed world-wide, and cause major damage to global agricultural (1). Despite there being over 4,000 species, only about 400 are known as significant pests (2). Aphids are effective pests partially because they do not require sexual reproduction and can use parthenogenesis to quickly increase their numbers (3). Another aspect of aphid biology that makes them an effective pest is their host range. Although many aphids are very specialized herbivores, only feeding on a few related species, some species feed on many taxa of plants (4). *Myzus persicae* is one of these polyphagous pests, feeding on over 40 different families, including Solanaceae (5).

Along with causing direct feeding damage, aphids are important plant virus vectors, representing over 50% of all known insect vectors for plant viruses (6)(7,8). Increasing evidence has shown that plant viruses alter vector host finding, dispersal, and inoculation through changes in host physiology, however the underlying mechanisms are largely unknown (9,10)(11). Recent evidence suggests that viruses may also directly affect aphid biology (12–15). *Rhopalosiphum padi* aphids carrying *Barley yellow dwarf virus* (BYDV), a virus from the genus *Luteovirus*, prefer non-infected hosts, while virus free *R. padi* prefer infected hosts (12). In these experiments, the authors determined this interaction was due to direct impacts on the aphid and not due to virus induced changes in host plant quality by purifying BYDV virions and feeding them to aphids in artificial diet before conducting aphid bioassays.

Although their lifestyle or host may change, all aphids depend on *Buchnera aphidicola* as their primary obligate endosymbiont (16). *Buchnera* provide the aphid with essential amino acids and nutrients that are limited in the aphid's diet (17–20), and because of this aphids can no longer survive without *Buchnera*. For example, when *Buchnera* is reduced by using antibiotics,

studies have shown lower body mass, lower fecundity, and changes to feeding behavior (21,22). Essential components of the *Buchnera* genome have been lost (23,24) as *Buchnera* co-diversified with aphids over time (16,25,26). Because of this *Buchnera* also depends on aphids for survival, living inside special aphid cells, known as “bacteriocytes”. Together, however, this mutualistic duo wreaks havoc on agriculture across the globe.

Previous studies have speculated *Buchnera* may have a role in aphid transmission of plant viruses (26–30). Specifically, the *Buchnera* chaperone protein GroEL, a homologue from *Escherichia coli* (31), has been implicated in transmission for a number of viruses (26,27,30,32–35). Direct interactions are thought to be unlikely due to the spatial separation of bacteriocytes and circulating virions (26). However, GroEL from *Buchnera* is found in aphid saliva and has been shown to trigger plant defenses and reduce aphid fecundity using transgenic plants expressing GroEL (36,37). Virus-induced changes in plant defense or nutrients may alter aphid-*Buchnera*-plant interactions, highlighting the need for additional research on virus-vector-*Buchnera* interactions (38).

Potato leafroll virus (PLRV) is a positive sense ssRNA virus and the type member of the genus *Potyvirus* (family *Potyviridae*). PLRV is phloem limited and transmitted in a circulative nonpropagative form. This means the virus particles will travel across the gut membrane on specific receptors into the insect hemolymph. From here it will traverse to the salivary gland and duct so that it may be injected back into the phloem tissue (9,39). Previous studies have shown that aphid vectors prefer to settle on plants infected with PLRV and that insect vectors have higher fecundity when feeding on these plants compared to controls (5,40–42). Additionally, a recent study investigating aphid small RNAs (sRNAs), found that *M. persicae* who fed upon PLRV infected plants and purified PLRV diets had significantly altered

sRNA profiles, manipulated immune response, and differentially expressed *Buchnera* sRNAs that are associated with tRNAs (43). The role of *Buchnera* tRNA associated sRNAs is largely unclear, as their expression is conserved in divergent *Buchnera* taxa (44), they are differentially expressed during aphid development (45) and when aphids feed on different host plant diets (46). In consequence, the mechanisms mediating virus-aphid-*Buchnera* interactions are largely unknown. Recently we demonstrated PLRV induces changes in plant nutrients and defenses in infected host plant, (47) however, the impacts of these changes on symbiont-aphid interactions are unknown. To address this lack of knowledge we examined changes in the transcriptome of *M. persicae* with and without PLRV, *Buchnera* titer, and changes in aphid and *Buchnera* transcripts from aphids feeding on PLRV-infected plants. By providing evidence that nonpropagative circulative plant viruses can affect insect vectors through changes in the transcriptome and alter *Buchnera* titer, our study will contribute to growing knowledge of the insect microbiome at a plant-insect interface.

Methods:

Plant and insect growth conditions

Solanum tuberosum were propagated using leaf-bud cutting from cv. Désirée (48) in laboratory experiments. Plants were grown in growth chambers under controlled conditions (25/23°C day/night with a photoperiod of 16/8 h day/night). Non-viruliferous and viruliferous aphid clones of a potato-adapted red strain of *Myzus persicae* were reared under controlled conditions (25/23°C day/night with a photoperiod of 16/8 h day/night) on healthy potato. All experiments were conducted in the same environmental chambers and conditions, so there were no

environmental differences in treatments (25/23°C day/night with a photoperiod of 16/8 h day/night).

Pathogen infection

Agrobacterium tumefaciens (LBA4404) containing the infectious clone of PLRV (49) was grown at 28°C in LB broth (+10 mM MgSO₄), with kanamycin (50 µg/mL), carbenicillin (100 µg/mL) and rifampicin (50 µg/mL) for selection. After 24 hours, bacteria were centrifuged to concentrate and resuspended in 10mM MgCl₂. One week old *S. tuberosum* were inoculated at an optical density (OD) of 0.70. Three weeks post infection, tissue was collected from all plants, RNA was extracted using the SV Total Isolation Kit as per manufacturer's instructions (Promega, Madison, WI, USA), and cDNA was synthesized using 1500 ng of total RNA and random hexamers (20ng/µL) with the SMART® MMLV as per manufacturer's instructions (Takara Bio USA, Mountain view, CA, USA). cDNAs were used in PCRs with PLRV specific primers (F-5'ATGAGTACGGTCGTGGTT-3' and R-5'CTATTTGGGGTTTTGCAAAGC-3'). A set of uninfected potato cuttings were grown at the same time as the plants above to serve as controls. After systemic plant infection was verified plants were immediately used in experiments.

RNAseq, qRT-PCR, and qPCR aphid experiments

Five adult aphids were placed on the first fully expanded leaflet of three infected and on three healthy plant. After 24 hours, all adults were removed and 20 larvae were left to develop. Seven days later all aphids were at the same developmental stage and 10 young aphids were collected into a tube from each plant (N=3 plants with 10 aphids per plant) and immediately frozen in liquid nitrogen until use in RNAseq experiments. The entire experiment was repeated a second

time for confirmation of RNAseq results using qRT-PCR and to examine the titer of the bacterial symbiont, *Buchnera*, in aphids. For this experiment 5 aphids were collected for RNA extraction and 5 aphids were collected for DNA extractions from each plant. We also prepared 6-7 plants for each treatment instead of 3 (N = 6-7 with 5 aphids per plant), however all other methods were the same.

RNA and DNA isolation from aphids

RNA was extracted from aphid tissue collected in the first two experiments as described above. The RNA concentration and purity were measured using a NanoDrop. The integrity of RNA was confirmed using the Bioanalyzer 2100 system (Agilent, Santa Clara, CA, USA). DNA was extracted from aphid tissue collected in the second experiment using Cetyl trimethylammonium bromide. The integrity of DNA was confirmed using an agarose gel. The DNA concentration and purity were measured using a NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA).

Library Preparation, and Sequencing.

Sequencing libraries were prepared using a multiplexing library protocol (50). Briefly, oligo(dT) 25 Dynabeads were used to purify mRNA, which was then fragmented, and the first-strand cDNA was synthesized using random primers, dNTP, and reverse transcriptase. The second-strand was synthesized using a dUTP mix, DNA Polymerase I, and RNase H, ends repaired, and adenylated. The cDNA fragments were ligated to adapters, selectively enriched by PCR, and purified using the AMPure XP beads. The library quality was assessed using the Agilent Bioanalyzer 2100 system and sequenced using an Illumina HiSeq 2000 instrument.

Read mapping, differential expressed gene (DEG) analysis, and Gene Ontology (GO) classification

RNA-Seq data were analyzed using RStudio (Version 1.1.383) and Bioconductor according to Anders et al. (2013) with some modifications (48) (See Supplementary Figure S1). Sequence quality was determined, trimmed, and poor-quality reads removed using ShortRead (52) and FastQC (53). Reads were mapped to the *Myzus persicae* clone G006 genome v2.0 from AphidBase (54) using TopHat2 (55). Mapped reads were assigned to genes and counted with HTSeq (56) and normalized by size factors obtained from the negative binomial-based DESeq2 package (57). Gene annotation files for both were downloaded from NCBI. After normalization, clusterization profiles of the samples were assessed by hierarchical clustering (with Euclidean distance metric and Ward's clustering method) and principal component analysis (PCA). Differentially expressed genes (DEGs) between infected and control treatments were identified using DESeq2 (57). Genes with False Discovery Rate (FDR)-corrected p-values ≤ 0.1 were classified as differentially expressed. For Gene ontology (GO) analysis, Blast2GO software (58) was utilized for annotation as previously described (59).

qRT-PCR

cDNA was synthesized using random hexamer (20ng/ μ L) and quantitative RT-PCR (qRT-PCR) was performed. Transcript abundance was quantified for the *M. persicae* genes, *HSP68-like* (MYZPE13164_G006_v1.0_000070430.1) and *M. persicae* cuticle protein5-like (*MPCP5-like*) (MYZPE13164_G006_v1.0_000133030.2), and for the *Buchnera* genes, *argE* (*BUMPUSDA_CDS00542*), *dnaK* (*BUMPUSDA_CDS00441*), and *groEL*

(*BUMPU*SDA_CDS00567), using gene specific primers (Supplementary Table S1). qRT-PCR reactions were carried out using SYBR green PCR master mix (Applied Biosystems, Carlsbad, CA, USA), in an CFX384 instrument (Bio-Rad, Hercules, CA, USA). Three technical replicates were performed for each individual sample, and a digital pipette was used for all pipetting. Relative transcript abundance was calculated utilizing a standard curve produced from 10-fold series dilution of cDNA synthesized from 1000 ng/μL of total RNA according to the standard curve method (Applied Biosystems, Carlsbad, CA, USA). Technical replicates of raw CT values were averaged and transcripts of interest were normalized to the house-keeping transcript ribosomal protein L7 *rpl7* for aphids or the 50S ribosomal subunit gene *rpIN* for *Buchnera*, as previously described (60–62).

***Buchnera* titer**

Buchnera titer here is defined as the ratio of *Buchnera* single copy genes to aphid single copy genes. To determine *Buchnera* titer in whole aphid bodies we used quantitative PCR (qPCR) and measured the ratio of a single copy *Buchnera* gene (*rplN*) to a single copy aphid gene (*RPL7*). qPCR reactions were carried out using SYBR green PCR master mix (Applied Biosystems, Carlsbad, CA, USA), in the CFX384 instrument (Bio-Rad, Hercules, CA, USA). Reactions were performed in triplicate for each sample, and the average was used for analysis. Relative abundance was calculated utilizing a standard curve produced from 10-fold serial dilution of DNA.

Statistical analyses

RNAseq data analyses were performed as described above. All statistical analyses for qRT-PCR and qPCR were determined using a $P < 0.05$. Analysis of variance (ANOVA) was used to determine significant difference in transcript abundance. For *Buchnera* titer single factor ANOVA was used to determine difference in relative abundance. The statistical analyses were performed using JMP 8 software (SAS Institute, Cary, NC, USA).

Results:

Differential gene expression in the presence of PLRV.

Over 150 million paired-end reads were obtained through Illumina sequencing, with an average of 125,815, 247 100-bp reads per library (Table 1, Fig 1A). About 73% of the reads mapped to the *M. persicae* reference genome, with around only 27% being uniquely mapped (Table 1, Fig 1B). In order to examine biological variability, a principal component analysis (PCA) of the normalized count data was performed (Fig 1C). The first component of variance separated samples by treatments and accounted for 54% of the variance. Hierarchical clustering confirmed PCA results in visual representation of DEG expression (Fig 1D). The transcriptome of viruliferous *M. persicae* was compared to the transcriptome of virus-free *M. persicae* using the negative binomial-based DESeq2 (57). Overall, 96 differentially expressed genes (DEGs) were detected using an FDR adjusted p-value ≤ 0.05 and log2 fold change (FC) ≥ 0.5 , however, by relaxing our FDR adjusted p-value to ≤ 0.1 we were able to include 38 additional DEGs (134 DEGs total included; Fig 1E; Supplementary Table S2). The presence of PLRV in the aphid vector caused a down-regulation of 70 genes and an up-regulation of 64 genes (Fig 1F; FDR

adjusted p-value ≤ 0.1 and log2 fold change (FC) ≥ 0.5). Overall, 0.8 % of the aphid genome was significantly impacted by the presence of PLRV.

Functional roles of differentially expressed genes

Gene ontology (GO) enrichment analyses were performed with the DEGs from each treatment to identify functions and pathways disturbed in aphids carrying PLRV. One or more gene ontology terms were assigned to each transcript from biological processes, molecular functions, and cellular compartments term using Blast2GO functional gene annotation (Conesa et al. 2005). The 134 DEGs were assigned to functional GO terms within the three categories, including 125 biological processes, 118 molecular functions, and 148 cellular compartments. Of the 134 DEGs, 53 (39.55% of total DEGs) were classified as “uncharacterized proteins.” The majority of DEGs assigned to biological processes were categorized as metabolic processes (41%), cellular-protein processes (11%), and oxidation-reduction processes (11%) (Fig 2A). As for DEGs assigned to molecular functions, almost half were associated with catalytic activity (33%) or nucleic acid binding (21%) (Fig 2B). Within the cellular component category, 24% were related to the membrane and 24% were related to intracellular locations (Fig 2C).

Next each DEGs was annotated using a single Blast2GO consensus description. Many of the genes up-regulated in PLRV aphids were related to cuticle formation and development (16%) and catalytic activity (16%), however the majority of up-regulated transcripts were uncharacterized (31%; Fig 3A). The largest groups of down-regulated genes in PLRV aphids were related to histones (10%), catalytic activity (10%), transmembrane transport (9%), proteolysis or protein ubiquitination (7%), and nucleic acid binding and metabolic processes (7%; Fig 3B). A significant proportion of the down-regulated transcripts in PLRV aphids were also uncategorized (47%). The most highly expressed DEGs included transcripts related to

cuticle formation and development and 4C1-like cytochrome P450s (Table 2). The most down-regulated transcripts in PLRV aphid were related to histones and histone modifying proteins (Table 3).

Validation of aphid transcripts via RT-qPCR

To validate the RNAseq analysis a separate experiment was performed using the same experimental design and the transcript abundance of one up-regulated gene (MYZPE13164_G006_v1.0_000133030.2 (*MPCP5-like*)) and one down-regulated gene (MYZPE13164_G006_v1.0_000070430.1 (*HSP68-like*)) was measured using qRT-PCR (bolded genes in Table 2 and 3). Consistent with the RNA-seq data, abundance of the *MPCP5-like* transcript was significantly higher in viruliferous *M. persicae* compared to virus-free controls (10.045, 2.017, relative expression respectively; $p = 0.019$; Fig. 4A). Abundance of the *HSP68-like* transcript was significantly lower in viruliferous *M. persicae* when compared to virus-free controls (1.44, 5.51, relative expression respectively; $p < 0.01$) (Fig 4A-B).

The impact of PLRV on *Buchnera aphidicola* titer

Buchnera has been previously implicated in transmission of PLRV and other luteoviruses (26,28,30,34,63), however *Buchnera* titer and coding sequence transcripts have not been examined in aphids carrying PLRV. From our experiments, *Buchnera* titer was ~1.5 times higher for virus-free aphids compared to aphids carrying PLRV (ratios 6.42, 4.20 respectively; $p = 0.037$; Fig 5A). To investigate the potential mechanisms mediating decreases in *Buchnera* titer we measured abundance of two transcripts related to stress, *dnaK* (64,65) and *groEL* (65,66), and one transcript related to metabolism, *argE* (45). Abundance of all three transcripts were reduced in aphids carrying PLRV compared to controls. Viruliferous aphids had 36.68% less *argE*

transcripts ($p = 0.026$), 16.67% less *groEL* transcripts ($p = 0.024$), and 18.77% less *dnaK* transcripts ($p = 0.046$) compared to that of the virus free aphids (Fig 5B-D).

Discussion:

The main focus of this paper was to examine the effect that *Potato leafroll virus* has on the transcriptome of *M. persicae*, and their primary endosymbiont *Buchnera aphidicola*. The largest category of known up-regulated transcripts in viruliferous aphids compared to controls were related to the cuticle and cuticle development. Insect cuticles are largely composed of a protein matrix embedded with chitin filaments (67). Cuticle proteins (CPs) have been shown to be involved in general development, molting, transmission of non-persistent viruses, and insecticide resistance through changes cuticle permeability (33,68–71). In *Acyrtosiphon pisum*, 19 CPs were found to be regulated by photoperiodism and suspected to be involved in the transition from asexual to sexual production (72). Further, cuticle proteins have been implicated as potentially facilitating transmission of *Barley yellow dwarf virus* (BYDV-GPV), *Cereal yellow dwarf virus* (CYDV-RPV), and *Turnip yellows virus* (TuYV), three related Luteoviridae viruses (73–75). Whilst we cannot know the function of changes in CP transcripts in PLRV-aphid interactions from these experiments, these genes represent promising targets for further investigation.

In addition to many cuticle related proteins, five *cytochrome P450s* genes were significantly up-regulated in viruliferous aphids compared to controls. Cytochrome P450s play important roles in hormones and pheromones metabolism but are more famous for their roles in the metabolism of insecticides and host plant chemicals. Polyphagous insects, like *M. persicae*, encounter many different hosts and tend to have high numbers P450-based metabolism of

allelochemicals compared to more specialized aphids (76). Previous work has shown that a *cytochrome 450 gene (CYP6CY3)* was found to increase nicotine tolerance and aphid host adaptation (77,78). It has been previously hypothesized that upregulation of p450s could help insect vectors tolerate less desirable hosts which could be beneficial to the virus (79).

Transcripts encoding a heat shock protein (HSP68-like) was among the most down-regulated in viruliferous aphids compared to controls. HSP68 is a member of the HSP70 family, which are important chaperone proteins that are known to be up-regulated in response to stress. One study found that the *HSP70* from *Bemisia tabaci* is up-regulated after acquisition of *Tomato yellow leaf curl virus* (TYLCV) (80). They went on to show that HSP70 protein can directly interact with TYLCV using *in vitro* studies and that they co-localize together in insect midgut cells using *in situ* hybridization. The authors suggest HSP70 may play an inhibitory role in virus transmission, as transmission was reduced when whiteflies were fed HSP70 antibodies. Because *HSP68-like* transcripts were down-regulated in aphids carrying PLRV in our study, it's tempting to speculate that this may increase PLRV transmission. Porras et al. demonstrated that BYDV-PAV, a strain that is only transmitted by *Rhopalosiphum padi* (bird-cherry oat aphid), up-regulated the abundance of three *HSP70* transcripts in the aphid vector. The authors found BYDV infection increases plant surface temperature and aphid heat tolerance, suggesting a protective role of HSP70 proteins in virus-aphid-plant interactions (81). Although it is not known if PLRV increases plant surface temperature and vector heat tolerance, it has been shown that potato plants kept at higher temperatures are more susceptible to PLRV than compared to lower temperatures (82). Also aphid acquisition and transmission at higher temperatures has previously resulted in higher transmission rates compared to lower temperatures (83), however at very high

temperatures differences were reduced (84). It is not known how decreases in *HSP68-like* transcripts in aphids carrying PLRV in our study may alter aphid heat tolerance.

Several histone genes were down-regulated in viruliferous *M. persicae* compared to controls in this study. Histones are involved in DNA organization and regulation of gene expression (85), but have also been shown to be induced in response to stress and starvation (86). Histone depletion can lead to changes to an open chromatin configuration and large scale shifts in expression, however it should be noted histone depletion is also associated with DNA damage (87). In a recent study investigating small RNAs (sRNAs), *M. persicae* who fed upon PLRV infected plants and purified PLRV diets had significantly altered sRNA profiles and immune responses. Ultimately, the significance of this response is unknown, and further investigation is necessary to understand potential impacts of the downregulation of these genes.

In this study there was a significant reduction of *Buchnera* titer and *Buchnera* gene expression of three genes (*dnaK*, *groEL*, and *argE*) in aphids carrying PLRV compared to control aphids. In general, gene regulation at the mRNA level in *Buchnera* is thought to be minimal because *Buchnera* transcription factors are reduced (88) and very few transcriptional responses had been observed previously (17). Only two transcription initiation factors ($\sigma 32$ and $\sigma 70$), the heat shock and housekeeping transcription factors, respectively, remain in *Buchnera* Myzus's genomes (89) similar to other *Buchnera* taxa (90). The housekeeping sigma factor ($\sigma 70$) initiates transcription of *argE* which is regulated by the repressor *argR* when bound to arginine in *Escherichia coli* (91). Similar to other *Buchnera* taxa *Buchnera* Myzus's genomes (89) has lost the repressor *argR* so it is unclear how this gene is down-regulated in virus infected aphids compared to un-infected aphids. The other two *Buchnera* genes (*dnaK* and *groEL*) that were down-regulated in this study in aphids carrying PLRV compared to control aphids are

associated with the heat shock regulon (90). Moreover, these *Buchnera* genes still retain recognizable σ_{32} promoter sites up-stream of *dnaK* and *groEL* in the *Myzus Buchnera* G006 genome (NCBI Reference Sequence: NZ_MJNC01000001; Supplemental Table 3) similar to other *Buchnera* taxa (90). The σ_{32} heat shock response is highly conserved in bacteria and is initiated in response to stress, such as heat shock or other environmental stressors that destabilize proteins (92). In general, compared to free-living bacteria, *Buchnera* only modestly up-regulates genes that still retain the upstream σ_{32} promoter sites during heat shock (90). In this study it is unclear how PLRV is either directly or indirectly dampening *Buchnera*'s expression of *dnaK* and *groEL* and if it is through a similar mechanism that is also down-regulating the aphid's stress response genes including *Hsp70*.

A decrease in *Buchnera* titer has previously been associated with different aphid clones (93), plant diets (16), increasing aphid nymphal age (94,95), and heat shock (95–97). For example, a mutation in the promoter region of the heat shock gene (*ibpA*) results in the reduction of *Buchnera* titer (96). Given that the experiments in this study were conducted in the same environment at the same temperature it is highly unlikely that the control aphids experienced heat shock compared to virus infected aphids. Instead, we hypothesize that the virus PLRV is reducing *Buchnera*'s ability to up-regulate genes that are associated with the heat shock regulon and this may lead to increased stress and lysing of *Buchnera* cells and ultimately a reduction of *Buchnera* titer. For example, most obligate pathogens and symbionts, including *Buchnera*, overexpress the protein GroEL during non-heat shock conditions to rescue misfolded proteins (98). Alternatively, as PLRV-infected plants have higher concentrations of free amino acids (47) and we cannot discount the indirect impacts of host plant changes on *Buchnera* titer in our experimental design, similar to Zhang et al. (16) where a change in host plant diet influenced

Buchnera titer. *Buchnera* sRNA's that are hypothesized to regulate *Buchnera* gene expression at the post-transcriptional level have been observed to be differentially expressed when aphids feed on host plants that vary in essential amino acids (46). In turn, aphids carrying PLRV may obtain higher levels of essential amino acids from virus infected plants and as such *Buchnera* genes that are involved in arginine biosynthesis, such as *argE*, are down-regulated compared to control aphids feeding on un-infected plants with lower amounts of essential amino acids.

In other insect-plant pathogen systems plant pathogens are known to modulate obligate symbiont titer. For example, in whiteflies *Portiera* titer is modulated by the co-occurrence of its facultative symbiont *Rickettsia* and the Tomato Yellow Leaf Curl Virus (99). In a second system, the obligate symbionts *Carsonella* and *Profftella* of the psyllid *Diaphorina citri* decrease in titer when the plant pathogen *Ca. Liberibacter asiaticus* infects *D. citri* male adults, whereas no significant difference was found in infected female adults (100,101). In adult females' ovaries, *Carsonella* and *Profftella* titer increased when infected by *Ca. L. asiaticus*. The latter authors hypothesized that endosymbionts titer increases as a part of the psyllid's immune response and/or response to altered plant nutrition by *Ca. L. asiaticus* infection (100).

In general, this work improves our understanding of the relationships that exist between hosts, viruses, vectors, and endosymbionts, but it also opens up more questions regarding the complexity and depth of these relationships. Aphids and bacterial endosymbionts may benefit from relationships with plant-infecting viruses indirectly or directly but additional studies are needed. Although it is known that *Buchnera* titer and gene expression responses vary with aphid lineages (102), it is not known how this is impacted by long term associations with plant-infecting viruses. In regions where virus pressure is high or where poor hosts dominate, aphids may more often be associated with plant infecting viruses. Given the mounting evidence of virus

manipulation of insect vectors, this could have lasting impacts on the population structures of these insect vectors and their obligate endosymbiont.

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Author contributions

CLC conceived the project. MFP and CLC designed the research. MFP and CLC performed research and analyzed the data. AKH, MFP, and CLC interpreted the data. AKH, MFP, and CLC wrote the article.

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Figure Legends

Fig 1. Overview of *Myzus persicae* transcriptome after *Potato leafroll virus* (PLRV) acquisition. (A) Number of paired-end reads generated for each library by Illumina HiSeq sequencing. The dashed line represents the average of paired-end reads from all 6 libraries. (B) Proportion of uniquely mapped, multimapped, and unmapped reads obtained for each library. Reads were mapped in the *Myzus persicae* clone G006 genome (AphidBase). (C) Principal component analysis of normalized count data from all samples. (D) Hierarchical clustering analysis of normalized count data z-scores exhibited by differentially expressed genes (DEGs) within each sample. (E) Volcano-plots of $-\log_{10}p$ and $\log_2FC$ exhibited by each gene in viruliferous aphids compared to controls. Up- and down-regulated genes are presented in red and green, respectively. (F) Numbers of up- and down-regulated DEGs in viruliferous aphids in comparison to control aphids. DEGs were identified using DESeq2 and defined by $|\log_2FC| \geq 0.5$ and false discovery rate (FDR)-corrected p-value ≤ 0.1 . Control (aphids without virus); Viruliferous (aphids carrying PLVR). FC, fold-change; p, FDR-corrected p-value.

690

691 **Fig 2. Blast2GO Gene Ontology of DEGs arranged by functional categories.** (A) Biological
 692 processes (BP), (B) Molecular Function (MF), and (C) Cellular Component (CC). The predicted
 693 gene functions of differentially expressed genes as assigned by Blast2GO at level 2-3 in each
 694 aforementioned category. Each DEG may be assigned to one or more GOterm, with a total of
 695 391 GOterms from the three functional groups assigned to the 134 DEGs.

696 **Fig 3. Blast2GO annotation for up-regulated DEGs and down-regulated DEGs in aphids**
 697 **carrying PLRV compared to controls.** The consensus description predicted by Blast2GO of
 698 the (A) 64 up-regulated DEGs and the (B) 70 down-regulated DEGs.

699

700 **Fig 4: Relative transcript abundance of two genes in *Myzus persicae* with and without**
 701 ***Potato leafroll virus* (PLRV).** (A) A cuticle related protein (MPCP5-like) was significantly up-
 702 regulated in expression in individuals with PLRV compared to virus free controls. (B) A
 703 predicted heat shock protein (HSP68-like) was significantly down-regulated in expression in
 704 individuals with PLRV compared to controls. Transcripts were measured relative to a
 705 housekeeping gene *RPL7*. Significant differences were calculated using an ANOVA (* $P < 0.05$;
 706 Error bars represent $\pm$ SEM).

707 **Fig 5. *Buchnera aphidicola* titer and transcript changes in *Myzus persicae* with *Potato***
 708 ***leafroll virus* (PLRV).** (A) Ratio of a single copy *Buchnera* gene *rpIN* relative to a single copy
 709 aphid gene *RPL7*, demonstrates *M. persicae* with PLRV have a decreased *Buchnera aphidicola*
 710 titer relative to control aphids. (B-D) *Buchnera* transcripts *groEL*, *dnaK*, and *argE* relative to the
 711 *Buchnera* gene *rpIN* housekeeping gene. All three transcripts were down-regulated in

viruliferous *M. persicae* compared to virus free controls. Significant differences were calculated using an ANOVA (* $P < 0.05$; Error bars represent $\pm$ SEM).

Tables

Table 1: RNAseq Stats.

Sample Name	Total paired-end reads	Total alignments	Aligned	Unique paired	Non-unique paired
Control 33	40,676,571	30,990,077	76.17%	28.52%	47.67%
Control 34	18,942,570	13,440,120	70.80%	25.89%	45.07%
Control 35	22,651,445	16,478,122	72.49%	25.93%	46.82%
PLRV 36	25,343,473	18,306,992	72.12%	26.30%	45.94%
PLRV 37	23,743,718	17,496,940	73.63%	27.15%	46.54%
PLRV 38	23,533,704	17,410,337	73.78%	27.05%	46.93%

Table 2: Most highly up-regulated *M. persicae* genes that were characterized* in aphids carrying PLRV compared to controls. DEGs determined by adjusted p value < 0.1 and described by Blast2GO. Gene ID corresponds to MYZPE13164_G006_v1.0_XXXXXXXXXX.X found on AphidBase.org. Regulation of bolded transcripts were validated in a separate experiment.

*20 uncharacterized genes were up-regulated (See Supplementary Table S2)

Putative function	Gene ID	Blast2GO consensus description	p-val	log2
Cytochrome P450	000087490.2	cytochrome P450 4C1-like	1.30E-05	2.34
	000113270.1	cytochrome P450 4C1-like	1.90E-05	2.21
	000087490.3	cytochrome P450 4C1-like	3.10E-09	2.16
	000111320.1	cytochrome P450 6k1-like	1.10E-04	0.95
Cuticle related	000133030.2	Adhesion plaque protein, chitin binding	4.60E-05	2.24
	000133030.1	Adhesion plaque protein, chitin binding	1.90E-04	1.91
	000086070.1	endocuticle glycoprotein in abdomen	3.00E-07	1.05
	000079280.1	osiris 20-like	1.80E-06	0.95
	000103820.1	Adhesion plaque protein, chitin binding	6.60E-06	0.88
	000103820.2	Adhesion plaque protein, chitin binding	1.50E-06	0.87
	000079260.1	osiris 18	9.40E-05	0.85
	000084640.1	glycine and glutamine-rich	1.40E-05	0.77
	000047580.1	Myzus persicae tentative cuticle protein	2.60E-04	0.74
Kinase Inhibitor repressors	000156640.1	52 kDa repressor of kinase inhibitor-like	7.10E-05	0.88
	000156640.2	52 kDa repressor of kinase inhibitor-like	3.70E-04	0.79
	000156640.4	52 kDa repressor of kinase inhibitor-like	3.50E-04	0.78
Kinases	000073070.1	alpha- kinase 1-like	2.20E-06	0.78
	000137500.2	serine threonine- kinase (NEK3)	2.20E-06	0.76
Hydrolase	000181580.1	N-acetylmuramoyl-L-alanine amidase-like	4.40E-04	0.93
Transcription factor	000125820.1	transcription factor A2 (mab3-liked)	1.10E-05	1.94
Zinc Transport	000174630.1	39S ribosomal mitochondrial	8.60E-05	0.77
Membrane	000137380.1	histidine-rich glycoprotein	3.00E-05	0.90
Cell organization	000090710.1	cytoskeleton-regulatory complex (pan1-like)	4.80E-04	1.268
	000021370.2	microtubular process (CFA58-like)	4.70E-04	1.265

000189110.1 actin reorganization (WAS-like) 4.50E-04 0.83

Table 3: Most highly down-regulated *M. persicae* transcripts that were characterized* in aphids carrying PLRV compared to controls. DEGs determined by adjusted p value < 0.1 and described by Blast2GO. Gene ID corresponds to MYZPE13164_G006_v1.0_XXXXXXXXXX.X found on AphidBase.org. Regulation of bolded transcripts were validated in a separate experiment.

*33 uncharacterized genes were down-regulated (See Supplemental Table S2)

Putative function	Gene	Blast2GO consensus description	p-value	log2
Histones	000100490.1	histone H3	5.06E-05	-2.58
	000100610.1	histone H3	2.69E-04	-2.14
	000100770.1	histone H3	1.04E-04	-1.74
	000100600.1	histone H2A	1.38E-04	-1.75
	000092680.1	histone H2A-like	9.76E-05	-1.47
	000100590.1	histone H2B-like	7.37E-05	-1.18
	000100620.1	histone H4	3.02E-04	-0.96
Histone Modifying	000163990.2	glycine-rich DOT1-like	7.33E-05	-0.81
	000163990.1	glycine-rich DOT1-like	1.97E-04	-0.76
ubiquitination	000119640.3	E3-ubiquitin ligase RNF19B-like	6.08E-06	-0.88
	000119640.1	E3-ubiquitin ligase RNF19B-like	1.42E-05	-0.81
	000119640.2	E3-ubiquitin ligase RNF19B-like	2.34E-05	-0.78
Hydrolase	000133360.1	serine carboxypeptidase	1.10E-04	-1.58
	000083200.2	Arylsulfatase B-like	4.69E-04	-1.11
	000083200.1	Arylsulfatase B-like	3.25E-04	-1.02
	000200070.1	Thioesterase (THEM6-like)	1.13E-04	-0.82
Response / Immunity	000071560.1	Protease inhibitor (Papain inhibitor)	2.91E-04	-2.46
	000070430.1	Heat shock 68-like	4.85E-04	-1.88
	000193260.2	G- coupled receptor Mth-like 3	2.03E-04	-0.80
Transport	000029670.1	dynein intermediate chain-like	1.90E-05	-1.01
	NRF6	Lipid transport (NRF6-like)	5.18E-05	-0.85
	000203490.1	zinc finger C3H1 type-like 2-A	1.03E-04	-0.86
	000072950.2	Sugar transport (TRET1-like)	6.74E-06	-0.81
nucleic acid metabolism	000036830.1	DNA integration (pol poly retrotransposon-related)	1.86E-04	-0.89
	000012610.1	mRNA catabolic process (BRISC/BRCA1-A complex-Like)	5.14E-04	-0.84

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752 **Supporting Information**

753 Supplementary Figure S1: Explanation of process

754 Supplementary Table S1: Primer Table

755 Supplementary Table S2: RNAseq significance list

756 Supplementary Table S3: Conserved σ_{32} -10 and -35 binding sites in *Escherichia coli* and

757 *Buchnera* taxa

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Figure 1: Overview of *Myzus persicae* transcriptome after *Potato leafroll virus* (PLRV) acquisition.

Figure 2: Blast2GO Gene Ontology of DEGs arranged by functional categories.

Figure 3: Blast2GO annotation for the up-regulated DEGs and down-regulated DEGs in aphid carrying PLRV compared to controls.

Fig 4: Relative transcript abundance of two genes in *Myzus persicae* with and without *Potato leafroll virus* (PLRV). (A) A cuticle related protein (MPCP5-like) was significantly up-regulated in expression in individuals with PLRV compared to virus free controls. (B) A predicted heat shock protein (HSP68-like) was significantly down-regulated in expression in individuals with PLRV compared to controls. Transcripts were measured relative to a housekeeping gene *RPL7*. Significant differences were calculated using an ANOVA (* $P < 0.05$; Error bars represent $\pm$ SEM).

- **Fig 5. *Buchnera aphidicola* titer and transcript changes in *Myzus persicae* with *Potato leafroll virus* (PLRV).** (A) Ratio of a single copy *Buchnera* gene *rpIN* relative to a single copy aphid gene *RPL7*, demonstrates *M. persicae* with PLRV have a decreased *Buchnera aphidicola* titer relative to control aphids. (B-D) *Buchnera* transcripts *groEL*, *dnaK*, and *argE* relative to the *Buchnera* gene *rpIN* housekeeping gene. All three transcripts were down-regulated in viruliferous *M. persicae* compared to virus free controls. Significant differences were calculated using an ANOVA (* $P < 0.05$; Error bars represent $\pm$ SEM).

Figure S1: Explanation of process