An aphid symbiont confers protection against a specialized RNA virus, another increases vulnerability to the same pathogen

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Abstract

Insects often harbor heritable symbionts that provide defense against specialized natural enemies, yet little is known about symbiont protection when hosts face simultaneous threats. In pea aphids (Acyrthosiphon pisum), the facultative endosymbiont Hamiltonella defensa confers protection against the parasitoid, Aphidius ervi, and Regiella insecticola protects against aphid-specific fungal pathogens, including Pandora neoaphidis. Here we investigated whether these two common aphid symbionts protect against a specialized virus A. pisum virus (APV), and whether their anti-fungal and anti-parasitoid services are impacted by APV infection. We found that APV imposed large fitness costs on symbiont-free aphids and these costs were elevated in aphids housing H. defensa. In contrast, APV titers were significantly reduced and costs to APV infection were largely eliminated in aphids with R. insecticola. To our knowledge, R. insecticola is the first aphid symbiont shown to protect against a viral pathogen, and only the second arthropod symbiont reported to do so. In contrast, APV infection did not impact the protective services either R. insecticola or H. defensa. To better understand APV biology, we produced five genomes and examined transmission routes. We found that moderate rates of vertical transmission, combined with horizontal transfer through food plants, were the major route of APV spread, although lateral transfer by parasitoids also occurred. Transmission was unaffected by facultative symbionts. In summary, the presence and species identity of facultative symbionts resulted in highly divergent outcomes for aphids infected with APV, while not impacting defensive services that target other enemies. These findings add to the diverse phenotypes conferred by aphid symbionts, and to the growing body of work highlighting extensive variation in symbiont-mediated interactions.

Introduction

Aphids (Hemiptera: Aphididae) are plant sap-feeding insects that interact with a range of microbial mutualists and pathogens. Almost all aphid species carry the obligate nutritional symbiont*, Buchnera aphidicola,* which is maternally transmitted and upgrades the nutritional profile of plant phloem (Douglas 2009). Nine facultative, heritable symbionts have also been identified in different species that can confer conditional benefits including defense against parasitic wasps and fungal pathogens (Oliver et al. 2010, Oliver and Martinez 2014, Vorburger 2014, Guo et al. 2017). Aphids frequently encounter viruses in the environment, including economically-important plant viruses that they vector (Brault et al. 2010) and pathogenic viruses specialized on aphids. The latter include single-stranded DNA viruses in the family Parvoviridae (Piccovirales) and positive-sense ssRNA viruses in the families Picornaviridae and Dicistroviridae (Picornavirales) (Van der Wilk et al. 1997, Moon et al. 1998, van Munster et al. 2003, Ryabov 2007, Ryabov et al. 2009, Asgari and Johnson 2010, Liu et al. 2016). However, little is known about the interactions among the many protective symbionts in aphids and pathogenic viruses.

Acyrthosiphon pisum virus (APV), is a picorna-like virus that persistently infects the pea aphid, *Acyrthosiphon pisum* (Van den Heuvel et al. 1997). The 10 kb APV genome contains two open reading frames (P1, P2) encoding a protease, helicase, RNA-dependent RNA polymerase and capsid protein (Van der Wilk et al. 1997). While primarily detected in epithelial cells of the gut and salivary glands, APV has also been weakly detected in the ovaries of pea aphids (Van den Heuvel et al. 1997, Lu et al. 2020). APV is vertically transmitted at moderate rates, and can be horizontally transferred from aphids to plants (Van den Heuvel et al. 1997, Lu et al. 2020), but no studies have shown aphid acquisition of APV from plants. APV exerts variable effects on aphid growth, survival and reproduction (Van den Heuvel et al. 1997, Lu et al. 2020), but how pea aphid genotype and facultative symbionts influence APV infection has not previously been investigated.

Hamiltonella defensa (Yersiniaceae: γ-Proteobacteria) is one of the most studied facultative symbionts in pea aphids because certain strains confer high levels of resistance against parasitoid wasps like*Aphidius ervi* (Hymenoptera: Braconidae) (Oliver and Higashi 2019). Protective strains of *H. defensa* further host specific variants of a bacteriophage named APSE which as a provirus expresses virulence genes that have been implicated in disabling parasitoid development (Oliver et al. 2009, Brandt et al. 2017, Lynn-Bell et al. 2019, Rouïl et al. 2020, Boyd et al. 2021). *H. defensa* strains infected by APSE-3 confer high levels of protection (> 85% of parasitized aphids survive) while strains infected by APSE-2 or APSE-8 provide moderate protection (40-60%) (Weldon et al. 2013, Oliver and Higashi 2019). Aphid genotype also contributes to resistance to parasitoids (Martinez et al. 2018). Another facultative symbiont associated with pea aphids, *Regiella insecticola* , is closely related to *H. defensa* (Patel et al. 2019) and confers protection against specialist entomopathogenic fungi like *Pandora neoaphidis*(Entomophthorales) (Scarborough et al. 2005, Parker et al. 2013). Levels of protection conferred by *R. insecticola* also vary with symbiont strain and host genotype (Parker et al. 2017).

We recently discovered an APV in certain pea aphid lines maintained in our laboratory. In this study, we assembled the genome of this APV and conducted assays that examined transmission and fitness effects on aphids by generating genetically homogeneous lines that controlled aphid and *Buchnera* genotypes while manipulating facultative symbiont presence and APV infection. We report that aphid fitness was adversely affected in aphids without facultative symbionts, effects which were worsened in aphid carrying *H. defensa* . In contrast, negative fitness impacts were ameliorated in aphids hosting *R. insecticola* . Overall, our results identify heretofore unknown interactions between facultative symbionts and a pathogenic virus that strongly influence host fitness.

Methods

Aphid, parasitoid and fungus cultures: *A. pisum* is cyclically parthenogenetic which enables clonal lines to be produced and maintained in the laboratory with continued exposure to a long-day-length photoperiod. Lines used in this study were reared as earlier described (Oliver et al. 2003) on *Vicia faba* Broad Windsor seedlings in Percival biological incubators at 20 ± 1° C on a 16 h light (L):8 h dark (D) photoperiod. Lines hosting particular symbionts were established by isolating a single parthenogenetic female in a petri dish with *V. fava* leaves and allowed to reproduce. Offspring were then regularly screened for all known pea aphid facultative symbionts using previously published PCR-based diagnostics (Russell et al. 2013, Martinez et al. 2014). An *A. ervi* culture was established from commercially produced (Syngenta Bioline Ltd.) and field-collected wasps from Dane County, WI USA, which are maintained on susceptible pea aphid lines lacking facultative symbionts as previously reported (Oliver et al. 2003). Adult parasitoids were kept at 20 ± 1° C under a 16 h L: 8 h D h photoperiod and provided a diet of honey and water. *P. neoaphidis* genotype ARSEF 2588 used in this study originated from the USDA-ARS Collection of Entomopathogenic Fungal Culture and was maintained on susceptible aphid lines (WI-48 and 5D-AB). Desiccated fungal cadavers were stored at 4°C with low humidity for no more than twelve weeks within an airtight container. Prior to experimental use, cadavers were rehydrated to induce sporulation (described in later section).

APV identification and sequencing: APV was first discovered in our laboratory during an RNAseq study that compared five clonal lines that hosted different strains of *H. defensa* (Chevignon, Unpublished

data). Following this discovery, we established a qPCR assay to screen for the presence of APV in all of our laboratory cultures. Briefly, individual aphids were chilled on ice for 5 min and homogenized in lysis buffer (10mM Tris-Cl pH 8.2, 1mM EDTA, 25mM NaCl) in 0.2 mL tubes. Samples were then centrifuged at 4°C for 20 min at 2200 x g. After centrifugation, 11 µl of supernatant was transferred to new 0.2 mL tubes which served as a template for complimentary DNA (cDNA) synthesis using SuperScript IV First-Strand Synthesis Reaction Kit (Invitrogen®). APV was the detected by real-time quantitative PCR (qPCR) using specific primers: APV P1 diagF (ACCATCCGAACTTGAACA GG) and APV P1 diagR (TGAAAGAACAACG-CCTGTGA). Ten µl reactions were run using Quantabio PerfeCTa SYBR Green FastMix chemistry and 0.5 µM of each primer on an Analytik Jena qTower^3 thermal cycler. Cycling conditions were 95° C for 5 min; 40 cycles of 95° C for 10 s, 58° C for 10s, 72° C for 10 s, and a final extension at 72° C for 2min. For the 12 pea aphid lines testing positive for APV we Sanger sequenced a ˜890 bp variable region of APV to examine strain variation. Using cDNA template generated as described above, we PCR-amplified a portion of the P1 ORF (Fig. 1A) using APVp1seqF (5' GATTGCGGTTTTCCATTTGT 3') and APVp1seqR (5' GGGGTTTTGCCCTATAGCAT 3'). PCRs were carried out in a 30 µl mixture using EconoTaq Plus master mix (Lucigen, USA) that included 15 µl of Taq polymerase, 0.3 µM of forward and reverse primers, 50 ng of template DNA and nuclease-free water up to 30 µl. The PCR conditions were 95 °C for 3 min (1 cycle), 95 °C for 30s, 60°C for 30 s and 72 °C for 1min (35 cycles), followed by 72 °C for 7 min. The amplified products were visually examined with electrophoresis using a 1% agarose gel, with DNA products purified using a cycle pure Kit (Omega Bio-Tek, USA) before submission to Eurofins Genomics for sequencing.

The RNAseq data set that originally identified APV in some of our cultures was used to assemble complete genomes for five of the infected laboratory lines. In brief, this data set was generated by extracting total RNA from 4$^{\text{th}}$ instar aphids from five clonal lines (three biological replicates consisting of five individual aphids per line) using the mirVana miRNA Isolation Kit (Ambion, Thermo Fisher Scientific). After DNase treatment using the TURBO DNA-free Kit (Ambion, Thermo Fisher Scientific) and ethanol precipitation in the presence of glycogen, RNA templates were quality checked by the Georgia Genomics and Bioinformatics Core using an Agilent 2100 Bioanalyzer (Agilent Technologies) and Fragment Analyzer (Advanced Analytical). cDNA libraries were then synthesized using the Kapa Stranded RNA-seq Library Preparation Kit (Kapa Biosystems) and 75 bp paired-end sequenced using Illumina NextSeq (150 Cycles). APV genomes were assembled by pooling*A. pisum* unaligned reads with APV reads for de novo assembly with SPAdes v3.15.3 (Prjibelski et al. 2020) using the parameter rnaviral. The first APV genome that was sequenced (NC_003780; Van der Wilk et al. 1997) was used as a reference to identify APV contigs using Minimap2 v2.22 with parameter 'x splice' (Li 2018). The resulting APV genomes were then aligned using MAFFT v7.450 (Kuraku et al. 2013) in Geneious Prime v2022.0.2 (https://www.geneious.com) with standard parameters and compared to other sequenced APVs including: 1 from Europe (AF024514) (Van der Wilk et al. 1997), six from China (MH301282 to MH301287) and four related RNA viruses; YYSMMV1 (*Sitobion miscanthi* virus 1; MK733235), two rosy apple aphid virus isolates (Riboviria; DQ286292, MW929927), and one avian-associated Riboviria (MT138201). The same viruses were also used to construct a maximum-likelihood phylogenetic tree using PhyML v3.3.20180621 (Guindon et al. 2010) implemented in Geneious Prime (v2022.0.2 https://www.geneious.com) with substitution model HK85 and100 bootstraps.

We further screened all lab colonies identified by qPCR to be APV infected by sequencing an 890 bp region of the P1 ORF using APVp1seqF (5' GATTGCGGTTTTCCAT TTGT 3') and APVp1seqR (5' GGGGTTTTGCCCTATAGCAT 3'). PCRs were carried out in a 30 µl mixture using EconoTaq Plus master mix (Lucigen, USA) that included 15 µl of Taq polymerase, 0.3 µM of forward and reverse primers, 50 ng of template DNA and nuclease-free water up to 30 µl. The PCR conditions were 95 °C for 3 min (1 cycle), 95 °C for 30s, 60°C for 30 s and 72 °C for 1min (35 cycles), followed by 72 °C for 7 min. Resulting products were visually examined on 1% agarose gels and then purified using the cycle pure Kit (Omega Bio-Tek, USA) before Sanger sequencing (Eurofins).

Experimental aphid lines: We engineered aphid lines that varied in color, facultative symbiont status, and APV infection while controlling for aphid and *Buchnera* genotypes (Table 1). Across five aphid genotypes, each naturally susceptible to *A. ervi* and*P. neoaphidis* , we first produced 8 lines that were either green or

red color morphs that either lacked facultative symbionts or hosted*R. insecticola* or *H. defensa* (Parker et al. 2013, Doremus et al. 2018). Lines hosting symbionts were generated by either microinjecting *H. defensa* or *R. insecticola* into aphids with no facultative symbionts or by selectively eliminating particular facultative symbionts with antibiotics without harming *Buchnera*(Doremus and Oliver 2017). We then infected aphids from each of the above lines with APV to produce a total of 16 lines (8 non-infected lines (-APV), and 8 infected lines (+APV) (Table 1). +APV lines were created by either: 1) collecting hemolymph from +APV aphids lacking facultative symbionts and injecting it into aphids from each of our -APV lines or 2) allowing aphids from our APV- lines to feed on a diet inoculated with a homogenate prepared from +APV aphids (Van den Heuvel et al. 1997). For the latter, 8-10 4^{th} instar +APV aphids were homogenized in 500 µl of aphid diet (Febvay et al. 1988) in a 1.5 mL centrifuge tube. The homogenate was then spun down at 6000 x g for 1 min to remove aphid debris followed by mixing the supernatant with ˜1 ml of aphid diet. The mixture was then sandwiched between two stretched parafilm layers on a 35mm x 10mm petri dish followed by the addition of 10-15 second instar uninfected aphids that were allowed to feed for 2 days. The aphids were then transferred to a fresh *V. faba* plant to develop into adults. Each line used in experiments was then generated from a single parthenogenetic female that was maintained for a minimum of 8 generations before use any assay. We confirmed bacterial symbiont and APV infection regularly after line creation and before all experimental assays using previously described PCR-based diagnostics.

Table 1. Experimental aphid lines established for use in this study. Aphid line	**Table 1. Experimental aphid lines established for use in this study.** Aphid color	**Table 1. Experimental aphid lines established for use in this study.** Injection donor or cured	**Table 1. Experimental aphid lines established for use in this study.** Facultative symbiont status	**Table 1. Experimental aphid lines established for use in this study.** Expected resistance to wasp (W) or fungus (F)	**Table 1. Experimental aphid lines established for use in this study.** APV infection status
ND18	Green		none	Low W	Negative
ND18+APV			none		Positive
ND18.H3		MM12	*H. defensa*/APSE-3	High W	Negative
ND18.H3+APV		MM12	*H. defensa*/APSE-3		Positive
5D-AB	Pink		none	Low W	Negative
5D-AB+APV			none		Positive
5D-AB.H2		82B	*H. defensa*/APSE-2	Moderate W	Negative
5D-AB.H2+APV		82B	*H. defensa*/APSE-2		Positive
LSR1-AB	Pink	Cured	none	Low F	Negative
LSR1-AB+APV		Cured	none		Positive
LSR1.Ri			*R. insecticola*	High F	Negative
LSR1.Ri+APV			*R. insecticola*		Positive
WI246-8	Pink		none	Low W	Negative
WI246-8+APV			none		Positive
WI576N-27	Green		none	Low W	Negative
WI576N-27+APV			none		Positive

APV transmission assays: To measure maternal transmission rates and whether *H. defensa* impacts vertical transmission, we reared APV-infected adult aphids of the same genotype with (ND18.H3) or without (ND18) *H. defensa* individually in petri dishes (55mm x 15mm) containing a single *V. faba* leaf. Adult aphids were monitored for the production of offspring approximately every 30 min. Since APV can be transferred from infected aphids to plants during feeding we replaced fava leaves every 2-3 hours. Nymphs were collected 1-30 min after birth then surface sterilized in a 1% bleach solution and transferred to a new fava leaf containing Petri dish. Newborn aphids were individually reared to adulthood to prevent possible aphid to aphid horizontal transmission through the leaves. We allowed the first-generation cohort to produce offspring and develop into third-fourth instar nymphs before screening for APV infection using the diagnostics previously described. Fisher's Exact Test was used to compare rates of maternal APV transmission among lines. To rule out rapid horizontal transmission in our Petri dish arenas that would potentially inflate estimates of vertical transmission, we also conducted a control assay, mimicking the conditions described above by allowing single APV+ adults to feed on a single *V. faba* leaf in a Petri dish. Adults were allowed to feed continuously for 1 h before removing them and any offspring they produced. We then added 8-10 second instar -APV aphids, which were allowed to feed for 30 min on the leaves previously fed upon by +APV before being separated and reared individually in petri dishes with a fresh *V. faba* leaf. These aphids were then allowed to develop into fourth instars and screened by PCR for the presence of APV.

Horizontal transmission of APV through plants was assessed by placing a single *V. faba* in cup cages with three +APV aphids (donor) and three -APV- aphids (recipient) which were distinguished by using 4 different donor and recipient lines lacking facultative symbionts that differed in color (pink or green morphs). Cup cage arenas were maintained at 20° C under 16 h light (L): 8 h dark (D) photoperiod. Eight third or fourth instar donor and recipient aphids were then collected after 1 or 3 weeks and screened by qPCR as described above to assess APV infection status. We also conducted assays to determine if oviposition by *A. ervi* could horizontally transfer APV from infected to uninfected aphids. A female *A. ervi* was allowed to oviposit into an +APV aphid and then immediately moved to a separate arena and allowed to oviposit into three -APV aphids in rapid succession. The three parasitized -APV aphids were identified by the order in which oviposition occurred and then placed into separate petri dishes with a single *V. faba* leaf. We allowed parasitized APV- recipient aphids to develop into fourth instars before screening them for APV infection as above.

Fitness measures: Aphid fecundity in different lines of +APV and -APV aphids was estimated by allowing cohorts of five fourth instar aphids to develop into adults on a single *V. faba* (equals 1 replicate). The number of offspring produced in each cup cage was carefully removed and counted every 3 or 4 days. In total, there were 9 replicates for each aphid line. Aphid mortality was also recorded and used to assess 50% survivorship. Aphid reproduction was analyzed using Analyses of Variance (ANOVA) with Tukey's HSD to compare means among aphid lines. Aphid survival data was fit to a lognormal distribution to estimate 50% survival time.

Enemy challenge assays: Cohorts of 20 aphids that were 48-72 h old (second instars) were singly parasitized by a mated *A. ervi*female and then placed onto a fresh *V. faba* plant in a cup cage (=1 replicate). A total of 8 replicates were conducted for each experimental aphid line (160 parasitized aphids per line). After parasitism, cup cages were maintained at 20° C under a 16 h light:8 h dark photoperiod. Ten days post-parasitism, we recorded the number of aphids that survived, mummified (a pupating wasp), or both aphid and wasp died (dual mortality) (Oliver et al. 2012). Results were then analyzed by logistic regression analyses. Since parasitoid fitness is often linked to host health, we measured hind tibia length to estimate the size of *A. ervi* eclosing from APV+ and APV- aphids, which served as a proxy for wasp quality (Godfray and Godfray 1994, van Lenteren 2003). One day old adult *A. ervi* were frozen overnight at -20°C and then dried at 60°C for 24 hours before measuring hind tibia length using an Olympus SZX16 stereomicroscope equipped with CellSens software (v. 1.4.1). Wasp tibia length was analyzed by One-way Analyses of Variance (ANOVA) to compare mean tibia length between APV+ and APV- aphid lines.

To assess whether APV infection affects fungal protection conferred by*R. insecticola* , we challenged aphids with *P. neoaphidis* as previously described (Weldon et al. 2020). Ten cohorts of ten 9-day old (early adult)

aphids (total 100 aphids) from each *R. insecticola*experimental line (Table 1) were then exposed to two sporulating aphid cadavers placed in a 35 mm diameter deep Petri dish with 1.5% agar for 90 minutes. Fungal plates were inverted over aphids to mimic a natural spore shower and rotated every fifteen minutes between replicates to normalize spore exposure. Each cohort was then placed onto a fresh *V. fava* plant and kept at 20°C with 100% humidity (via an unvented cup lid) for 24 hours under 16:8 L:D hour light cycle. After 24 hours, the unvented lid was replaced with a vented lid. Aphids were monitored every twenty-four hours for ten days post-exposure for aphid survival, dual mortality (aphid and pathogen), and fungal sporulation. The results were analyzed using logistic regression.

APV and symbiont abundance: We estimated APV and symbiont abundance by measuring genome copy number of each. Briefly, +APV and -APV adult aphids from a given experimental line were placed in separate cup cages with a fresh *V. faba* plant and allowed to reproduce for approximately 24 hours. Thereafter, all adults were removed and offspring were allowed to develop. Aphids were then sampled at 2, 4, 8 and 16 days old. APV genome copy number was then estimated by generating cDNA templates from 6-8 aphids at each time point (biological replicates) as described above followed duplicate qPCR (technical replication) for each sample using APV-specific primers and reaction conditions as described above. APV genome copy number per sample was then estimated by plotting the data against a standard curve generated by serial dilution of a plasmid containing the APV amplicon and normalized using a single copy aphid gene (*Eφ-1a*). Relative genome copy number for *H. defensa* was similarly determined at the same time points using previously reported primers that amplify a region of the *H. defensa dnaK* gene (Weldon et al. 2013, Martinez et al. 2014) while relative genome copy number for *R. insecticola* was determined using primers designed during this study (Reg_dnaK_Q_F: 5'-TGGTGCAGCAAAAAGTG AAG-3' and Reg_dnaK_Q_R: 5'-CACCCATGGTTTCAATACCC-3') that amplify a region of the *R. insecticola dnaK gene. Cycle conditions for the R. insecticola primers were* 95° C for 5 min; 40 cycles of 95° C for 10 s, 60° C for 10 s, 72° C for 10 s, and a final extension at 72° C for 2 min. Relative abundance of each symbiont was then determined by the $2^{-(\Delta\Delta T)}$method (Livak and Schmittgen 2001). Results were log10 transformed, and the distributions of symbiont titers in each experimental line at each time point were checked for normality using the Goodness-of-fit test. Transformed titers were then compared using ANOVA with Tukey's post-hoc Honest Significant Difference (HSD) test. Both analyses as well as all other statistical tests performed during the study were performed using JMP Pro v. 14.0 (SAS Institute Inc., Cary, NC).

Results:

Discovery of APV in several laboratory lines of pea aphids: We discovered that APV infected some laboratory-held aphid cultures through an RNAseq study (Chevignon, Unpublished data), in which a substantial portion of total reads (15%-26%) mapped to an APV genome in three clonal lines of *A. pisum* named AS3, AS3AB and ZA17 that hosted different strains of *H. defensa* (Table S1). Less than 0.2% of reads also mapped to APV in two other lines named A2C and NY26 that hosted other strains of *H. defensa* (Table S1). After developing a PCR-based diagnostic assay, rescreening confirmed infection of the AS3, AS3AB and ZA17 lines, but did not detect APV in the A2C or NY26 lines which suggested low level infection at the time we made the RNAseq libraries had been lost. PCR screening all of the other aphid cultures in the laboratory indicated that 39% (23/59) carried APV infection. Sequencing a domain within the APV P1 open reading frame suggested the APVs present in our laboratory were very similar with only a few single nucleotide polymorphisms (SNPs) identified. The RNAseq data we generated further enabled us to assemble complete genomes for the APVs in the AS3, AS3AB, ZA17, A2C and NY26 lines. Alignment to other APV genomes in public databases showed high overall similarity (Fig. 1A). A distance matrix computed from amino acid sequences (Table S2) and a maximum likelihood phylogeny (Fig. 1B) indicated the APVs from our laboratory were nearly identical to one another (>99.6-100%) but less similar (< 92.6%) to several APV isolates from China.

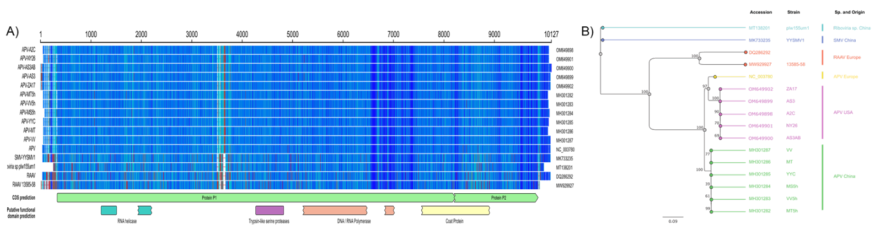


Figure 1 : A) MAFFT Whole genome alignment of APV isolates. B) Maximum likelihood phylogeny of APV isolates.

APV is both vertically and horizontally transmitted: We first assessed the efficacy of vertical transmission by determining the proportion of offspring infected females produce that are also infected. Using APV+ aphids (ND18 genotype), we observed that 30-40% of offspring each female produced carried the virus with no significant differences detected between aphids with or without *H. defensa* (Table 2A). This finding clearly indicated that maternal transmission occurs at moderate rates, but also showed most progeny are not infected. We thus examined two mechanisms for horizontal transmission. We first tested transmission from +APV aphids to -APV aphids feeding on the same host plant using aphid lines that differed in color, including recipient lines with *H. defensa* or *R. insecticola* . Half or more of the -APV aphids were infected after 1 week while nearly all were infected after 3 weeks (Table 2B). The presence of *H. defensa* or*R. insecticola* did not prevent aphid acquisition of APV. The second assay tested whether *A. ervi* could horizontally transmit APV by first ovipositing into +APV aphids without facultative symbionts and then being allowed to oviposit into three -APV aphids with or without *H. defensa* .

Only 3 of the 48 (6%) recipient aphids were infected: two ND18 aphids that had no facultative symbionts and one ND18.H3 aphid hosting *H. defensa/* APSE3 (Table S3). We also noted the order of attack (1-3) in recipient aphids which showed that each of the aphids that were infected by a wasp were first in the order of attack.

Table 2A. Maternal transmission rate of APV from aphids with and without *H. defensa*

Line	Symbiont infection	Symbiont infection	Mother	% Offspring with APV (n=11)	% Offspring with APV (n=11)	% Offspring with *H. defensa* (n=10)	% Offspring with *H. defensa* (n=10)	Within group Fisher's Exact	Within group Fisher's Exact	Overall infection rate
ND18 APV+	Ham-	Ham-	1	36%	36%	-	-	$P =$ 0.450	$P =$ 0.450	31%
			2	27%	27%	-	-			
			3	45%	45%	-	-			
			4	9%	9%	-	-			
			5	36%	36%	-	-			
ND18 APV+	Ham+	Ham+	1	45%	45%	100%	100%	$P =$ 0.597	$P =$ 0.597	38%
			2	18%	18%	100%	100%			
			3	45%	45%	100%	100%			
			4	36%	36%	100%	100%			
			5	45%	45%	100%	100%			

Table 2B: Horizontal transmission of APV by aphids feeding on host plant. Donor (color)	**Table 2B:** Horizontal transmission of APV by aphids feeding on host plant. Donor (color)	**Table 2B:** Horizontal transmission of APV by aphids feeding on host plant. Recipient (color)	**Table 2B:** Horizontal transmission of APV by aphids feeding on host plant. Recipient (color)	**Table 2B:** Horizontal transmission of APV by aphids feeding on host plant. Recipient (color)	**Table 2B:** Horizontal transmission of APV by aphids feeding on host plant. Replicate	**Table 2B:** Horizontal transmission of APV by aphids feeding on host plant. Replicate	**Table 2B:** Horizontal transmission of APV by aphids feeding on host plant. Infection at wk 1 # infected / # tested	**Table 2B:** Horizontal transmission of APV by aphids feeding on host plant. Infection at wk 1 # infected / # tested	**Table 2B:** Horizontal transmission of APV by aphids feeding on host plant. Infection at wk 3 # infected / # tested	**Table 2B:** Horizontal transmission of APV by aphids feeding on host plant. Infection at wk 3 # infected / # tested
ND18 +APV (Green)	ND18 +APV (Green)	WI246-8 UI (Pink)	WI246-8 UI (Pink)	WI246-8 UI (Pink)	1	1	6/8	6/8	8/8	8/8
					2	2	4/8	4/8	8/8	8/8
					3	3	6/8	6/8	8/8	8/8
WI246-8+APV (Pink)	WI246-8+APV (Pink)	WI576N-27 UI (Green)	WI576N-27 UI (Green)	WI576N-27 UI (Green)	1	1	4/8	4/8	8/8	8/8
					2	2	7/8	7/8	8/8	8/8
					3	3	5/8	5/8	8/8	8/8
WI246-8+APV (Pink)	WI246-8+APV (Pink)	ND18 UI (Green)	ND18 UI (Green)	ND18 UI (Green)	1	1	7/8	7/8	8/8	8/8
					2	2	6/8	6/8	8/8	8/8
					3	3	7/8	7/8	8/8	8/8
WI246-8+APV (Pink)	WI246-8+APV (Pink)	ND18+**H3** (Green)	ND18+**H3** (Green)	ND18+**H3** (Green)	1	1	4/8	4/8	8/8	8/8
					2	2	5/8	5/8	7/8	7/8
					3	3	5/8	5/8	8/8	8/8
ND18+APV (Green)	ND18+APV (Green)	LSR1-AB (Pink)	LSR1-AB (Pink)	LSR1-AB (Pink)	1	1	4/8	4/8	8/8	8/8
					2	2	6/8	6/8	8/8	8/8
					3	3	8/8	8/8	7/8	7/8
ND18+APV (Green)	ND18+APV (Green)	LSR1-**Ri** (Pink)	LSR1-**Ri** (Pink)	LSR1-**Ri** (Pink)	1	1	8/8	8/8	7/8	7/8
					2	2	7/8	7/8	8/8	8/8
					3	3	4/8	4/8	8/8	8/8

R. insecticola **reduces fitness costs associated with APV infection while** ***H. defensa*** **increases costs:** Across all five aphid genotypes, lifetime fecundity assays showed that APV-infected aphids without facultative symbionts produce fewer progeny than non-infected aphids (Table 3). APV infection also reduced aphid longevity in all genotypes except WI576N-27 (Table 3B). Since *R. insecticola* and *H. defensa* confer protection against specialized fungi and parasitoids (Scarborough et al. 2005, Parker et al. 2013), we next asked if either affected the fitness costs associated with APV infection. We found that aphids hosting *R. insecticola*(genotype LSR1) produced far more offspring and lived longer than control lines without *R. insecticola* when infected by APV (Table 3C). The cumulative fecundity of the *R. insecticola* subline with a persistent APV infection was nearly identical to that of the *R. insecticola* subline without APV. In the absence of APV, we did not observe significant reductions in fecundity or longevity in aphids of harboring *R. insecticola* compared to symbiont-free controls (Table 3C).

In contrast to *R. insecticola* , aphids hosting *H. defensa* /APSE-3 (ND18.H3) or *H. defensa* /APSE-2 (5D-AB.H2) exhibited even larger reductions in fecundity and longevity relative to those with only APV infection (Table 3D, E). Thus, *R. insecticola*reduced the fitness costs of APV infection while *H. defensa*increased them. In the absence of APV, both *H. defensa* lines exhibited reductions in fecundity and longevity when compared to controls without facultative symbionts (Table 3D, E).

Table 3: Aphid fecundity and 50% survival in the absence of enemy challenge.

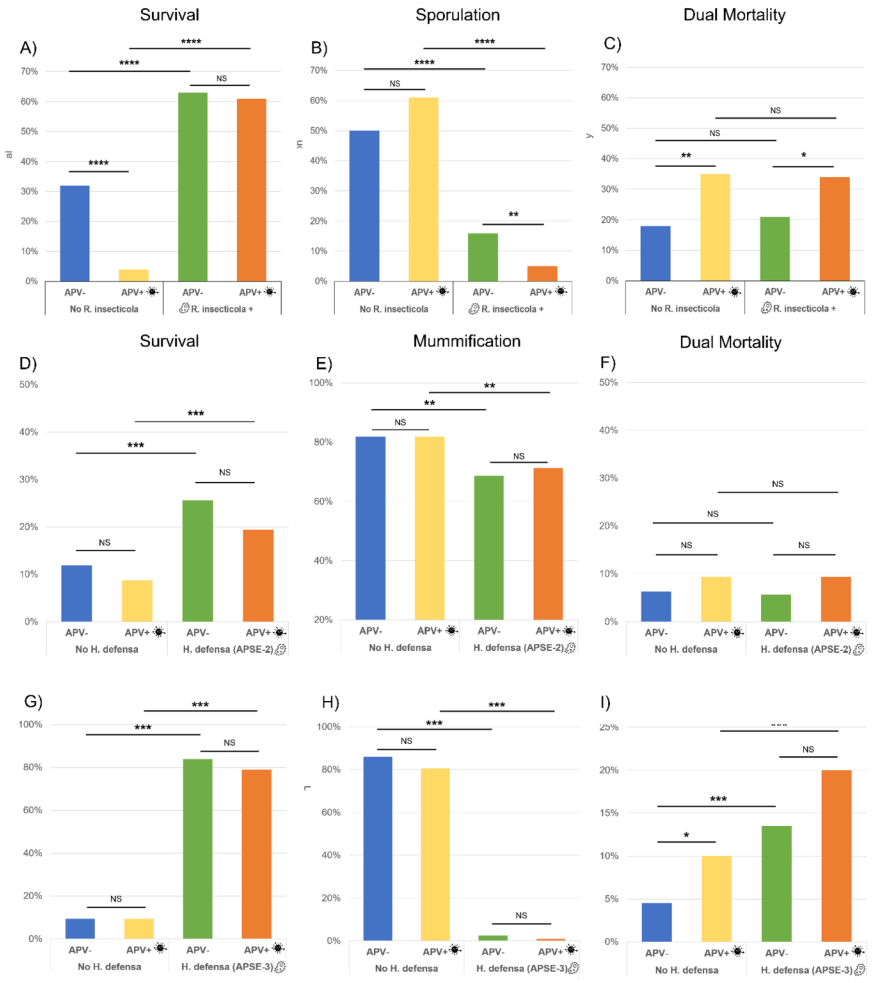
Panel
A
B
C
D
E

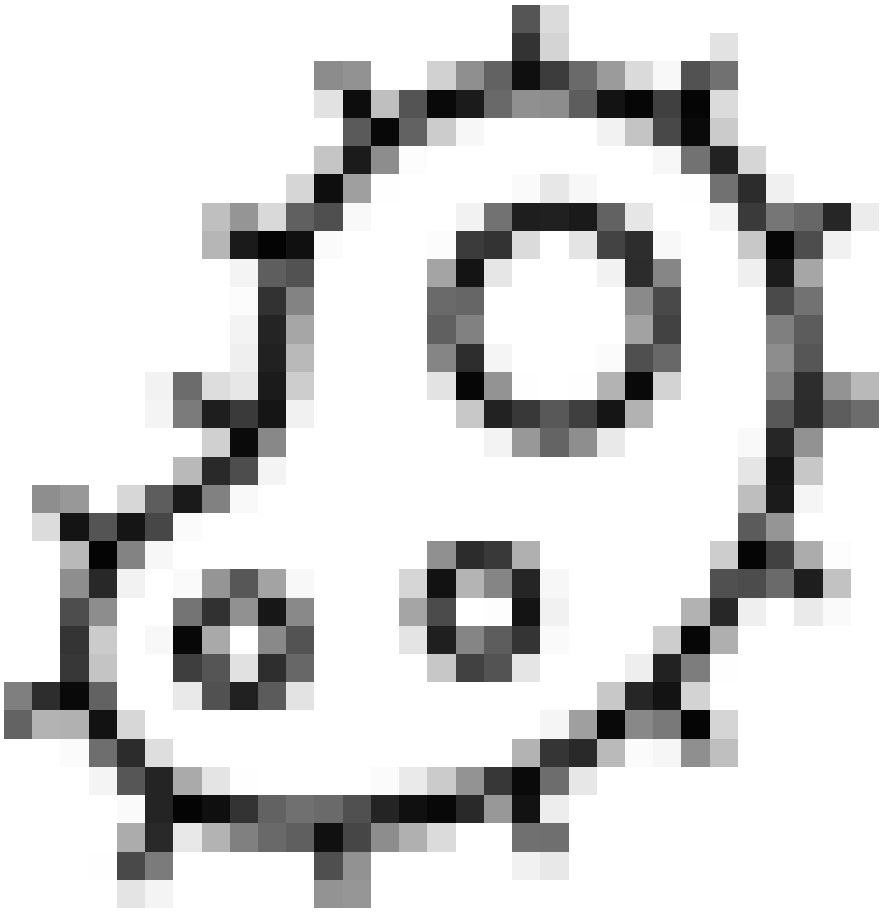
[α] Fecundity between ND18 lines was compared using Tukey's post hoc test. Letter after value denotes sig. dif. at $P < 0.05$

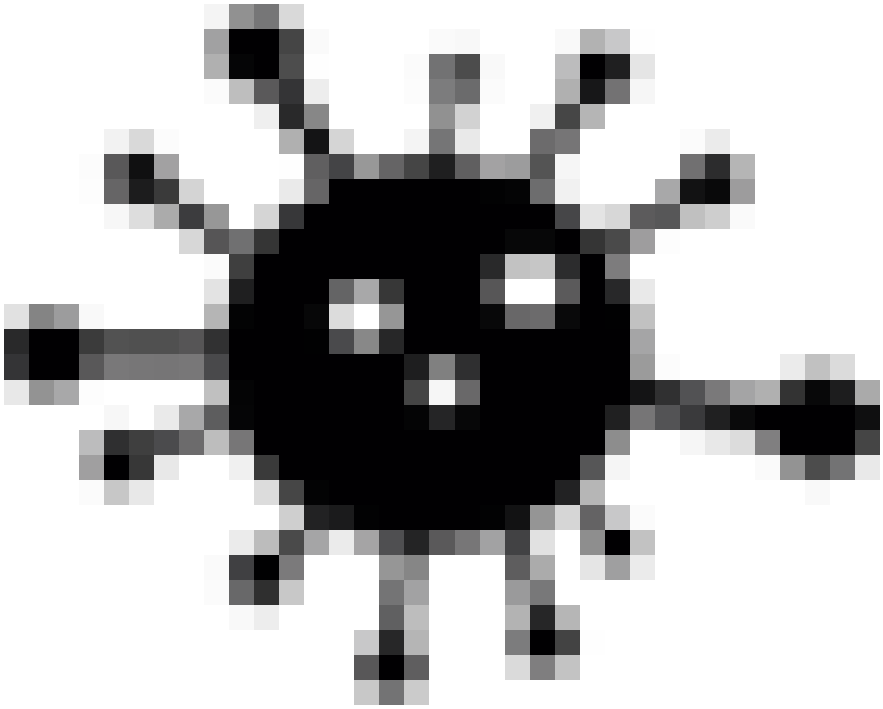
APV infection does not alter symbiont-mediated protection against other mortality agents: We next examined whether the protective effects of *R. insecticola* against *P. neophidis* or *H. defensa* against *A. ervi* were also influenced by virus co-infection. Results strongly indicated the protective effects of*R. insecticola* against *P. neophidis* were not reduced by APV infection as measured by much higher aphid survival and lower fungal sporulation when compared to aphids without *R. insecticola* (Fig 2A, B, Table S4). However, APV infection of aphids without *R. insecticola,* resulted in lower survival and higher fungal sporulation rates than infection with *P. neophidis* alone (Fig. 2A-C). The high-level protective effects of *H. defensa* /APSE-3 (ND18.H3) and moderate protective effects of *H. defensa* /APSE-2 (5D-AB.H2) against *A. ervi* were also not lowered by APV infection (Fig. 2D-I; Table S5). For aphid lines without facultative symbionts, aphid survival was very low, while successful wasp development (mummification) was high, but these did not generally differ between APV positive and negative lines (Fig. 2D-I; Fig S1; Table S5). The one exception to this latter trend was the WI246-8 line, which produced fewer mummies if infected with APV (W1246-8+APV) but this outcome was also associated with more aphids dying while also not producing a

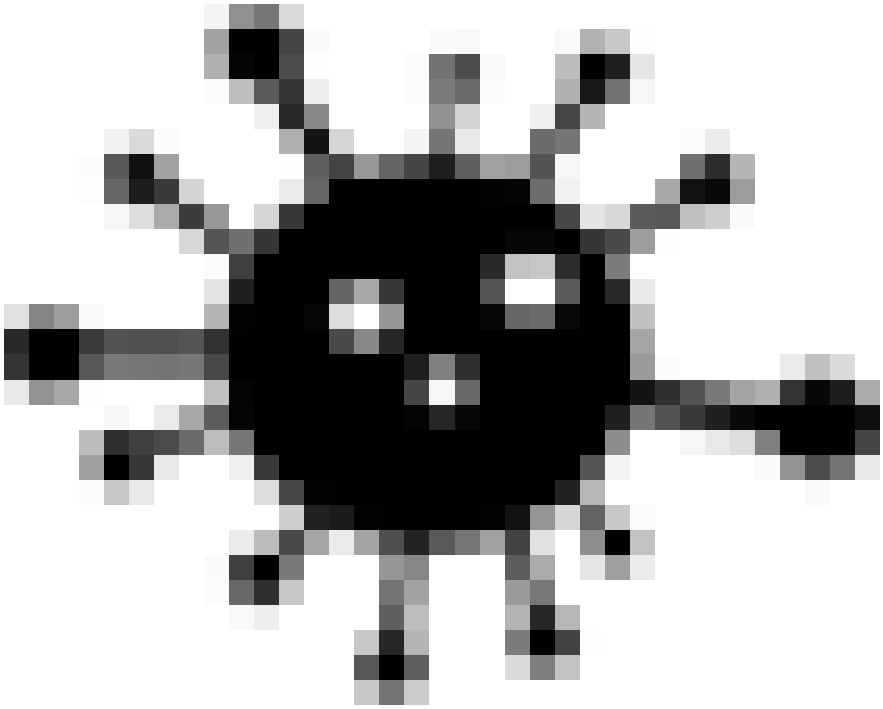
parasitoid (dual mortality) rather than an increase in aphid survival (Table S5; Fig. S1E, F).

Since parasitoid fitness is known to be influenced by host quality, we also examined whether *A. ervi* developing in hosts infected by APV exhibited reduced fitness by estimating the size of emerging female wasp offspring. We found that smaller female *A. ervi* were produced from APV-infected aphids in three of the four assayed aphid lines that lacked facultative symbionts (Table 4A, B). We did not measure the size of emerging wasps from aphids hosting *H. defensa* /APSE-3 because very few mummies were produced due to the high level of protection this strain confers. However, we did measure the size of female wasp offspring that developed in aphids hosting *H. defensa* /APSE-2. Interestingly, no differences were detected between the size of wasps that emerged from aphids with *H. defensa*/ APSE-2 that were persistently infected with APV versus aphids that were not virus infected (Table 4B).









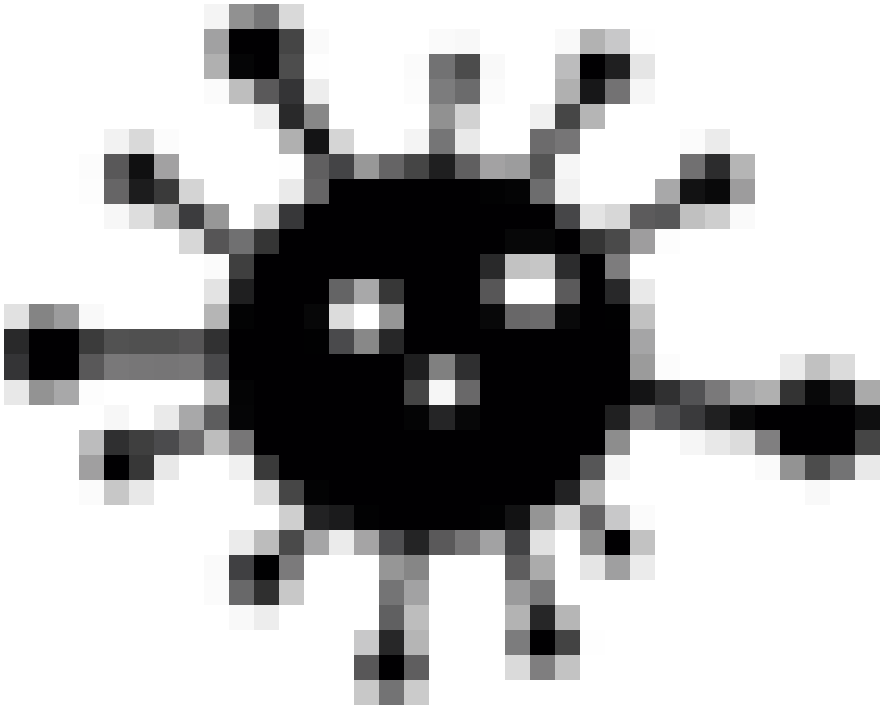


Figure 2: Aphid survival, fungal sporulation and dual mortality following challenge by the fungal pathogen *P. neoaphidis* (A-C). Outcomes of fungal exposure were contrasted between aphids with and without APV ( ) as well as aphids with and without *R. insecticola* ( ). Aphid survival, mummification and dual-mortality following parasitism by the wasp *A. ervi* (D-I). Parasitism outcomes were contrasted between aphid with and without APV ( ) as well as between aphids with and without *H. defensa* ( ). Brackets above indicate contrasts between sublines. Asterisk(s) above bars indicate significant differences (NS = $P > 0.05$; * = P [?] 0.05; ** = P [?] 0.01; *** = P [?] 0.001; **** = P [?] 0.0001).

Table 4: Mean (±SE) length of right hind tibia of female adult *A. ervi* produced from (A) APV infected and APV free aphids lacking *H. defensa* and (B) female adult *A. ervi* produced from APV infected and APV free aphids with or without *H. defensa*/APSE-2.

A) Line	Infection Status	*N*	Mean Tibia Length (μm) ± SE	*DF*	*t*-value	*p*-value
ND18	APV- / No Ham	30	911.92 ± 8.41	57.34	2.38	0.021*
	APV+ / No Ham	30	881.96 ± 9.31			
WI246-8	APV- / No Ham	30	878.69 ± 8.27	46.43	3.41	0.001*
	APV+ / No Ham	20	833.32 ± 10.44			
WI576N-27	APV- / No Ham	30	903.20 ± 8.71	42.42	0.95	0.346

	APV+ / No Ham	23	899.09 ± 11.97			
B)						
5D-AB	APV- / No Ham	23	865.38 ± 12.00 **a**	3	3.26	0.025*
5D-AB+APV	APV+ / No Ham	20	816.87 ± 12.87 **b**			
5D-AB.H2	APV- / APSE-2	21	860.62 ± 12.56 **ab**			
5D-AB.H2+APV	APV+ / APSE-2	22	862.23 ± 12.27 **ab**			

* Indicates a significant difference by One-Way ANOVA.

$^{\beta}$ Tukey's post hoc test was used to compare wasp tibia length emerging from parasitized 5D-AB sublines. Letter after value denotes sig. dif. at $P < 0.05$.

APV titers are lower in *R. insecticola* harboring aphids but not in those with *H. defensa*:

Given that *R. insecticola* ameliorated APV infection costs and*H. defensa* increased them, we hypothesized that the former reduces virus infection load, while the latter does not. We also investigated whether APV influenced facultative symbionts titers. Focusing first on the facultative symbionts, we compared their relative abundance in aphids that were persistently infected with APV to aphids that were not from day 2 when nymphs were second instars to day 16 when they were mature adults. *R. insecticola* progressively increased in abundance in both APV-infected and non-infected aphids although relative abundances were significantly higher in the latter until day 16 when they were similar (Fig. 3A). *H. defensa* with APSE-2 also progressively increased in abundance with aphid age, while *H. defensa* with APSE-3 exhibited little change in abundance until day 16 (Fig. 3B, C). However, no differences in these trajectories were found between APV-infected and non-infected aphids. We also measured relative abundances of APSE-2 and APSE3, which only modestly increased with aphid age and also exhibited almost no differences between APV-infected and non-infected aphids (Fig. 3D, E). We thus concluded that APV infection overall had modest effects on the relative abundance of *R. insecticola* an no effect on *H. defensa* abundance.

We then compared APV abundance in persistently infected aphids that either hosted these facultative symbionts or were symbiont-free. For the LSR aphid genotype, APV titer progressively increased with aphid age but titers were significantly lower at days 8 and 16 aphids in aphids with*R. insecticola* (LSR1.Ri) compared to controls lacking the facultative symbiont (LSR1-AB)(Fig. 4A). For the ND18 and 5D-AB lines, APV titers more rapidly increased than was observed in the LSR1-AB line, but exhibited no differences between aphids that hosted *H. defensa/* APSE-3 or *H. defensa/* APSE-2 versus no symbionts (Fig. 4B, C). Aphid genotype had little impact on APV infection trajectories (Fig. 4).

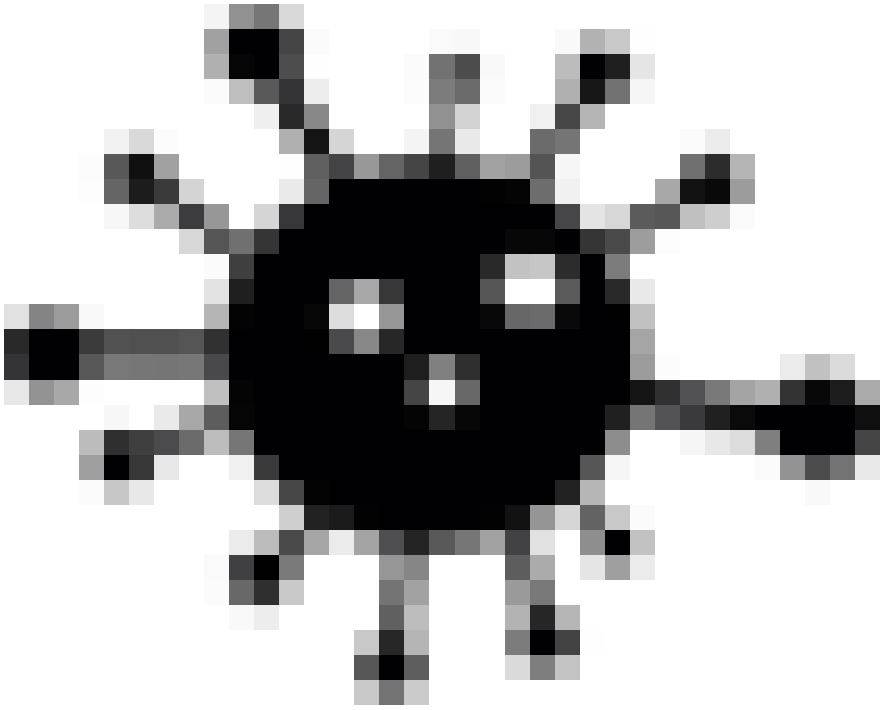


Figure 3 : The relative abundance (±SE) of *R. insecticola*(A) or *H. defensa* strains carrying either APSE-3 (B & D) and APSE-2 (C & E) in the presence and absence of APV across aphid development. Symbiont abundance was estimated using the single copy gene*dnak* and normalized using the housekeeping gene $\epsilon\varphi$*1-a* . The relative abundance (±SE) of the bacteriopahges APSE-3 and APSE-2 were estimated using the structural P1 gene and normalized to the number of*H. defensa dnaK*. Asterisks indicate significant differences (* $P < 0.05$).

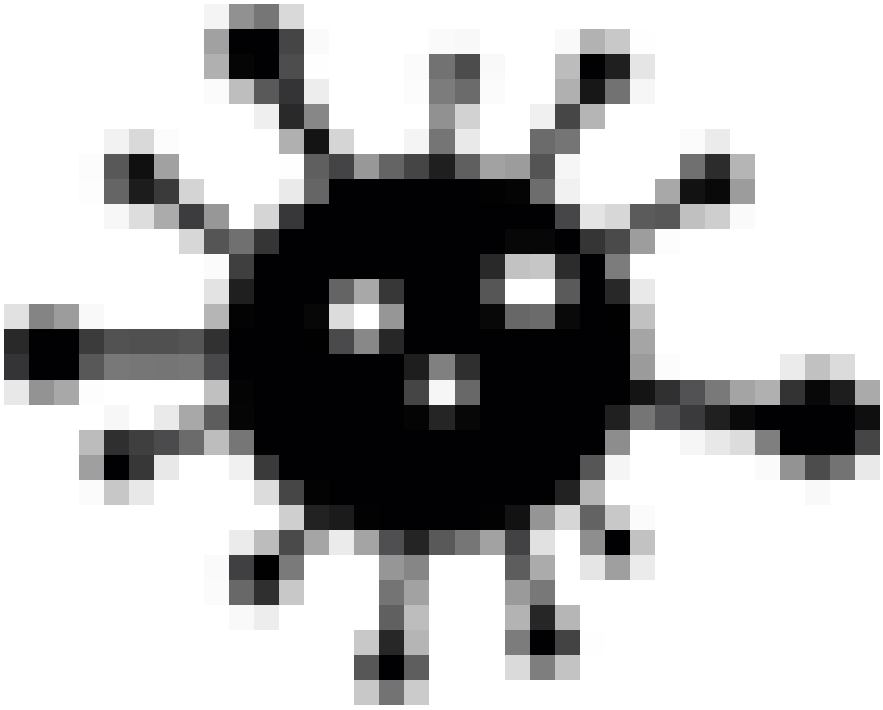


Figure 4: APV abundance (±SE) in aphid lines in the presence and absence of *R. insecticola* (A), *H. defensa* /APSE-3 (B) and *H. defensa* /APSE-2 (C). Viral abundance was estimated amplyfying a fragment of the single copy gene P1 and normalized to the aphid the housekeeping gene *Eφ1-α* . Viral abundnace in symbiont-free lines were compared using ANOVA. Viral abundance in the presence and absence of symbionts at each time point were compared using t-test. Asterisk(s) above bars indicate significant differences (NS $=P > 0.05$; * = P [?] 0.05; ** = P [?] 0.01; *** = P [?] 0.001).

Discussion

R. insecticola greatly reduces fitness costs associated with APV infection, but *H. defensa* exacerbates costs

Persistent infection by APV was previously reported to reduce pea aphid fitness (Van den Heuvel et al. 1997, Lu et al. 2020). However, these studies did not control for aphid genotype or the presence of facultative symbionts, which occur in most pea aphids and are known to confer protection to specialized natural enemies (Russell et al. 2013, Oliver et al. 2014). Here, we generalize prior findings by showing that persistent APV infections reduced aphid fecundity and survival across multiple pea aphid genotypes lacking facultative symbionts (Table 3). In aphids without facultative symbionts, we also found that APV titer exhibited similar trajectories over aphid lifespan, which is consistent with the infection costs we observed (Fig. 4).

When we examined our experimental lines with and without two common and closely related protective facultative symbionts *H. defensa* and*R. insecticola,* we found that the fitness of aphids with persistent APV infections varied dramatically depending on which symbiont was present. In aphids carrying *R. insecticola* , costs to persistent infection with APV were largely eliminated (Fig 2A-B, Table 3). Not only were fitness estimates similar between *R. insecticola* carrying aphids with and without APV, but aphids with both APV and *R. insecticola* produced statistically similar numbers of offspring compared to the control line (no APV or symbiont). APV abundance was also lower in aphids with *R. insecticola* versus those without this

symbiont, although significantly so only in older aphids (Fig. 4A). Taken together, these results indicate that *R. insecticola* provides substantial protection against infection with APV.

To our knowledge, *R. insecticola* represents only the second heritable symbiont known to confer protection against viral pathogens. Some strains of the ubiquitous *Wolbachia* symbiont confer protection against specialized RNA viruses in natural hosts (Hedges et al. 2008, Teixeira et al. 2008, Pimentel et al. 2021). Though *Wolbachia's* pathogen blocking mechanisms remain poorly understood, and may vary between natural and novel associations, hypotheses include immune priming, resource competition, or modification of the host cell environment (Terradas and McGraw 2017, Lindsey et al. 2018). Associations between anti-viral *Wolbachia* strains introduced into important insect vectors are actively being researched and applied in real-world efforts to mitigate human disease such as dengue (Nazni et al. 2019, O'Neill et al. 2019). Hence, having a second heritable symbiont with anti-viral properties in a system with unparalleled *in vivo* experimental protocols and developing*in vitro* ones (Brandt et al. 2017, Patel et al. 2019) provides excellent opportunities to develop an additional model of anti-viral symbiosis. One caveat to our study is that we only examined a single strain of *R. insecticola* in one aphid background. However, this is by far the most common of only two strains recovered from recent surveys of N. American pea aphids on alfalfa (Peng et al. 2022). And given prior findings that *R. insecticola* improves pea aphid fitness in the presence of specialized fungal pathogens (Parker et al. 2013), and when coinfecting aphids alongside costly strains of the facultative symbionts *H. defensa* and *Spiroplasma*(Mathé-Hubert et al. 2019, Weldon et al. 2020), this appears to be a common phenotype associated with *R. insecticola* .

In contrast to *R. insecticola* , APV infection costs were significantly exacerbated in aphid lines carrying *H. defensa*(Table 3C & D) and *H. defensa* did not influence APV abundance (Fig. 2D-I & Fig. 4). While symbionts that protect hosts receive the most interest, those that enhance pathogen infection are nonetheless important for natural symbiont maintenance and disease dynamics (Graham et al. 2012, Amuzu et al. 2018).

The presence of APV does not impact symbiont defensive phenotypes

Little is known about the performance of defensive symbionts when challenged with simultaneous threats (Hrček et al. 2016, Smith et al. 2021). Here, we found aphids carrying *H. defensa* were similarly susceptible to parasitism by *A. ervi* regardless of APV infection and APV had no effect on *R. insecticola* conferred protection against *P. neoaphidis* (Fig 2). While protection levels conferred by defensive symbionts are known to vary depending on abiotic factors (Guay et al. 2009, Doremus et al. 2018, Higashi et al. 2020), host genotypes (Vorburger and Gouskov 2011, Łukasik et al. 2013, Parker et al. 2017, Weldon et al. 2020), symbiont strain (Cayetano et al. 2015, McLean et al. 2018, Oliver and Higashi 2019, McLean et al. 2020), or co-occurrence with other symbionts (Weldon et al. 2020), our results indicate that APV does not alter defensive phenotypes. In contrast, APV had variable impacts on endogenous defenses against these specialized enemies. Aphid lines free of facultative symbionts were equally susceptible to parasitism by *A. ervi* with and without APV, but those challenged with the fungus *P. neoaphidis* performed significantly worse when APV was present (Fig. 2, Fig. S1). The latter suggests that the aphid immune system may not be able to effectively respond to simultaneous pathogen challenges. This result also indicates that the anti-fungal benefits of carrying *R. insecticola* were greater when APV was present. Aphids carrying *R. insecticola* are likely to benefit from both enhanced anti-fungal benefits and tolerance to APV, spreading at the expense of symbiont-free aphids or those with

H. defensa.

Facultative symbionts did not influence APV transmission

We found that vertical transmission rates of APV were about 35% and not impacted by *H. defensa* (Table 2A). This rate is similar with prior reports for APV (Lu et al. 2020) and other aphid viruses (Laubscher and Von Wechmar 1992, van Munster et al. 2003). It was also previously reported that feeding by aphids resulted in the horizontal transfer of APV to plants, with the virus persisting up to 7 days without replication in plant tissues (Lu et al. 2020). Here, we confirmed lateral transmission of APV through food plants by showing that that APV-free aphids readily acquired the virus through phloem feeding on plants previously

fed on by APV+ aphids (Table 2B). We further showed that APV acquisition rates were not affected by the presence of *H. defensa* or *R. insecticola* . Thus, despite reducing APV abundance and improving tolerance to infection as described above, *R. insecticola* did not impede APV acquisition. We also found that parasitoids can transfer APV via oviposition, a previously undescribed route of transmission for pathogenic viruses. Rates of wasp-mediated transfer of APV were low and only occurred when oviposition occurred immediately following contact with a virus-infected aphid. Parasitoids have been shown to move *H. defensa*horizontally among black bean aphids (Gehrer and Vorburger 2012) suggesting microbial transfer via the contaminated ovipositors of wasps may be an underappreciated route of microbe exchange among multicellular eukaryotes.

Together these results show moderate vertical transmission and horizontal transfer through food plants are the major routes of APV spread. That APV can promote aphid feeding and colonization by modulating plant defenses (Lu et al. 2020) suggests this virus may employ tactics that facilitate its spread as seen for plant viruses vectored by sap-feeding insects (Roossinck 2015).

The effects of APV may extend to higher trophic levels

Host infection with viral pathogens can impact parasitoid fitness (Flick et al. 2016, Dupont et al. 2020). We found that wasps developing from APV+ aphids were significantly smaller than those from virus-free controls in three of four lines lacking *H. defensa* . In the single*H. defensa* line we examined, wasps that survived symbiont defenses were similar in size regardless of APV infection. This result is perplexing given that aphids with both *H. defensa* and APV exhibited the poorest fitness overall (Table 4D, E) combined with earlier studies finding that wasps emerging from aphids with *H. defensa* were smaller (Dion et al. 2011, Schmid et al. 2012) .

Conclusions

Little is known about the ecology of APV in natural aphid populations. But given the infection costs identified here and elsewhere, along with the rapid spread of this virus through multiple mechanisms, outbreaks may occur which significantly alter aphid population dynamics with effects that reverberate through the food web (Laubscher and Von Wechmar 1993, Ban et al. 2008, Jiang et al. 2014, Gupta et al. 2017, Dupont et al. 2020). Absent other factors, APV outbreaks would likely select for aphids carrying *R. insecticola* , and against those with *H. defensa* , which would reduce the populations' potential to respond to subsequent increases in parasitism pressure, while enhancing protection against fungal pathogens. Alternatively, high parasitism rates, which select for *H. defensa* (Oliver et al. 2008, Smith et al. 2015, Hrček et al. 2016, Ives et al. 2020) potentially limit this aphid's capacity to respond to APV outbreaks. Finally, of practical concern, we note from recent experience that APV infections spread readily in pea aphids held under common laboratory conditions. While APV infections did not influence symbiont-mediated protective phenotypes, they did impact fitness measures in the absence of enemy challenge and hence have the potential to impact a range of lab-based studies. We note that the low rates of vertical transmission, combined with PCR-based screening, allow for the ready elimination of APV from valuable experimental lines without the need to discard them.

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Author Contributions

C.H.V.H., S.E.A., G.C., and K.M.O. conceived and design experiments. C.H.V.H, S.E.A., G.C, K.K.L., V.P., and W.L.N provided technical assistance with experiments. C.H.V.H., V.P., and G.C., K.M.O. analyzed the data. C.H.V.H., M.R.S., and K.M.O. wrote the paper.

Data Accessibility

Datasets on aphid parasitism, fecundity and survivorship assays will be made available in the Dryad Digital Repository. Sequences generated for a portion of the APV P1 domain that were generated from the 12 laboratory aphid lines were submitted to GenBank under the accession numbers OM649898 - OM649902. Assembled APV genomes were submitted into Genbank under the accession numbers OM649898 - OM649902. SRA data generated from RNAseq used to assemble the APV genomes were deposited under BioProject PRJNA803168.

References:

Amuzu, H. E., K. Tsyganov, C. Koh, R. I. Herbert, D. R. Powell, and E. A. McGraw. 2018. Wolbachia enhances insect-specific flavivirus infection in *Aedes aegypti* mosquitoes. Ecology and Evolution 8:5441-5454.

Asgari, S., and K. N. Johnson. 2010. Insect Virology. Caister Academic.

Ban, L., E. Ahmed, V. Ninkovic, G. Delp, and R. Glinwood. 2008. Infection with an insect virus affects olfactory behavior and interactions with host plant and natural enemies in an aphid. Entomologia experimentalis et applicata 127:108-117.

Boyd, B. M., G. Chevignon, V. Patel, K. M. Oliver, and M. R. Strand. 2021. Evolutionary genomics of APSE: a tailed phage that lysogenically converts the bacterium *Hamiltonella defensa* into a heritable protective symbiont of aphids. Virology journal 18:219.

Brandt, J. W., G. Chevignon, K. M. Oliver, and M. R. Strand. 2017. Culture of an aphid heritable symbiont demonstrates its direct role in defense against parasitoids. Proceedings of the Royal Society B: Biological Sciences 284:20171925.

Brault, V., M. Uzest, B. Monsion, E. Jacquot, and S. Blanc. 2010. Aphids as transport devices for plant viruses. Comptes rendus biologies 333:524-538.

Cayetano, L., L. Rothacher, J.-C. Simon, and C. Vorburger. 2015. Cheaper is not always worse: strongly protective isolates of a defensive symbiont are less costly to the aphid host. Proceedings of the Royal Society B: Biological Sciences 282:20142333.

Chevignon, G., B. M. Boyd, J. W. Brandt, K. M. Oliver, and M. R. Strand. 2018. Culture-facilitated comparative genomics of the facultative symbiont *Hamiltonella defensa* . Genome Biology and Evolution 10:786-802.

Dion, E., F. Zele, J. C. Simon, and Y. Outreman. 2011. Rapid evolution of parasitoids when faced with the symbiont-mediated resistance of their hosts. Journal of evolutionary biology 24:741-750.

Doremus, M. R., and K. M. Oliver. 2017. Aphid heritable symbiont exploits defensive mutualism. Applied environmental microbiology 83.

Doremus, M. R., A. H. Smith, K. L. Kim, A. J. Holder, J. A. Russell, and K. M. Oliver. 2018. Breakdown of a defensive symbiosis, but not endogenous defences, at elevated temperatures. Molecular ecology 27:2138-2151.

Douglas, A. E. 2009. The microbial dimension in insect nutritional ecology. Functional Ecology 23:38-47.

Dupont, C., A. Michiels, C. Sochard, N. Dardenne, S. Meyer, V. Brault, Y. Outreman, and A. Sentis. 2020. Virus mediated trophic interactions between aphids and their natural enemies. Oikos 129:274-282.

Febvay, G., B. Delobel, and Y. Rahbé. 1988. Influence of the amino acid balance on the improvement of an artificial diet for a biotype of*Acyrthosiphon pisum* (Homoptera: Aphididae). Canadian Journal of Zoology 66:2449-2453.

Flick, A. J., M. A. Acevedo, and B. D. Elderd. 2016. The negative effects of pathogen-infected prey on predators: a meta-analysis. Oikos 125:1554-1560.

Gehrer, L., and C. Vorburger. 2012. Parasitoids as vectors of facultative bacterial endosymbionts in aphids. Biology letters 8:613-615.

Godfray, H. C. J., and H. Godfray. 1994. Parasitoids: behavioral and evolutionary ecology. Princeton University Press.

Graham, R. I., D. Grzywacz, W. L. Mushobozi, and K. Wilson. 2012. *Wolbachia* in a major African crop pest increases susceptibility to viral disease rather than protects. Ecology letters 15:993-1000.

Guay, J.-F., S. Boudreault, D. Michaud, and C. Cloutier. 2009. Impact of environmental stress on aphid clonal resistance to parasitoids: role of*Hamiltonella defensa* bacterial symbiosis in association with a new facultative symbiont of the pea aphid. Journal of Insect Physiology 55:919-926.

Guindon, S., J.-F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology 59:307-321.

Guo, J., S. Hatt, K. He, J. Chen, F. Francis, and Z. Wang. 2017. Nine facultative endosymbionts in aphids. A review. Journal of Asia-Pacific Entomology 20:794-801.

Gupta, V., C. Stewart, S. Rund, K. Monteith, and P. Vale. 2017. Costs and benefits of sublethal Drosophila C virus infection. Journal of evolutionary biology 30:1325-1335.

Hedges, L. M., J. C. Brownlie, S. L. O'Neill, and K. N. Johnson. 2008. *Wolbachia* and virus protection in insects. Science 322:702-702.

Higashi, C. H., B. T. Barton, and K. M. Oliver. 2020. Warmer nights offer no respite for a defensive mutualism. Journal of Animal Ecology 89:1895-1905.

Hrček, J., A. H. C. McLean, and H. C. J. Godfray. 2016. Symbionts modify interactions between insects and natural enemies in the field. Journal of Animal Ecology 85:1605-1612.

Ives, A. R., B. T. Barton, R. M. Penczykowski, J. P. Harmon, K. L. Kim, K. Oliver, and V. C. Radeloff. 2020. Self-perpetuating ecological–evolutionary dynamics in an agricultural host–parasite system. Nature Ecology & Evolution 4:702-711.

Jiang, J.-X., Y.-B. Bao, X.-Y. Ji, and N.-F. Wan. 2014. Effect of nucleopolyhedrovirus infection of Spodoptera litura larvae on host discrimination by *Microplitis pallidipes* . Biocontrol Science Technology 24:561-573.

Kuraku, S., C. M. Zmasek, O. Nishimura, and K. Katoh. 2013. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Research 41:W22-W28.

Laubscher, J., and M. Von Wechmar. 1992. Influence of aphid lethal paralysis virus and Rhopalosiphum padi virus on aphid biology at different temperatures. Journal of Invertebrate Pathology 60:134-140.

Laubscher, J., and M. Von Wechmar. 1993. Assessment of Aphid lethal paralysis virus as an apparent population growth-limiting factor in grain aphids in the presence of other natural enemies. Biocontrol Science Technology 3:455-466.

Li, H. 2018. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 34:3094-3100.

Lindsey, A. R. I., T. Bhattacharya, I. L. G. Newton, and R. W. Hardy. 2018. Conflict in the Intracellular Lives of Endosymbionts and Viruses: A Mechanistic Look at *Wolbachia* -Mediated Pathogen-blocking. Viruses 10:141.

Liu, S., D. Vijayendran, Y. Chen, and B. C. Bonning. 2016. Aphis glycines virus 2, a novel insect virus with a unique genome structure. Viruses 8:315.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$CT method. Methods 25:402-408.

Lu, H., J. Zhu, J. Yu, X. Chen, L. Kang, and F. Cui. 2020. A symbiotic virus facilitates aphid adaptation to host plants by suppressing jasmonic acid responses. Molecular Plant-Microbe Interactions 33:55-65.

Łukasik, P., H. Guo, M. Van Asch, J. Ferrari, and H. Godfray. 2013. Protection against a fungal pathogen conferred by the aphid facultative endosymbionts *Rickettsia* and *Spiroplasma* is expressed in multiple host genotypes and species and is not influenced by co-infection with another symbiont. Journal of evolutionary biology 26:2654-2661.

Lynn-Bell, N. L., M. R. Strand, and K. M. Oliver. 2019. Bacteriophage acquisition restores protective mutualism. Microbiology 165:985-989.

Martinez, A. J., M. R. Doremus, L. J. Kraft, K. L. Kim, and K. M. Oliver. 2018. Multi-modal defences in aphids offer redundant protection and increased costs likely impeding a protective mutualism. Journal of Animal Ecology 87:464-477.

Martinez, A. J., S. R. Weldon, and K. M. Oliver. 2014. Effects of parasitism on aphid nutritional and protective symbioses. Molecular ecology 23:1594-1607.

Mathé-Hubert, H., H. Kaech, P. Ganesanandamoorthy, and C. Vorburger. 2019. Evolutionary costs and benefits of infection with diverse strains of Spiroplasma in pea aphids*. Evolution 73:1466-1481.

McLean, A., J. Hrček, B. Parker, H. Mathé-Hubert, H. Kaech, C. Paine, and H. Godfray. 2020. Multiple phenotypes conferred by a single insect symbiont are independent. Proceedings of the Royal Society B 287:20200562.

McLean, A. H., B. J. Parker, J. Hrček, J. C. Kavanagh, P. A. Wellham, and H. C. J. Godfray. 2018. Consequences of symbiont co-infections for insect host phenotypes. Journal of Animal Ecology 87:478-488.

Moon, J. S., L. L. Domier, N. K. McCoppin, C. J. D'Arcy, and H. Jin. 1998. Nucleotide Sequence Analysis Shows that Rhopalosiphum padi Virus Is a Member of a Novel Group of Insect-Infecting RNA Viruses. Virology 243:54-65.

Nazni, W. A., A. A. Hoffmann, A. NoorAfizah, Y. L. Cheong, M. V. Mancini, N. Golding, G. M. Kamarul, M. A. Arif, H. Thohir, and H. NurSyamimi. 2019. Establishment of *Wolbachia* strain w*AlbB*in Malaysian populations of *Aedes aegypti* for dengue control. Current biology 29:4241-4248. e4245.

O'Neill, S. L., P. A. Ryan, A. P. Turley, G. Wilson, K. Retzki, I. Iturbe-Ormaetxe, Y. Dong, N. Kenny, C. J. Paton, and S. A. Ritchie. 2019. Scaled deployment of *Wolbachia* to protect the community from dengue and other *Aedes* transmitted arboviruses. Gates open research 2.

Oliver, K. M., J. Campos, N. A. Moran, and M. S. Hunter. 2008. Population dynamics of defensive symbionts in aphids. Proceedings of the Royal Society B: Biological Sciences 275:293-299.

Oliver, K. M., P. H. Degnan, G. R. Burke, and N. A. Moran. 2010. Facultative Symbionts in Aphids and the Horizontal Transfer of Ecologically Important Traits. Annual review of entomology 55:247-266.

Oliver, K. M., P. H. Degnan, M. S. Hunter, and N. A. Moran. 2009. Bacteriophages encode factors required for protection in a symbiotic mutualism. Science 325:992-994.

Oliver, K. M., and C. H. Higashi. 2019. Variations on a protective theme: *Hamiltonella defensa* infections in aphids variably impact parasitoid success. Current Opinion in Insect Science 32:1-7.

Oliver, K. M., and A. J. Martinez. 2014. How resident microbes modulate ecologically-important traits of insects. Current Opinion in Insect Science 4:1-7.

Oliver, K. M., K. Noge, E. M. Huang, J. M. Campos, J. X. Becerra, and M. S. Hunter. 2012. Parasitic wasp responses to symbiont-based defense in aphids. BMC Biology 10:11.

Oliver, K. M., J. A. Russell, N. A. Moran, and M. S. Hunter. 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proceedings of the National Academy of Sciences 100:1803-1807.

Oliver, K. M., A. H. Smith, and J. A. Russell. 2014. Defensive symbiosis in the real world–advancing ecological studies of heritable, protective bacteria in aphids and beyond. Functional ecology 28:341-355.

Parker, B. J., J. Hrček, A. H. McLean, and H. C. J. Godfray. 2017. Genotype specificity among hosts, pathogens, and beneficial microbes influences the strength of symbiont-mediated protection. Evolution 71:1222-1231.

Parker, B. J., C. J. Spragg, B. Altincicek, and N. M. Gerardo. 2013. Symbiont-mediated protection against fungal pathogens in pea aphids: a role for pathogen specificity? Applied environmental microbiology 79:2455-2458.

Patel, V., G. Chevignon, A. Manzano-Marín, J. W. Brandt, M. R. Strand, J. A. Russell, and K. M. Oliver. 2019. Cultivation-Assisted Genome of Candidatus *Fukatsuia symbiotica* ; the Enigmatic "X-Type" Symbiont of Aphids. Genome Biology and Evolution 11:3510-3522.

Pimentel, A. C., C. S. Cesar, M. Martins, and R. Cogni. 2021. The Antiviral Effects of the Symbiont Bacteria *Wolbachia* in Insects. Frontiers in immunology 11:3690.

Prjibelski, A., D. Antipov, D. Meleshko, A. Lapidus, and A. Korobeynikov. 2020. Using SPAdes De Novo Assembler. Current Protocols in Bioinformatics 70:e102.

Roossinck, M. J. 2015. Plants, viruses and the environment: ecology and mutualism. Virology 479:271-277.

Rouïl, J., E. Jousselin, A. Coeur d'acier, C. Cruaud, and A. Manzano-Marín. 2020. The Protector within: Comparative Genomics of APSE Phages across Aphids Reveals Rampant Recombination and Diverse Toxin Arsenals. Genome Biology and Evolution 12:878-889.

Russell, J. A., S. Weldon, A. H. Smith, K. L. Kim, Y. Hu, P. Łukasik, S. Doll, I. Anastopoulos, M. Novin, and K. M. Oliver. 2013. Uncovering symbiont-driven genetic diversity across North American pea aphids. Molecular ecology 22:2045-2059.

Ryabov, E. V. 2007. A novel virus isolated from the aphid Brevicoryne brassicae with similarity to Hymenoptera picorna-like viruses. Journal of general virology 88:2590-2595.

Ryabov, E. V., G. Keane, N. Naish, C. Evered, and D. Winstanley. 2009. Densovirus induces winged morphs in asexual clones of the rosy apple aphid, *Dysaphis plantaginea* . Proceedings of the National Academy of Sciences 106:8465-8470.

Scarborough, C. L., J. Ferrari, and H. Godfray. 2005. Aphid protected from pathogen by endosymbiont. Science 310:1781-1781.

Schmid, M., R. Sieber, Y. S. Zimmermann, and C. Vorburger. 2012. Development, specificity and sublethal effects of symbiont-conferred resistance to parasitoids in aphids. Functional ecology 26:207-215.

Smith, A. H., P. Łukasik, M. P. O'Connor, A. Lee, G. Mayo, M. T. Drott, S. Doll, R. Tuttle, R. A. Disciullo, A. Messina, K. M. Oliver, and J. A. Russell. 2015. Patterns, causes and consequences of defensive microbiome dynamics across multiple scales. Molecular ecology 24:1135-1149.

Smith, A. H., M. P. O'Connor, B. Deal, C. Kotzer, A. Lee, B. Wagner, J. Joffe, S. Woloszynek, K. M. Oliver, and J. A. Russell. 2021. Does getting defensive get you anywhere?—Seasonal balancing selection, temperature, and parasitoids shape real-world, protective endosymbiont dynamics in the pea aphid. Molecular ecology 30:2449-2472.

Teixeira, L., Á. Ferreira, and M. Ashburner. 2008. The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in Drosophila melanogaster. PLoS biology 6:e1000002.

Terradas, G., and E. A. McGraw. 2017. Wolbachia-mediated virus blocking in the mosquito vector *Aedes aegypti* . Current Opinion in Insect Science 22:37-44.

Van den Heuvel, J., H. Hummelen, M. Verbeek, A. Dullemans, and F. Van der Wilk. 1997. Characteristics of Acyrthosiphon pisum virus, a newly identified virus infecting the pea aphid. Journal of Invertebrate Pathology 70:169-176.

Van der Wilk, F., A. Dullemans, M. Verbeek, and J. Van den Heuvel. 1997. Nucleotide sequence and genomic organization of Acyrthosiphon pisum virus. Virology 238:353-362.

van Lenteren, J. C. 2003. Quality control and production of biological control agents: theory and testing procedures. Cabi Publishing.

van Munster, M., A. Dullemans, M. Verbeek, J. Van Den Heuvel, C. Reinbold, V. Brault, A. Clerivet, and F. Van Der Wilk. 2003. Characterization of a new densovirus infecting the green peach aphid*Myzus persicae* . Journal of Invertebrate Pathology 84:6-14.

Vorburger, C. 2014. The evolutionary ecology of symbiont-conferred resistance to parasitoids in aphids. Insect Science 21:251-264.

Vorburger, C., and A. Gouskov. 2011. Only helpful when required: a longevity cost of harboring defensive symbionts. Journal of evolutionary biology 24:1611-1617.

Weldon, S., J. Russell, and K. Oliver. 2020. More is not always better: coinfections with defensive symbionts generate highly variable outcomes. Applied environmental microbiology 86.

Weldon, S., M. Strand, and K. Oliver. 2013. Phage loss and the breakdown of a defensive symbiosis in aphids. Proceedings of the Royal Society B: Biological Sciences 280:20122103.

Supplemental Information

Table S1: Percentage of RNAseq reads mapped to reference genome.

References	**A2C**	**NY26**	**AS3**	**AS3AB**	**ZA17**
H. defensa	0.471%	0.155%	0.167%	0.002%	0.098%
B. aphidicola	1.795%	1.744%	1.247%	1.126%	1.319%
APV	0.126%	0.009%	25.851%	25.913%	14.970%
A. pisum	86.123%	88.434%	54.201%	58.878%	71.272%
Unmapped	11.485%	9.658%	18.533%	14.080%	12.341%

Table S2: Amino Acid similarity matrix table among APV (grouped by region) and related viruses.

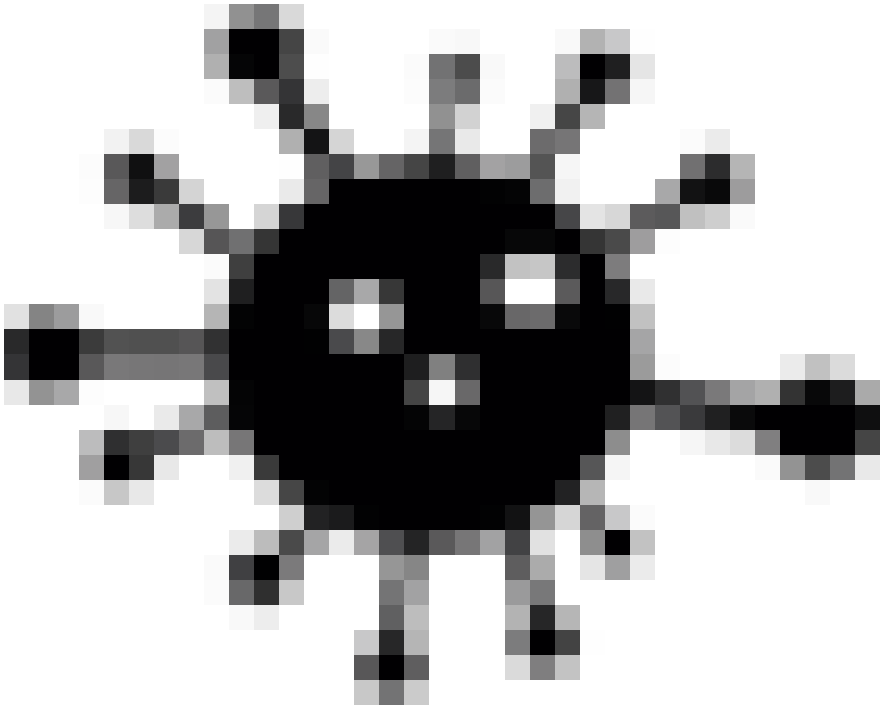

Table S3: *A. ervi* can facilitate the transfer of APV between aphids

Aphid recipient line & infection status	**Parasitism group**	**Parasitism sequence (Ct value)**	**Parasitism sequence (Ct value)**	**Parasitism sequence (Ct value)**
		1st aphid	**2nd aphid**	**3rd aphid**
ND18 APV- / Ham-	A	Negative	Negative	Negative
	B	Negative	Negative	Negative
	C	Negative	Negative	Negative
	D	Negative	Negative	Negative
	E	POSITIVE (Ct=22.09)	Negative	Negative
	F	Negative	Negative	Negative
	G	Negative	Negative	Negative
	H	POSITIVE (Ct=21.85)	Negative	Negative
ND18 APV- / Ham+	A	Negative	Negative	Negative
	B	Negative	Negative	Negative
	C	POSITIVE (Ct=23.92)	Negative	Negative
	D	Negative	Negative	Negative
	E	Negative	Negative	Negative

Aphid recipient line & infection status	Parasitism group	Parasitism sequence (Ct value)	Parasitism sequence (Ct value)	Parasitism sequence (Ct value)
	F	Negative	Negative	Negative
	G	Negative	Negative	Negative
	H	Negative	Negative	Negative

Negative – Aphid not infected with APV indicated by diagnostic qPCR

Positive – Aphid infected with APV indicated by diagnostic qPCR

Table S4: Logistic regression analysis of fungal assays.

Comparison	Line	Contrast	Variable	LRT (L-R ChiSq; Prob>ChiSq)
A	LSR01.LSR	Regi-/APV- vs Regi-/APV+	Survival	$F_{\text{Regi-APV+}} = 29.595$, $P < 0.0001$
			Sporulation	$F_{\text{Regi-APV+}} = 2.455$, $P = 0.117$
			Dual Mortality	$F_{\text{Regi-APV+}} = 7.521$, $P = 0.006$
B	LSR01.LSR	Regi-/APV- vs Regi+/APV-	Survival	$F_{\text{Regi+APV-}} = 19.594$, $P < 0.001$
			Sporulation	$F_{\text{Regi+APV-}} = 27.108$, $P < 0.0001$
			Dual Mortality	$F_{\text{Regi+APV-}} = 0.287$, $P = 0.592$
C	LSR01.LSR	Regi+/APV+ vs Regi+/APV-	Survival	$F_{\text{Regi+APV+}} = 0.085$, $P = 0.771$
			Sporulation	$F_{\text{Regi+APV+}} = 6.736$, $P = 0.009$
			Dual Mortality	$F_{\text{Regi+APV+}} = 4.269$, $P = 0.039$
D	LSR01.LSR	Regi+/AVP+ vs Regi-/APV+	Survival	$F_{\text{Regi-APV+}} = 84.894$, $P < 0.0001$
			Sporulation	$F_{\text{Regi-APV+}} = 80.219$, $P < 0.0001$
			Dual Mortality	$F_{\text{Regi-APV+}} = 0.022$, $P = 0.882$

Table S5: Logistic regression analysis of parasitism assays.

Comparison	Line	Contrast	Variable	LRT (L-R ChiSq; Prob>ChiSq)
A	ND18	Ham-/APV- vs Ham-/APV+	Survival	$F_{\text{Ham-APV+}} = 0.0003$, $P = 0.985$
			Mummification	$F_{\text{Ham-APV+}} = 2.028$, $P = 0.154$
			Dual Mortality	$F_{\text{Ham-APV+}} = 4.390$, $P = 0.036$
B	ND18	Ham-/APV- vs Ham+/APV-	Survival	$F_{\text{HAM+APV-}} = 169.615$, $P < 0.001$
			Mummification	$F_{\text{HAM+APV-}} = 304.468$, $P < 0.001$
			Dual Mortality	$F_{\text{HAM+APV-}} = 10.309$, $P = 0.001$
C	ND18	Ham+/APV+ vs Ham+/APV-	Survival	$F_{\text{Ham+APV+}} = 2.189$, $P = 0.139$
			Mummification	$F_{\text{Ham+APV+}} = 1.058$, $P = 0.304$
			Dual Mortality	$F_{\text{Ham+APV+}} = 3.373$, $P = 0.066$
D	ND18	Ham+/AVP+ vs Ham-/APV+	Survival	$F_{\text{HAM-APV+}} = 206.265$, $P < 0.001$
			Mummification	$F_{\text{HAM-APV+}} = 307.421$, $P < 0.001$
			Dual Mortality	$F_{\text{HAM-APV+}} = 7.517$, $P = 0.0006$
E	576N-27	APV- vs APV+	Survival	$F_{\text{APV+}} = 0.416$, $P = 0.519$
			Mummification	$F_{\text{APV+}} = 0.326$, $P = 0.568$
			Dual Mortality	$F_{\text{APV+}} = 3.320$, $P = 0.068$
F	246-8	APV- vs APV+	Survival	$F_{\text{APV+}} = 0.230$, $P = 0.632$
			Mummification	$F_{\text{APV+}} = 12.733$, $P = 0.0004$
			Dual Mortality	$F_{\text{APV+}} = 717.323$, $P < 0.0001$
G	5D-AB	Ham-/APV- vs Ham-/APV+	Survival	$F_{\text{Ham-APV+}}$ 0.848, $P < 0.357$

Comparison	Line	Contrast	Variable	LRT (L-R ChiSq; Prob>ChiSq)
			Mummification	$F_{\text{Ham-APV+}} = 0.059$, P = 0.808
			Dual Mortality	$F_{\text{Ham-APV+}} = 0.465$, P = 0.496
H	5D-AB	Ham-/APV- vs Ham+/APV-	Survival	$F_{\text{HAM+APV-}} = 169.615$, P < 0.001
			Mummification	$F_{\text{HAM+APV-}} = 7.482$, P < 0.006
			Dual Mortality	$F_{\text{HAM+APV-}} = 0.056$, P = 0.813
I	5D-AB	Ham+/APV+ vs Ham+/APV-	Survival	$F_{\text{Ham+APV+}} = 1.966$, P = 0.161
			Mummification	$F_{\text{Ham+APV+}} = 0.238$, P = 0.626
			Dual Mortality	$F_{\text{Ham+APV+}} = 1.638$, P = 0.201
J	5D-AB	Ham+/AVP+ vs Ham-/APV+	Survival	$F_{\text{HAM-APV+}} = 7.636$, P < 0.006
			Mummification	$F_{\text{HAM-APV+}} = 6.171$, P < 0.013
			Dual Mortality	$F_{\text{HAM-APV+}} = 0.130$, P = 0.718

Table S6: Full-factorial logistic regression model on the major effects of symbiont infection status, virus infection status and their interaction on aphid survival, mummification and dual mortality post parasitism for ND18 aphid sublines with and without APV and *H. defensa* /APSE-3.

		Survival	*Survival*	*Mummification*	*Mummification*	*Dual Mortality*	*Dual Mortality*
Variable	DF	**LRT X^2**	**Prob>Chisq**	**LRT X^2**	**Prob>Chisq**	**LRT X^2**	**Prob>Chisq**
Symbiont	1	0.83	0.363	2.48	0.115	7.35	**0.007**
APV	1	473.11	**< 0.0001**	646.49	**< 0.0001**	17.81	**< 0.0001**
Symbiont*Virus	1	0.40	0.526	0.39	0.534	0.60	0.438

Table S7: Full-factorial logistic regression model on the major effects of symbiont infection status, virus infection status and their interaction on aphid survival, mummification and dual mortality post parasitism for 5DAB aphid sublines with and without APV and *H. defensa* /APSE-2.

		Survival	*Survival*	*Mummification*	*Mummification*	*Dual Mortality*	*Dual Mortality*
Variable	DF	**LRT X^2**	**Prob>Chisq**	**LRT X^2**	**Prob>Chisq**	**LRT X^2**	**Prob>Chisq**
Symbiont	1	2.47	0.116	0.25	0.618	1.92	0.17
APV	1	0.01	0.936	0.02	0.901	0.17	0.68
Symbiont*Virus	1	17.79	**< 0.0001**	13.60	**0.0002**	0.003	0.96

Table S8: Full-factorial logistic regression model on the major effects of symbiont infection status, virus infection status and their interaction on aphid survival, fungal sporulation and dual mortality post *P. neoaphidis* exposure for LSR1 aphid sublines with and without APV and *R. insecticola* .

		Survival	*Survival*	*Mummification*	*Mummification*	*Dual Mortality*	*Dual Mortality*
Variable	DF	**LRT X^2**	**Prob>Chisq**	**LRT X^2**	**Prob>Chisq**	**LRT X^2**	**Prob>Chisq**
Symbiont	1	97.25	**< 0.0001**	101.83	0.153	0.10	**0.001**
APV	1	20.79	**< 0.0001**	2.04	**< 0.0001**	11.59	0.75
Symbiont*Virus	1	17.81	**< 0.0001**	9.19	**0.002**	0.26	0.61

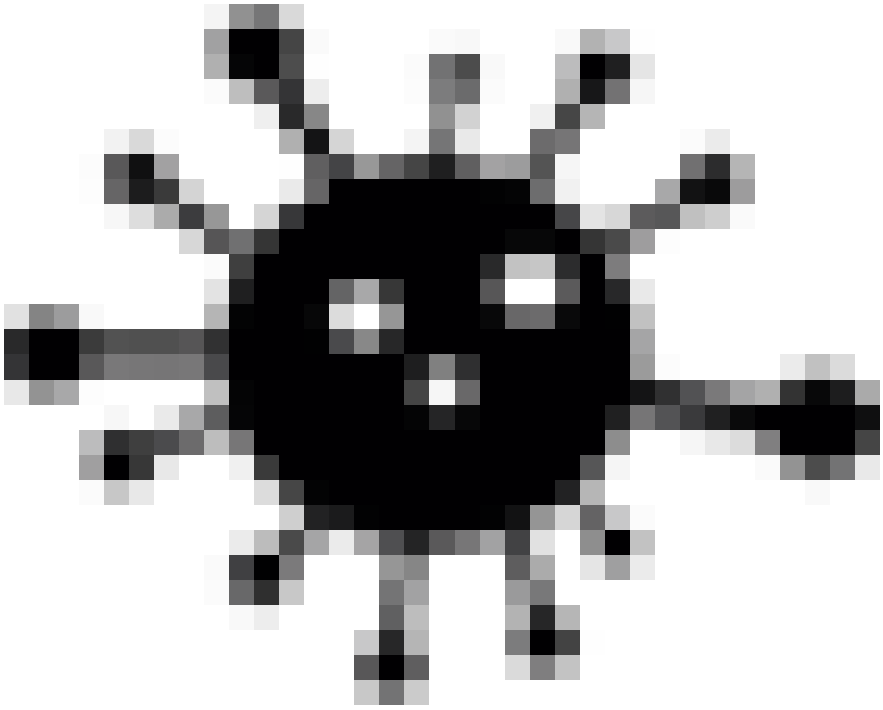

Figure S1: Aphid survival, mummification and dual-mortality of uninfected (UI) aphids lines 576N-27 and WI246-8 following parasitism by the wasp *A. ervi* . Parasitism outcomes were contrasted between aphid with and without APV. Asterisk(s) above bars indicate significant differences (NS = $P > 0.05$; * = P [?] 0.05; ** = P [?] 0.01; *** = P [?] 0.001; **** = P [?] 0.0001).

Hosted file

`APV Genome + Phylo.svg` available at https://authorea.com/users/492555/articles/575248-an-aphid-symbiont-confers-protection-against-a-specialized-rna-virus-another-increases-vulnerability-to-the-same-pathogen