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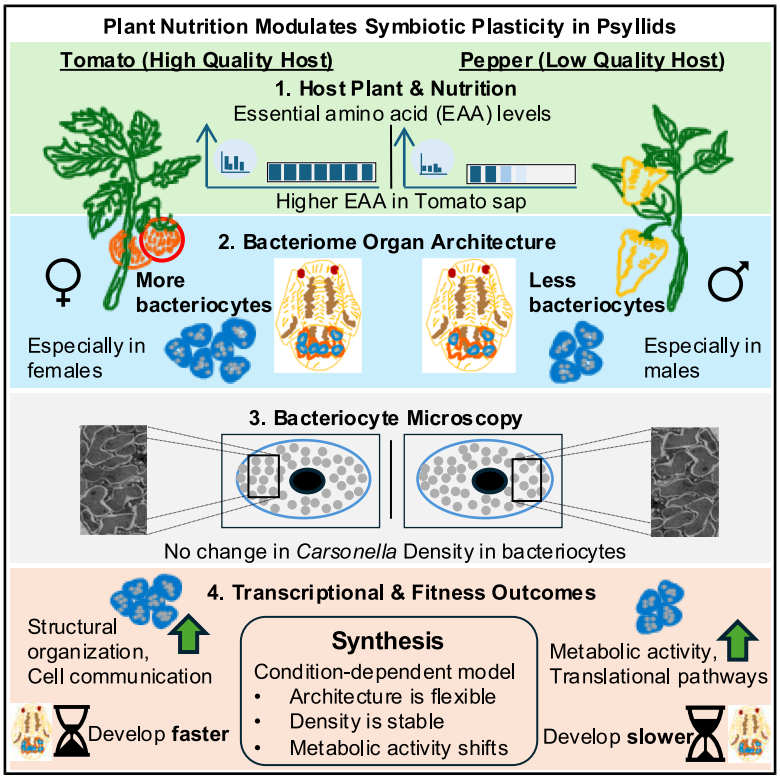
Host plant quality reshapes symbiotic organ architecture without altering symbiont density

Graphical abstract



Highlights

- Tomato phloem shows ~2.3× higher essential amino acid concentrations than pepper
- Tomato increases bacteriocyte number (especially females), not *Carsonella* density
- Bacteriome gene expression shifts are minor, affecting cell structure, and translation
- High plant quality raises fitness and drives flexible bacteriome architecture

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In brief

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Host plant quality reshapes symbiotic organ architecture without altering symbiont density

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SUMMARY

Obligate nutritional symbioses enable sap-feeding insects to overcome essential amino acid limitations, yet the extent to which these associations exhibit plasticity across host plants remains unclear. We tested whether host plant quality alters the *Bactericera cockerelli-Carsonella* symbiosis by comparing psyllids developing on tomato and pepper. Tomato sap contained significantly higher essential amino acid concentrations than pepper. Despite these nutritional differences, only 42 genes were differentially expressed in the bacteriome, a symbiotic organ composed of insect cells (bacteriocytes) that house *Carsonella*. Tomato-fed psyllids upregulated genes associated with cell communication and structural organization, whereas pepper-fed psyllids upregulated metabolic and translational pathways. We also observed that bacteriocyte number was higher on tomato and in females, while bacteriocyte symbiont density remained unchanged. Psyllid fitness was also higher on tomato. These findings support a condition-dependent model in which plant nutrition shapes bacteriome structural and transcriptional investment in symbiosis without altering symbiont density per bacteriocyte.

INTRODUCTION

Insect herbivores are generally highly specialized, feeding on a single host plant species or a narrow range of closely related taxa.[1] Psyllids (Hemiptera: Psylloidea), a group of sap-sucking insects, exemplify this pattern of specialization, as most species exhibit strong host plant specificity and rely on either a single plant species (monophagy) or a limited set of congeneric plant hosts (oligophagy) to feed and complete their development on.[2] This specialization is further shaped by the nutritional constraints of their exclusive diet of phloem sap.[3] Phloem sap presents significant nutritional challenges for insect herbivores. It is rich in sugars but low in nitrogen, particularly in the form of essential amino acids, the group of amino acids that animals cannot synthesize *de novo*.[3,4] Because amino acid concentrations and compositions vary substantially among plant species,[4,5] the nutritional environments experienced by oligophagous psyllids can differ markedly across hosts. It remains unclear how this nutritional variation affects the psyllid's nutritional metabolism, and how much metabolic flexibility is required to accommodate differences in essential amino acid profiles.

Obligate nutritional endosymbionts are widespread among sap-sucking insect herbivores and are essential for insect host development and reproduction because they supplement sap diets deficient in essential amino acids.[3] In psyllids, all species examined to date harbor the obligate endosymbiont *Carsonella*, which has co-diversified with its insect hosts over millions of years and encodes most essential amino acid pathways.[6,7] *Carsonella* is housed within an organ-like structure within the abdomen known as the bacteriome, which is composed of specialized insect host cells called bacteriocytes.[8] Within bacteriocytes, psyllids and *Carsonella* engage in a tightly integrated metabolic partnership, collaboratively synthesizing essential amino acids through pathways that combine symbiont-encoded functions with host genes, including host genes acquired via horizontal gene transfer.[9–11] This metabolic interdependence reflects both the extreme genome reduction of *Carsonella* and the long-term evolutionary relationship of this obligate mutualism.[6,7]

Similar metabolic collaborations between sap-feeding insect hosts and their obligate nutritional symbionts have independently evolved in other insect lineages as well.[12] Previous studies, primarily in aphids and to a lesser extent in whiteflies, show that variation in essential amino acid composition across host plant species and artificial diets can significantly influence insect fitness, obligate symbiont titer, bacteriocyte number, and bacteriocyte gene expression and methylation profiles. However, the direction and magnitude of these effects vary among systems and experimental designs, particularly with respect to diet type, specific amino acid manipulations, presence of facultative symbionts, and insect and symbiont genotypes.[13–24] Despite growing evidence for metabolic cooperation between psyllids and *Carsonella*,[9–11] it remains poorly understood whether, and how, oligophagous psyllids feeding on multiple host plants that vary in availability of essential amino acids in

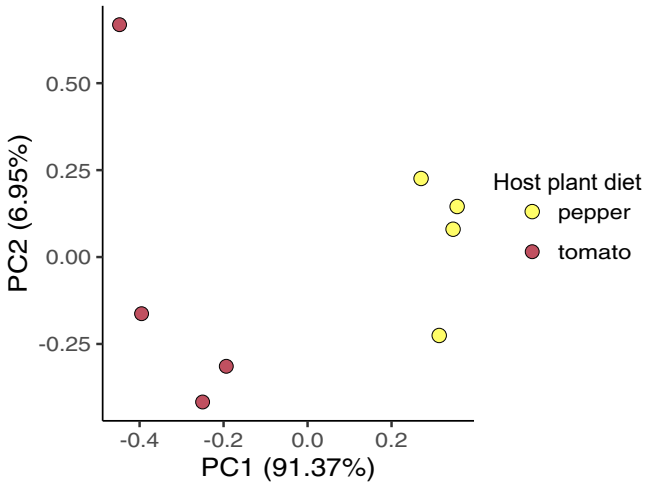

Figure 1. PCA plot comparing essential and non-essential amino acid concentrations from tomato and pepper phloem sap from plants infested with *B. cockerelli*

Four pooled biological replicates per plant species. Data are $\log_{1+}$ transformed amino acid concentrations, % variance explained by PC1 and PC2, and variables are scaled (mean-centered and variance-standardized).

sap modulate their interactions with *Carsonella* to meet insect host nutritional demands.

Bactericera cockerelli, the potato psyllid, is an oligophagous species that broadly feeds and develops on multiple host plant species primarily within the Solanaceae plant family.[25,26] Native to North America, *B. cockerelli* is a major agricultural pest of solanaceous crops, including potato and tomato, due to its ability to transmit the bacterial plant pathogen *Candidatus* Liberibacter psyllaurous (now formally recognized as *Candidatus* Liberibacter psyllidaureus) into crop plants causing disease.[27,28] Beyond its plant pathogenic effects, *Ca.* Liberibacter psyllidaureus can also function as a mutualistic facultative symbiont for the psyllid, complementing genetic gaps in *Carsonella*'s biosynthesis of arginine and enhancing psyllid host fitness under arginine limitation.[29]

Here, we examine whether plasticity exists in the *B. cockerelli-Carsonella* symbiosis by assessing how this association responds to feeding on two solanaceous host plants, tomato and pepper, which may vary in sap amino acid profiles.[30,31] Specifically, we test whether sap from tomato and pepper differs in amino acid concentrations during psyllid feeding and development, and whether host plant species is associated with changes in bacteriome gene expression. We further evaluate whether host plant species influences bacteriocyte number, *Carsonella* density, and psyllid fitness. Together, this work aims to elucidate how nutritional variation among host plant species shapes the regulation of an obligate insect-microbe mutualism.

RESULTS

For all trials, the same genotype of *B. cockerelli* (not infected with *Candidatus* Liberibacter psyllidaureus)[10] was used and maintained on either pepper or tomato for more than three generations prior to the start of the experiments. For all trials the study targeted the 5th instar to capture peak nutritional demands during the final stage of maturation before molting into a sexually mature adult while also ensuring the findings remain consistent with previous psyllid bacteriome transcriptomic research.[11,29]

Amino acid analyses of host plant species sap

Phloem sap amino acid profiles of *B. cockerelli* infested tomato and pepper were first compared using multivariate analyses. Multivariate analysis revealed a significant effect of plant species on overall amino acid profiles (PERMANOVA, $R^2 = 0.89$, $p = 0.026$), with clear separation between tomato and pepper sap biological replicates in the principal-component analysis (PCA) ordination (Figure 1; Table S1).

Univariate comparisons of individual amino acids and their derivatives showed that most of the compounds were present at significantly higher concentrations in tomato sap compared to pepper sap, including all essential amino acids (Welch's two-sample *t* test, FDR-adjusted $p < 0.05$) (Figure 2; Table S1). Several plant stress- and signaling-related metabolites, such as GABA, β-alanine, and ethanolamine, were especially enriched in tomato sap (up to ~7.5-fold) (Table S1). In contrast, a small subset of non-essential amino acids (e.g., glutamate, aspartate) and other amino acid derivatives (e.g., citrulline (Cit) and sarcosine) did not differ between host plant species (Welch's two-sample *t* test, FDR-adjusted $p > 0.05$) (Figure 2; Table S1).

Few genes are differentially expressed in psyllid bacteriomes across host plant species

For RNA-sequencing, an average of 22,962,325 ($N = 4$) and 19,887,561 ($N = 4$) trimmed, high-quality reads per biological replicate were successfully mapped to *B. cockerelli's* genome for the tomato and pepper 5th instar bacteriome samples, respectively (Table S2; Figure S1). Only 42 genes out of 12,160 annotated *B. cockerelli* genes were significantly differentially expressed in bacteriomes between tomato and pepper host plant species. A total of 30 genes were significantly upregulated in bacteriomes of psyllids feeding on tomato plants, and a total of 12 genes were significantly upregulated in bacteriomes of psyllids feeding on pepper plants (Table S3).

Among the 30 genes significantly upregulated in bacteriomes of psyllids feeding on tomato compared to pepper, 77% had functional annotations based on BLASTp, GO terms, and/or Phyre2, while the remainder were hypothetical or transposon-related proteins (Figure 3[10]; Table S3). Annotated genes spanned several broad GO functional categories, including cell structure and motility, organ structure, membrane transport, and trafficking, protein quality control and regulation, and metabolism and biosynthesis (Figure 3; Table S3). Consistent with these annotations, STRING-based gene ontology enrichment analysis identified 32 significantly enriched biological process GO terms in tomato-fed psyllid bacteriomes. These were dominated by pathways related to cell communication and signaling such as cilia and microtubules, intracellular trafficking and signaling, photoreceptor-like programs, synapse- and junction-like regulation, and nutrient and ion transport (Figure S2; Table S4).

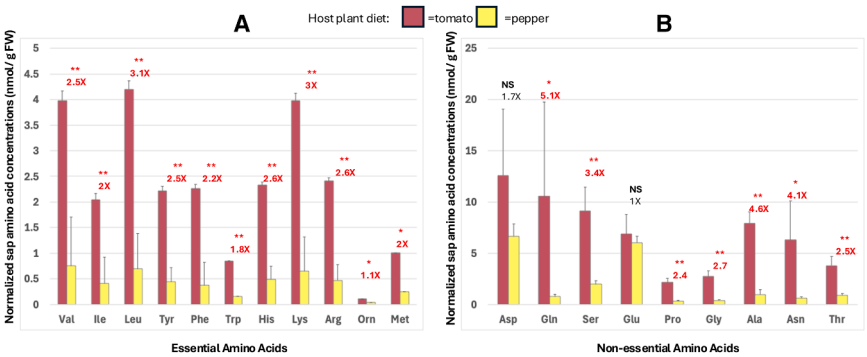

Figure 2. Free amino-acid concentrations from tomato and pepper phloem sap

(A and B) Normalized sap amino acid concentrations (nmol/g FW) for: (A) essential amino acids and (B) non-essential amino acids from tomato and pepper phloem sap. Significance values are from Welch's two-sample *t* test (N = 4 pooled biological replicates per plant species). Significance is indicated with red asterisks; ** indicates p adj <0.001; * indicates p adj <0.05; NS = not significant. Fold changes are from normalized sap concentrations (nmol/g FW) comparing tomato to pepper.

In contrast, only 12 genes were significantly upregulated in bacteriomes of psyllids feeding on pepper compared to tomato, of which 75% were functionally annotated and the remainder classified as hypothetical proteins (Figure 3; Table S3). Annotated genes were primarily associated with metal transport, gene regulation and signaling including RNA binding proteins, immune, endocrine, and membrane related genes such as the Toll pathway's Gram-positive serine protease (*GRASS*), a venom-like protease, the takeout-like (*TO*) protein, and a lipid binding protein (Figure 3; Table S3). STRING enrichment analysis revealed 13 significantly enriched biological process GO terms in pepper-fed psyllid bacteriomes, with enrichment of pathways related to high metabolic activity and protein synthesis such as ribosome biogenesis, RNA

upregulated on tomato diet	annotation	gene
(1) cell structure, division, and motility	dynein beta chain, ciliary-like	ANN06438
	thioredoxin domain-containing protein 9	ANN11735
	intraflagellar transport protein 57 homolog	ANN01224
	cilia- and flagella-associated protein 20-like	ANN22261
	coiled-coil domain-containing protein 81-like	ANN00669
	microtubule-associated protein Jupiter-like	ANN22111
	structural protein (nucleoporin ndc1)	ANN20732
(2) organ structure	collagen alpha-1(III) chain-like	ANN18245
	neural cell adhesion molecule; hemicentrin-2	ANN08496
(3) membrane transport and trafficking	sodium channel protein Nach-like	ANN06129
	beta-galactoside alpha-2,6-sialyltransferase	ANN07065
	otoferlin-like isoform	ANN10932
	ABC transporter G family member	ANN06355
(4) protein quality control and regulation	DDI1 homolog;	ANN21359
	parkin coregulated gene protein homolog	ANN10650
	hsp90 co-chaperone	ANN08066
	calmodulin-A-like	ANN04723
	protein croquemort-like isoform X1 [	ANN02604
	protein peptidyl-prolyl isomerization	ANN03264
(5) metabolism and biosynthesis	fatty acyl-CoA reductase wat-like	ANN13551
	cytochrome P450	ANN15891
	putative malate dehydogenase	ANN20489
	extracellular sulfatase Sulf	ANN19769
upregulated on pepper diet		
(1) metal transport	zinc transporter ZIP3	ANN10557
(2) gene regulation, signaling, cell division	RNA binding protein/rna/dna	ANN14486
	arrestin domain-containing protein	ANN20509
	P:regulation of mitotic nuclear division	ANN15081
(3) immune, endocrine, metabolism	venom protease-like	ANN07195
	Gram-Positive specific serine protease	ANN15907
	protein takeout like	ANN17290
	alpha-tocopherol transfer protein-like	ANN13992
	Lipid binding protein	ANN04535

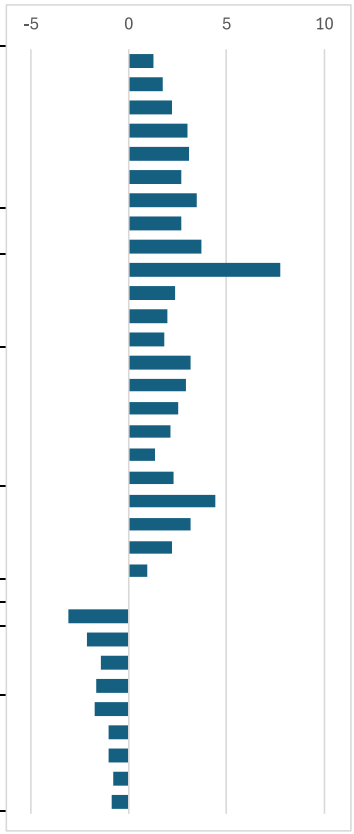

Figure 3. Annotated *Bactericera cockerelli* genes differentially expressed significantly in bacteriomes from tomato compared to pepper host plant diets

Significance is defined as FDR $\leq$0.05 and $\geq$ log2(1.5) fold change in normalized expression between the groups. Positive values = upregulated in tomato/downregulated in pepper; negative values = upregulated in pepper/downregulated in tomato. Genes are grouped by broad biological associations and annotations from BLAST, Phyre 2.0, or GO processes. Gene annotation IDs are from Kwak et al. 2023.[10] The bar graph to the right of genes represents log2 fold change in gene expression of pooled bacteriome biological replicates on tomato (N = 4) compared to pepper (N = 4).

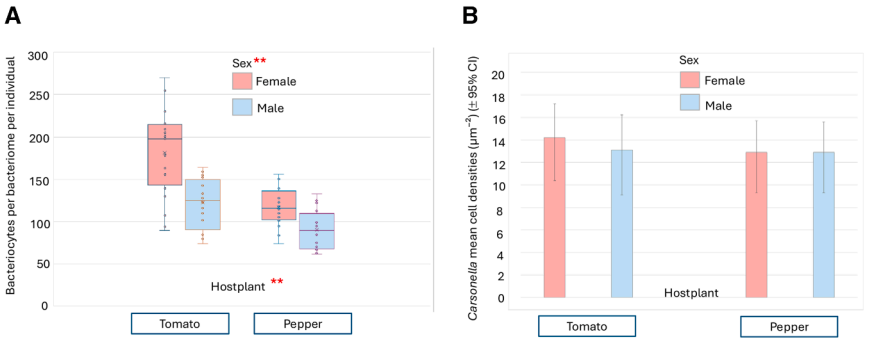

Figure 4. Psyllid bacteriocyte number and *Carsonella* density between tomato and pepper host plant species

(A) Box and whisker plots (interquartile range, horizontal line in bar represents mean) of bacteriocyte counts for individual 5th instar *B. cockerelli* males and females on two host plant species, tomato and pepper. Each black dot represents an individual psyllid, $N = 40$ individuals per host plant species, which consists of 20 males and 20 females. Significant factors are indicated with red asterisks; ** indicates $p < 0.001$.

(B) *Carsonella* cell densities (estimated marginal means ± 95% confidence intervals) of 5th instar female or male *B. cockerelli* feeding on either tomato or pepper plant species. Sex, plant species, and its interaction are non-significant based on a negative binomial mixed-effects model. $N = 22$, 17, 20, and 21 tiles for tomato female, tomato male, pepper female, and pepper male, respectively.

processing, chromatin assembly, and mitochondrial translation (Figure S2; Table S4).

Bacteriocyte cell count is higher in females and in tomato feeding psyllid bacteriomes

To assess the effects of host plant diet and sex on bacteriocyte abundance within *B. cockerelli* bacteriomes, we quantified the number of bacteriocytes per bacteriome in 5th instar psyllids feeding on either tomato or pepper plants ($N = 40$ psyllid individuals per host plant species; 50% female and male). Both sex and host plant had significant main effects on bacteriocyte number, with higher bacteriocyte counts observed in females compared to males and in psyllids feeding on tomato compared to pepper (sex, $F(79, 1) = 33.033$, $p < 0.001$), $\eta^2 = 0.303$; host plant, $F(79, 1) = 42.004$, $p < 0.001$, $\eta^2 = 0.356$) (Figure 4A). The interaction between sex and hostplant was also significant ($F(79, 1) = 4.755$, $p = 0.032$, $\eta^2 = 0.059$).

Carsonella cell density is the same across host plant and sex treatments

Using transmission electron microscopy (TEM) *Carsonella* cell density within 5th instar bacteriocytes was analyzed using a negative binomial generalized linear mixed model (GLMM) incorporating host plant (pepper vs. tomato), sex (female vs. male), and their interaction as fixed effects, with insect individual included as a random intercept to account for repeated tile measurements. The negative binomial model adequately captured the mean-variance relationship of the data, as no evidence of overdispersion was detected (dispersion ratio = 1.02, $p = 0.84$), and residual diagnostics based on DHARMa simulations showed no systematic deviations from model assumptions, with residuals displaying uniformity ($p = 0.44$) and no evidence of zero inflation ($p = 1.0$). *Carsonella* cell density did not differ significantly between host plant species (type III Wald $\chi^2 = 0.32$, $p = 0.57$), between sexes ($\chi^2 < 0.001$, $p = 0.99$), nor was there a significant host plant × sex interaction ($\chi^2 = 0.11$, $p = 0.74$). Thus, *Carsonella* abundance within bacteriocytes was consistent across pepper and tomato fed psyllids for both males and females (Figures 4B and 5).

Psyllid fitness is higher on tomato compared to pepper

To assess the impact of host plant species on the fitness of *B. cockerelli*, bioassays were conducted with *B. cockerelli* feeding and developing on a diet of either pepper or tomato. Results indicate that psyllids feeding on tomato exhibited significantly greater fitness compared to psyllids feeding on pepper, with a faster egg incubation time ($F(20,1) = 65.490$, $p < 0.001$, $\eta^2 = 0.766$), nymphal development time ($F(20,1) = 8.225$, $p = 0.010$, $\eta^2 = 0.291$), and total development time ($F(20,1) = 51.249$, $p < 0.001$, $\eta^2 = 0.719$; Figures 6D–6F). Other life history traits, including hatch rate, number of eggs laid, number of eggs hatched, adult emergence rate, total immatures, and total adults, were not significantly different between host plant diets (Figures 6A–6C and 6G–6I).

DISCUSSION

Host plant nutritional context shapes symbiotic investment

Host plant quality strongly shaped the nutritional landscape experienced by developing psyllids. Phloem sap from tomato plants contained substantially higher concentrations of amino acids than sap from pepper plants during immature development, with all essential amino acids elevated by an average of ~2.3-fold (Figure 2). Because essential amino acids must be synthesized *de novo* through the obligate *B. cockerelli-Carsonella* symbiosis, this difference in resource availability was expected to alter the metabolic demands placed on the partnership. On the higher-quality host (tomato), where essential amino acids are more abundant and overall fitness is elevated (Figure 6), psyllids appear able to allocate more resources toward expanding bacteriocyte number, potentially increasing total symbiotic output without altering per-bacteriocyte symbiont density (Figure 4). In contrast, on the lower-quality host (pepper), psyllids may compensate for essential amino acid limitation through heightened metabolic and translational activity (Figures 3 and S2) rather than through restructuring the symbiosis itself (Figure 4). Collectively, these findings point toward a condition-dependent model of symbiotic investment in psyllids, in which favorable nutritional environments promote structural expansion of bacteriocyte number potentially allowing more *Carsonella* symbionts per insect host, whereas nutritionally limited environments elicit metabolic compensation. Future work should explicitly test whether this represents an adaptive regulatory strategy for oligophagous psyllids.

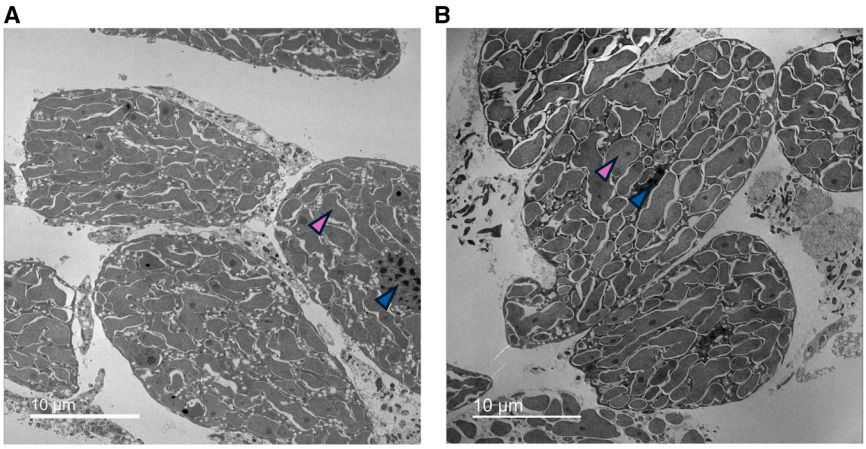

Figure 5. Bacteriocytes of psyllids feeding on tomato and pepper

Representative transmission electron microscopy (TEM) images of *Bactericera cockerelli* bacteriomes from (A) a 5th instar female psyllid feeding on tomato, and (B) a 5th instar male psyllid feeding on pepper. Dark regions in the center of the enlarged symbiotic insect cells (bacteriocytes) are the insect nuclei (examples indicated with blue arrowheads). *Carsonella* cells are visible as irregular shaped gray regions outlined in white (an artifact of the host derived membrane surrounding each cell) within the cytoplasm of the bacteriocytes (examples indicated with magenta arrowheads). Image taken at 1200× magnification. The scale bar in the images represents 10 μm.

Context-dependent responses across hemipteran systems

Work in the aphid, *Acyrthosiphon pisum*, has similarly demonstrated that bacteriocyte number can respond to variation in essential amino acid availability. However, studies using artificial diets have reported the opposite pattern to that observed here. For example, when specific essential amino acids were limited, aphids increased bacteriocyte number and upregulated genes associated with cell signaling and proliferation under the more nutritionally unsuitable conditions.[22,24] In contrast, when *A. pisum* fed on two natural host plant species that differed in amino acid profiles, bacteriocyte number and obligate symbiont titer remained unchanged based on TEM and quantitative PCR (q-PCR). Instead, aphids differentially expressed host collaborative symbiosis genes (some with differential methylation), potentially compensating at the transcriptional level for reduced host plant suitability and the associated decline in fitness.[23]

More broadly, most studies examining nutritional effects on insect-symbiont interactions, particularly in aphids, have focused on changes in obligate symbiont titer rather than bacteriocyte abundance. In artificial diet experiments, obligate symbiont titers generally increase with higher nitrogen or essential amino acid concentrations.[14,18] Yet this relationship is not universal. In whiteflies feeding on different plant species that varied in essential amino acid content, lower essential amino acid availability was associated with increased obligate symbiont titer.[19] Together, these contrasting patterns suggest that symbiotic responses to nutritional variation are context dependent, differing across taxa and between artificial and natural dietary conditions, and may involve shifts in bacteriocyte number, symbiont titer, and/or host transcriptional regulation depending on the ecological setting, including the presence of facultative symbionts or host plant switching.[16,17,21] However, some of the inconsistency in reported symbiont titer responses may also reflect methological limitations, as symbiont abundance is typically measured in studies using qPCR proxies of genome copy number. These measures can be influenced by both host and symbiont polyploidy, with extreme polyploidy documented in *Carsonella* and psyllid bacteriocyte cells.[32–35] Importantly, variation in host bacteriocyte and symbiont polyploidy has been observed across development, sex, and different conditions thereby introducing bias and complicating biological interpretation of gene copy number-based estimates of symbiont titer.[32,36]

Sex-specific allocation to the symbiosis

In addition to host plant effects, bacteriocyte number was consistently higher in females than in males at the 5th instar, regardless of plant species (Figure 4A). Sex-specific differences in bacteriocyte abundance are rarely evaluated in aphid or whitefly nutritional studies. For example, aphid experiments typically rely on female parthenogenic lineages to maximize generation speed, experimental tractability, and control for genotype. Nonetheless, sex-dependent patterns in obligate symbiont titers have been documented in developmental and stage specific studies in a variety of insect groups. In psyllid (*B. cockerelli*) adults, 16S rRNA based fluorescence *in situ* hybridization indicated no sex-based differences in bacteriome-associated *Carsonella* abundance, whereas qPCR suggested higher whole-body titers in adult females. This discrepancy may reflect the inclusion of *Carsonella* in female reproductive tissues in whole-body qPCR estimates, as well as differences in how each method is influenced by variation in symbiont polyploidy.[37] Interestingly, consistent with our findings that 5th instar females harbor more bacteriocytes per bacteriome (Figure 4), prior work shows that adult female psyllids (*B. cockerelli*, *Cacopsylla pyricola*, and *Aphalara calthae*) possess larger bacteriomes than males,[38] suggesting sex-based differences in bacteriome size and cellular composition. In species from several other insect systems with nutritional symbionts, such as mealybugs, lice, aphids, scales, and bed bugs, obligate symbionts are either absent, degenerate, and/or decline with age in males when compared to their female counterparts.[39–44] These patterns are often interpreted in the context of differential reproductive investment between sexes, given the maternal transmission of obligate symbionts. In contrast, although we observed greater bacteriocyte abundance in females, symbiont density within bacteriocytes did not differ between sexes at the 5th instar on either host plant (Figure 4B). This suggests that sex-specific variation at this developmental stage may be driven by differences in host cell allocation rather than changes in per-cell symbiont load.

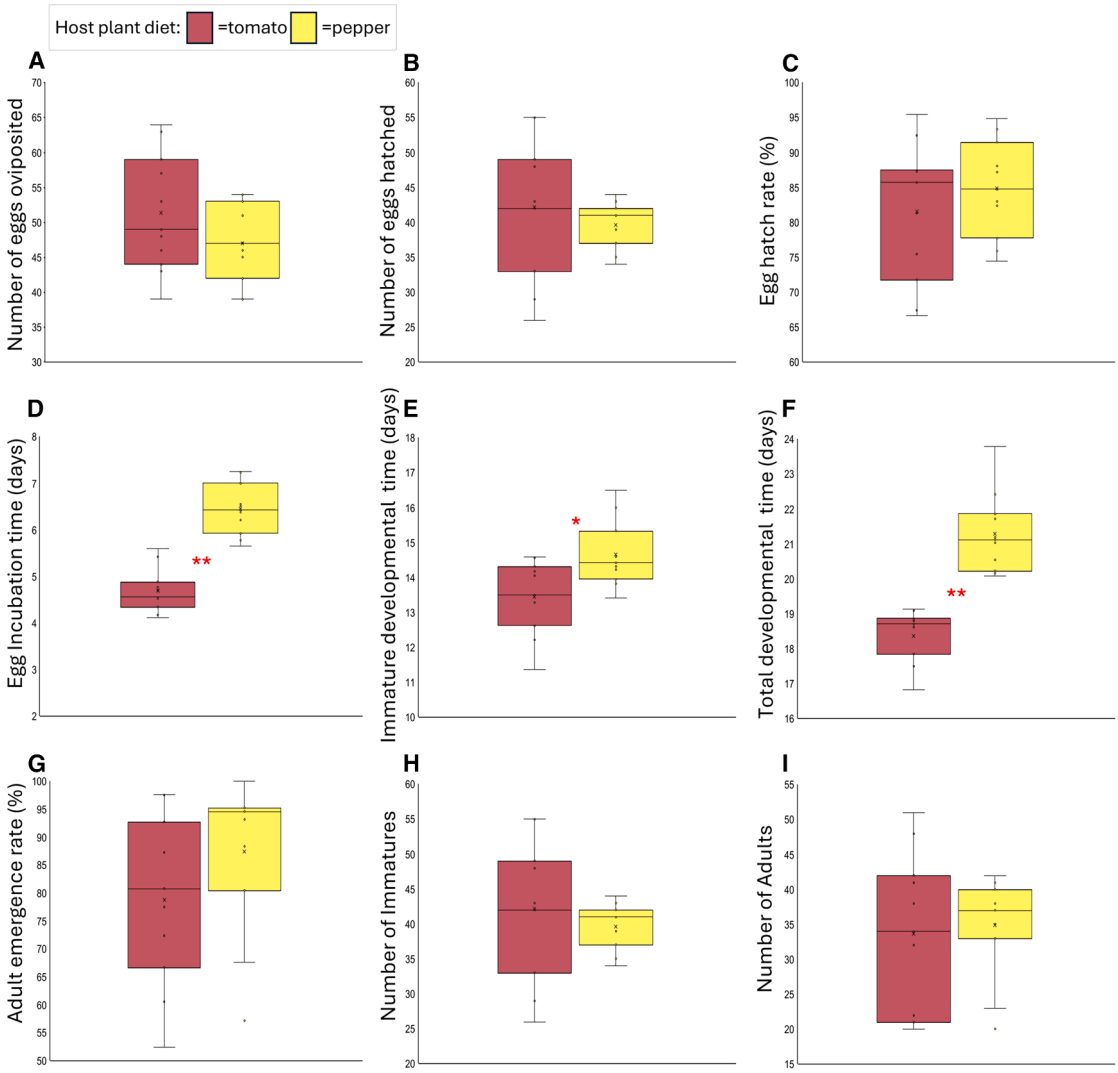

Figure 6. Life history parameters of psyllids feeding on tomato compared to pepper

Box and whisker plots (interquartile range, horizontal line in bar represents mean) of *Bactericera cockerelli* life history and developmental data (A–I) for psyllids developing on tomato compared to pepper. Each black dot represents an isofemale line (N = 11 isofemale lines per host plant species). Significant factors are indicated with red asterisks; one-way ANOVA, ** indicates $p < 0.001$; * indicates $p < 0.01$.

Transcriptional signatures of structural expansion and metabolic compensation

Surprisingly, given plant nutritional differences and differential psyllid fitness between host plant species only a small subset of psyllid genes were significantly differentially expressed in their symbiotic organ, the bacteriome (Figure 3). Insect genes directly involved in the collaborative biosynthesis of essential amino acids with *Carsonella* were not differentially regulated between host plant species. This contrasts with *Ca.* L. psyllidaureus based infection comparisons: a previous study of the same psyllid genetic line, differing only in infection with *Ca.* L. psyllidaureus and feeding on a common host plant (tomato) revealed fitness differences and identified ~366-fold more differentially expressed genes between infected and un-infected psyllids.[29] Many of these genes were linked to the nutritional symbiosis, including horizontally transferred genes in the psyllid genome that interact with *Carsonella* to support the biosynthesis of methionine, phenylalanine, and arginine. Further non-plant based, artificial diet experiments corroborated mRNA sequencing and metagenomic findings, demonstrating that *L. psyllidaureus* plays a mutualistic role in supplying arginine to the psyllid host and increases psyllid fitness.[29] In other systems such as *A. pisum* where their host plants varied in amino acid profiles, collaborative genes involved in ammonia recycling and transport were found to be differentially regulated in bacteriocytes.[23] In contrast, when *A. pisum* was fed an artificial diet deficient in tyrosine and phenylalanine, two essential amino acids important for development, no differential regulation was observed in bacteriocyte genes associated with the metabolism of tyrosine or phenylalanine, or in amino acid transport. Instead,

genes involved in proliferation, cell size control, and signaling were differentially expressed, accompanied by increases in both bacteriocyte number and size.[22] Together, these findings suggest that compensatory responses to nutrient limitation can vary substantially across biological contexts and systems.

Here bacteriomes from tomato-fed psyllids exhibited a broader upregulated transcriptional response than those from pepper-fed psyllids, with upregulated genes and enriched pathways related to cell communication, intracellular trafficking, and structural organization, whereas pepper-fed psyllids showed a smaller set of upregulated genes and enriched pathways primarily associated with metabolic activity, ribosome biogenesis, and protein synthesis (Figures 3 and S2). The enhanced cell signaling and trafficking in tomato-fed psyllid bacteriomes is consistent with their increased bacteriocyte number, as expansion of the bacteriocyte population would likely require greater intercellular coordination and maintenance inside these bacteriome organs. In contrast, on the less nutritious pepper, where psyllids exhibited lower fitness and fewer bacteriocytes, upregulation of metabolic and translational pathways suggests that, when structural expansion of bacteriocytes in the bacteriome is constrained, psyllids may compensate by increasing activity within existing bacteriocytes.

Notably, the upregulation of the *takeout-like* (*TO*) gene in pepper-fed psyllid bacteriomes is intriguing given that, in *Drosophila*, *takeout* functions within a circadian output pathway that integrates temporal and nutritional status cues to regulate feeding-related metabolism and behavior.[45] In *Drosophila*, the gene is expressed in the brain, antennae, and alimentary canal and is induced under starvation, consistent with a role in nutritional stress responses.[45] Although the function of *TO* in psyllids remains unknown, its elevated expression in bacteriomes of psyllids feeding on the lower-quality pepper host is consistent with a potential role in coordinating nutritional stress or feeding-related metabolic adjustments within the symbiosis with *Carsonella*. Supporting this interpretation, in *Candidatus* Liberibacter psyllidaureus infected psyllids from the same genetic line, where infection increases psyllid host fitness and *Candidatus* Liberibacter psyllidaureus provisions arginine to the psyllid, *TO* expression was significantly downregulated in bacteriomes compared to bacteriomes of uninfected individuals, consistent with reduced nutritional stress under improved nutrient conditions.[29]

Overall insights

Overall, our results demonstrate that host plant quality shapes symbiotic investment in *B. cockerelli* through coordinated changes in bacteriocyte abundance, transcriptional activity, and host performance, rather than through modulation of per-cell symbiont density. Elevated essential amino acid availability in tomato was associated with increased psyllid fitness by promoting faster development and expansion of bacteriocyte number, potentially boosting *Carsonella* abundance within the bacteriome organ as a whole, and was accompanied by transcriptional signatures consistent with enhanced cellular coordination and structural organization. In contrast, the nutritionally poorer pepper host elicited a more limited structural response of bacteriomes but greater expression of metabolic and translational pathways, suggesting compensation within existing bacteriocytes under nutritional constraint. Comparisons with other hemipteran systems highlight that responses to dietary variation are not uniform and may depend on ecological context, host-symbiont genomic integration, and the presence of additional microbial partners. Together, these findings support a condition-dependent model in which psyllids adjust the structural and physiological dimensions of their symbiosis in response to host plant nutritional landscapes. Elucidating the mechanistic basis and adaptive significance of this plasticity will be critical for understanding how oligophagous herbivores maintain symbiotic function across variable plant host environments.

Limitations of the study

Our study examined bacteriome traits under a limited set of genetic, developmental, nutritional, and environmental conditions, and future work is needed to assess their plasticity across a broader genotype, dietary, and ecological range. For example, facultative symbionts, such as *Candidatus* Liberibacter psyllidaureus in *B. cockerelli*, can influence insect host nutritional symbioses[29] and may further modify bacteriome dynamics, from the base-line observed here, when feeding on different hostplants that vary in amino acids. Additionally, this study used a single psyllid genotype, corresponding to the chromosomal genome sequence described in Kwak et al. (2023).[10] However, different psyllid haplotypes may exhibit variation in their symbiotic dynamics, as they can differ in both physiology and hostplant interactions.[46] Moreover, host plant species differ not only in nutrient availability but also secondary chemistry, potentially affecting insect-symbiont interactions as well.[12]

This study focused on the 5th instar, the final immature life-stage, because the demand for host-derived nutrients and symbiont provided essential amino acids is assumed to be at its peak to support the growth of the final molt and the transition to reproductive maturity. Also to ensure our results are comparable with existing bacteriome transcriptomic datasets for psyllids[11,29] and aphids,[23,47] we followed established protocols that prioritize the late immature stages, which are also the most tractable for bacteriome dissections and analyses. However, longitudinal studies across all life stages would better resolve developmental fluctuations in symbiosis. Finally, TEM in this study enabled precise, cell-specific quantification of *Carsonella*, while circumventing polyploidy-related biases common in gene-copy methods like qPCR. However, because microscopy approaches, like physiological assays, are limited by low throughput per insect individual sample sizes, incorporating additional non-gene copy related imaging techniques and developing new techniques not influenced by polyploidy would further strengthen these findings.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Dr. Allison K. Hansen (ahans003@ucr.edu).

Materials availability

Psyllid culture generated in this study are available upon request to the Lead Contact, Dr. Allison K. Hansen (ahans003@ucr.edu) with necessary permits.

Data and code availability

All raw RNA sequencing data are deposited in NCBI under BioProject ID: GenBank: PRJNA1424560 with BioSample accessions: GenBank: SAMN55388112 -SAMN55388119. Information regarding these data are available in Table S2. TEM raw and processed files are available via Zenodo doi: https://doi.org/10.5281/zenodo.18644712. RNAseq code is available on figshare: "Dataset_S9_RNAseq_Code", https://doi.org/10.25387/g3.1410985. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Contributors listed in alphabetical order. Conceptualization, A.K.H.; methodology, A.K.H., M.S.K., S.M., and S.S.R.; validation, A.K.H. and S.M.; formal analysis, A.K.H., S.M., and S.S.R.; investigation, A.K.H., M.S.K., S.M., and S.S.R.; resources, A.K.H.; data curation A.K.H. and S.M.; writing – original draft, A.K.H. and S.S.R.; writing – review and editing, A.K.H.; visualization, A.K.H., S.M., and S.S.R.; supervision; A.K.H.; funding acquisition, A.K.H. and S.S.R.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Bactericera cockerelli bacteriomes	This study	N/A
Lycopersicon esculentum sap	This study	N/A
Capsicum annuum sap	This study	N/A
Experimental models: Organisms/strains		
Bactericera cockerelli strain isoF-IL, western haplotype, CA	Kwak et al.[11]	N/A
Lycopersicon esculentum, variety Moneymaker	Eden Brothers, NC	N/A
Capsicum annuum, variety California Wonder	Totally Tomato, WI	#T03121
Oligonucleotides		
YDRAG Q-PCR- IGS-7F	Casteel et al.[48]	5′-ATAGCTCAGGCGGTTAGAGTG–3′
YDRAG Q-PCR- IGS-7R	Casteel et al.[48]	5′-CCTTGCCTGATTGAATGGTG-3′
Deposited data		
raw RNA sequencing data- NCBI BioProject ID: PRJNA1424560	This study	BioSample accessions: GenBank: SAMN55388112 -SAMN55388119
TEM raw and processed files- Zenodo	This study	https://doi.org/10.5281/zenodo.18644712
RNAseq code- Figshare	Pers and Hansen[49]	https://doi.org/10.25387/g3.1410985
Software and algorithms		
R	R Core Team[50]	(version 4.5.0)
FASTQC	Andrews[51]	v. 0.11.8
Trimmomatic	Bolger et al.[52]	v. 0.36
HISAT	Pertea et al.[53]	v. 2.1.0
StringTie	Pertea et al.[54]	v. 2.2.1
edgeR	Robinson et al.[55]	v. 4.2.0
BLASTp	Altschul et al.[56]	on-line interface, version updated on 10/2025
Phyre2	Powell et al.[57]	v. 2.2
STRING	Szklarczyk et al.[58]	v.12.0; on-line interface
FIJI/ImageJ	Schindelin et al.[59]	Version updated on 12/2026
SAMJ plugin	Garcia-Lopez-de-Haro et al.[60]	Version updated on 12/2026
IBM SPSS statistics	IBM SPSS Statistics[61]	v29.0.2.0
Other		
B. cockerelli reference genome	Kwak et al.[11]	GCA_024516035.1

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Psyllid rearing and their host plants

Two sub-colonies of psyllids, derived from the same parent colony and sharing the same genetic background of *Bactericera cockerelli* isoF-IL[10] were used for this study, which was determined to be the Western haplotype given the mitogenome has 100% sequence similarity to the reference Western haplotype *B. cockerelli* mitogenome (NC_030055.1[62]) using blastn.[10] Sub-colonies were reared for over three generations on either tomato (*Lycopersicon esculentum*), variety Moneymaker (Eden Brothers, NC), or sweet pepper (*Capsicum annuum*), variety California Wonder (Totally Tomato, WI) prior to the start of all trials in this study given host switching from the mother's natal plant can influence host plant performance in *B. cockerelli*[46] in addition to symbiosis dynamics in aphids.[21] Plants were grown in Soil Mix III[63] with 1.33 g of Osmocote (ICL Growing Solutions Americas, South Carolina) per ½-gallon of soil at 25°C under a 16L:8D light/dark cycle in an insect rearing room with TSL 2000 Full Spectrum grow lights (Mars Hydro, CA). Using the same methods detailed in Kwak et al.[29] and Casteel et al.[48] all psyllids for trials were confirmed to be free

of *Candidatus* Liberibacter psyllidaureus,[27] which is a plant pathogen/facultative insect symbiont that is associated higher psyllid fitness and differential gene expression of bacteriocytes.[29] For all trials, colonies were maintained on either four-week-old tomato or pepper plants at 25°C under a 16L:8D light/dark cycle in a rearing room in mesh insect cages. All data collected in trials was from fifth instar immatures; similar to prior bacteriome gene expression studies of *B. cockerelli*.[11,29]

METHOD DETAILS

Host plant sap amino acid analyses

Four-week old tomato or pepper plants, four plants per cage, were placed into insect mesh cages and inoculated with 200 one-week-old male and female adult *B. cockerelli* individuals per cage. Psyllids were removed after 24h of egg laying and immatures were allowed to develop on plants until they were fifth instar. Phloem sap was collected from both plant species using the EDTA method following a modified protocol of Tetyuk et al.[64] Briefly, for each plant species one top tier leaf at the petiole was used per plant and immediately weighed then placed into a test tube with 20 mM of K2-EDTA solution (N = 5 leaves per tube). Tubes in a tube rack were placed in a dish lined with wet paper towels and covered with a black plastic bag with slits for aeration and finally placed inside a light tight cabinet for 2 h to prevent phloem sealing. After incubation, leaves were thoroughly rinsed in nuclease-free water and five leaves per tube were placed into 1.4 mL of nuclease-free water. Tubes were incubated again in the humidified collection chambers for 4 h. Following the final round of incubation, the aqueous sap collection was immediately flash frozen by liquid nitrogen and stored at −80°C. Frozen samples were packed in dry ice and sent to UC Davis proteomics core facility for free amino acid analysis.

To ensure sufficient amino acid concentrations, sap from 10 leaves total per plant species were pooled together for one sample replicate (N = 4 pooled biological replicates per plant species treatment). Samples were taken to dryness in a Conoco Rotary Vacuum Evaporator, then reconstituted in 0.1N HCl. Then samples were precipitated with 10% SSA (sulfosalicylic acid) to a final concentration of 2% and frozen overnight. Next the supernatant was diluted with 100 nmol/mL AE-Cys Li diluent prior to the 50 μl injection. Free amino acids are separated using ion-exchange chromatography with a post-column ninhydrin reaction. Instrument/buffers and column are from Hitachi Tokyo, Japan while the ninhydrin is supplied by Fuji Film, USA. Calibration of the Hitachi 8080 was performed using amino acid standards (Sigma-Aldrich, St. Louis, MO). Absorbance was recorded at both 570 nm and 440 nm after the reaction with ninhydrin to determine the response factor for each individual amino acid and to quantify levels relative to the known amino acid standards. The included reference standard (AE-Cys) was used to correct for any variances in injection volume due to the autosampler.

RNA sequencing and differential gene expression analysis

Dissection of fifth instar psyllids was conducted as in Kwak and Hansen[11] to isolate the bacteriome from the psyllid abdomen. Briefly, fifth instar psyllids were immersed into RNase free Buffer A[65] under a dissection microscope and RNase-free insect pins were used to dissect the bacteriome from the insect abdomen. Each individual bacteriome was immediately transferred to RNAprotect Bacterial Reagent (QIAGEN, Germantown, MD, USA) to stabilize RNA and was stored at −80°C until total RNA was extracted (N = 60 pooled bacteriomes per biological replicate with a total of four pooled biological replicates per plant species treatment, tomato or pepper). Following bacteriome dissections, total RNA was extracted from each biological replicate (N = 8) using the Zymo quick-RNA Microprep Kit with DNAse treatment (Zymo Research, Irvine, CA, USA) followed by purification with the RNA Clean & Concentrator Kit-5 (Zymo Research, Irvine, CA, USA) following the manufacture's protocols. The quality and quantity of the purified RNA samples were assessed using the Agilent TapeStation (Agilent, Santa Clara, CA, USA) and Qubit 4.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) at the University of California, Riverside, Genomics Core Facility.

Strand-specific libraries were prepared from total RNA and sequenced on the Illumina NovaSeq S4 platform (paired-end 150bp reads) by DNA Technology Core at the University of California, Davis as in Kwak et al. (2025). The same RNAseq pipeline detailed in Pers and Hansen (2021)[49] was conducted here, and the detailed code can be found publicly on figshare: ''Dataset_S9_RNAseq_Code'' https://doi.org/10.25387/g3.1410985. Briefly raw RNA-seq reads were quality checked and trimmed with FASTQC v.0.11.8[51] and Trimmomatic v. 0. 36[52] with the following parameters: ILLUMINACLIP:TruSeq3-PE.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36. The trimmed reads were aligned using HISAT v. 2.1. 0[53] against the chromosomal assembly of *B. cockerelli*-isoF-IL for NCBI Accession number: GCA_024516035.1.[10] The mapped reads for each gene were quantified as raw read counts using StringTie v.2.2.1[54] using gff files annotated for *B. cockerelli* as in Kwak and Hansen.[11]

Bacteriocyte cell counting

To examine phenotypic differences in psyllid bacteriomes based on host plant diet the number of bacteriocytes within the psyllid individual bacteriome were determined. Entire bacteriomes from fifth instar immatures were dissected out with insect pins using a Leica Wild MZ8 stereo dissecting microscope at 6X magnification. Following dissection, the bacteriome was mounted on a glass slide under a coverslip. Bacteriocyte quantification was then performed using an Olympus BH2 compound light microscope at 25X magnification (N = 40 psyllid individuals per host plant species, where 50% of individuals were either male or female).

Quantification of *Carsonella* cell density by transmission electron microscopy

Carsonella abundance within bacteriocytes was quantified across host plant species using transmission electron microscopy (TEM). Bacteriomes were dissected from fifth instar immatures under a dissection microscope using fine insect pins as above. Dissected bacteriomes were immediately fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) at 4 °C for 24 h. Samples were rinsed in buffer, post-fixed in 2% osmium tetroxide, dehydrated through a graded ethanol series, and embedded in Spurr's resin at the University of California, Riverside Microscopy and Imaging Core Facilities.

Ultrathin sections (60 nm) were cut using a Leica UCT ultramicrotome and collected on 300-mesh copper grids. Sections were post-stained with 2% uranyl acetate for 5 min followed by Sato's lead stain for 1 min at the University of California, San Diego Electron Microscopy Core. TEM imaging was performed on a Talos L120C transmission electron microscope (Thermo Fisher Scientific, MA, USA) operated at 120 kV at the University of California, Riverside Microscopy and Imaging Core Facilities. For each insect individual, multiple non-adjacent sections were imaged to minimize repeated sampling of the same *Carsonella* cells. Images were acquired at a consistent magnification and calibration across all samples. Fields containing sectioning artifacts, ruptured bacteriocytes, or insufficient contrast were excluded prior to analysis. Image selection and exclusion criteria were applied without reference to host plant or sex treatment.

TEM images were analyzed using FIJI/ImageJ.[59] Calibrated images were subdivided into non-overlapping tiles of fixed area (10×10 μm; 100 μm^2). Tiles were selected systematically across bacteriocyte cytoplasm while avoiding cell boundaries and non-cytoplasmic regions to minimize spatial bias. *Carsonella* cells within each tile were identified and quantified using automated segmentation implemented with the SAMJ plugin.[60] Automated outputs were visually inspected, and segmentation accuracy was validated against manual counts on a subset of images to ensure consistency across host plant and sex treatments. *Carsonella* cell density was expressed as the number of cells per μm^2. Tiles were analyzed per host plant species, sampled from four individual insects ($N = 4$) with equal representation of males and females for both plant species ($N = 22, 17, 20, 21$ tiles for tomato female, tomato male, pepper female, and pepper male, respectively). Multiple tiles were sampled per individual, resulting in repeated measurements within individuals. All samples were prepared, processed, imaged, and analyzed using identical protocols to ensure comparability across treatments.

Fitness trials

Life history parameters were measured for fitness trials for *B. cockerelli* cultures feeding on tomato compared to pepper using similar methods as in Kwak et al.[29] Briefly, a five-day-old virgin female and male were paired in a vial for 6 h for mating. After a 6-h mating period, the male and female were separated, and the male was discarded. Each mated female was then placed on its own three-week-old natal host plant (tomato or potato) and the plant was covered with a mesh bag to contain the mated female and subsequent isofemale line. The mated female oviposited on its plant for five days and then was removed from its plant. The following life history and developmental data were collected for each isofemale line ($N = 11$ mated females per host plant species treatment, pepper or tomato): total eggs oviposited (the number of eggs laid), number of eggs hatched, hatching rate (the percentage of eggs that hatched), incubation time (the average period from the first egg appearance to the first immature emergence), immature development time (the average time period from the first immature appearance to the first adult emergence), total development time (the average time from the first egg appearance to the first adult emergence), adult emergence rate (the percentage of immatures that survived to adulthood), number of immatures, and number of adults.

QUANTIFICATION AND STATISTICAL ANALYSIS

Host plant sap amino acid analyses

Sample size = sap from 10 leaves total per plant species were pooled together for one sample replicate ($N = 4$ pooled biological replicates per plant species treatment). Amino acid concentrations were normalized to leaf fresh weight and are reported as amount per gram fresh weight. Amino acid concentrations in host plant sap were analyzed using both multivariate and univariate approaches in R (version 4.5.0).[50] Analyses were performed using the *tidyverse*, *vegan*, or *ggfortify* packages. Prior to analysis, amino acid concentrations were $\log_{1+}$ transformed using log1p to reduce skewness and accommodate zero values. All statistical analyses were conducted on transformed data unless otherwise stated. For multivariate analysis of amino acid profiles, overall differences in both non-essential and essential amino acid composition between hostplant species were assessed using principal component analysis (PCA) and permutational multivariate analysis of variance (PERMANOVA). PCA was performed on scaled variables (mean-centered and variance-standardized) using the prcomp function and was used solely for visualization of multivariate patterns. PERMANOVA was conducted using the adonis2 function in the vegan package, with plant species as the explanatory factor and Euclidean distance calculated from the transformed amino acid matrix. Statistical significance was assessed using 999 permutations under a reduced model. PERMANOVA results were interpreted as evidence of global differences in amino acid profiles between species rather than amino acid–specific effects. Homogeneity of multivariate dispersion between plant species was assessed using analysis of distances to group centroids (betadisper), and no significant differences in dispersion were detected.

For univariate amino acid and amino acid derivative comparisons differences in individual amino acid concentrations between plant species were tested using Welch's two-sample t-tests, which do not assume equal variances. For each amino acid, mean concentrations were calculated for each species from $\log_{1+}$ transformed data, and log fold changes were estimated as the difference in

mean transformed values between species. To account for multiple testing across amino acids, *p*-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure. Amino acids with FDR-adjusted *p*-values <0.05 were considered statistically significant.

For biological interpretation, fold changes were back-transformed from log fold changes using the exponential function, such that values > 1 indicate higher concentrations in tomato sap and values < 1 indicate higher concentrations in pepper sap.

RNA sequencing and differential gene expression analysis

Sample size = *N* = 60 pooled bacteriomes per biological replicate with a total of four pooled biological replicates per plant species treatment, tomato or pepper. Differentially expressed genes were identified by comparing tomato and pepper feeding psyllid bacteriomes using EdgeR[54] (version 4.2.0), with significance defined by FDR $\leq$0.05 and $\geq$1.5-fold change in normalized expression (i.e., log2 fold change between the groups). Multidimensional Scaling (MDS) analysis was used to qualitatively visualize normalized transcriptomic read profiles of each biological replicate and host plant species using R (version 4.2.0[50]). For genes that were differentially expressed, in addition to previous annotations from Kwak et al.,[10] BLASTp[56] was used to identify the top ten hits (E-value threshold <1e−5) to help identify putative gene functions. If the gene's BLASTp hit was "hypothetical" or "uncharacterized" Phyre2.2[57] was also conducted using default parameters to obtain further information on putative protein functions based on its predicted protein structure. Predicted protein structures with a high confidence score (>90%) were reported only. To determine if there were enriched Biological GO terms we used String v.12.0's on-line interface.[58] We imported *B. cockerelli*-isoF-IL's genome into String v.12.0 to construct a custom database for all analyses; *B. cockerelli*-isoF-IL (STRG0A21YYY).[10] The Functional Enrichment Function was used (values/ranks function) ranking RNAseq fold-change data with defaults set at high FDR stringency (1%) to determine enriched GO-terms.

Bacteriocyte cell counting

Sample size = *N* = 40 psyllid individuals per host plant species, where 50% of individuals were either male or female). The independent variables in this experiment were the host plant species fed on by the psyllid (tomato vs. pepper) and the sex of the psyllid individual (male or female), while the dependent variable was the bacteriocyte count. The Levene's Test of Equality of variance was conducted to ensure all variables had equal variances followed by a two-way ANOVA using IBM SPSS statistics v29.0.2.0.[61]

Quantification of *Carsonella* cell density by transmission electron microscopy

Sample size = *N* = 22, 17, 20, 21 tiles for tomato female, tomato male, pepper female, and pepper male, respectively. Tiles reside from a total of four individual insects (*N* = 4) with equal representation of males and females for both plant species. TEM statistical analyses were conducted in R (version 4.5.0).[50] *Carsonella* cell counts per tile were analyzed using generalized linear mixed models (GLMMs) fitted with the glmmTMB package. A negative binomial error distribution with a log link function was used to account for overdispersed count data. Host plant (pepper vs. tomato), insect sex (female vs. male), and their interaction were included as fixed effects. Individual insect identity was included as a random intercept to account for non-independence of multiple tiles sampled from the same individual. Tile area was included as a log-transformed offset to explicitly model *Carsonella* cell density (cells per μm^2), although tile area was constant across observations.

Model fit and assumptions were evaluated using the performance and DHARMa packages. Overdispersion was assessed using dispersion tests, and simulated residuals were examined for uniformity, patterns across fitted values, and evidence of zero inflation. Type III Wald chi-square tests were used to evaluate the significance of fixed effects using the car package. Estimated marginal means and 95% confidence intervals were calculated on the response scale (cells per μm^2) using the emmeans package. Pairwise comparisons of host plant effects were performed within each sex. Statistical significance was assessed at $\alpha = 0.05$.

Fitness trials

Sample size = *N* = 11 mated females (i.e., isofemale lines) per host plant species treatment, pepper or tomato. The independent variable in plant fitness trials was host plant species of the psyllids. The dependent variables compared between pepper and tomato reared psyllids include the life history parameters detailed above. The Levene's Test of Equality of variance was conducted to ensure all variables had equal variances followed by a one-way ANOVA using IBM SPSS statistics v29.0.2.0.[61]