Parasitoid gene expression changes after adaptation to symbiont-protected hosts

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Abstract:

Reciprocal selection between aphids, their protective endosymbionts, and the parasitoid wasps that prey upon them offers an opportunity to study the basis of their coevolution. We investigated adaptation to symbiont-conferred defense by rearing the parasitoid wasp *Lysiphlebus fabarum* on aphids (*Aphis fabae*) possessing different defensive symbiont strains (*Hamiltonella defensa*). After ten generations of experimental evolution, wasps showed increased abilities to parasitize aphids possessing the *H. defensa* strain they evolved with, but not aphids possessing the other strain. We show that the two symbiont strains encode different toxins, potentially creating different targets for counter-adaptation. Phenotypic and behavioral comparisons suggest that neither life history traits nor oviposition behavior differed among evolved parasitoid lineages. In contrast, comparative transcriptomics of adult female wasps identified a suite of differentially expressed genes among lineages, even when reared in a common, symbiont-free, aphid host. In concurrence with the specificity of each parasitoid lineages' infectivity, most differentially expressed parasitoid transcripts were also lineage-specific. These transcripts are enriched with putative venom toxins and contain highly expressed, potentially defensive viral particles. Together, these results suggest that wild populations of *L. fabarum* employ a complicated offensive arsenal with sufficient genetic variation for wasps to adapt rapidly and specifically to their hosts' microbial defenses.

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Introduction

Across all organisms, parasitism is a common and strong driver of evolution (Windsor 1998; Meyer et al. 2012). Host-parasite interactions can create intense reciprocal selection, resulting in antagonistic coevolution through adaptation and counter-adaptation between species (Woolhouse et al. 2002). If both host resistance and parasite infectivity show genotype-specificity, with neither player possessing genotypes that are universally resistant or infective, respectively, then coevolution can maintain genetic diversity in both opponents by favoring rare genotypes via negative frequency-dependent selection (Carius et al. 2001; Wilfert and Jiggins 2010; Schmid-Hempel 2011; Rouchet and Vorburger 2012). Laboratory-based experimental evolution in host-parasite systems provides an opportunity to observe these processes in action, and combined with next-generation sequencing it allows the study of the genomic basis of reciprocal counter adaptations in antagonistic coevolution (Schlötterer et al. 2015).

Parasitoid insects are a hyper-diverse and geographically widespread group that parasitize other arthropods (Godfray 1994). Selective pressures are acute in host-parasitoid systems because successful parasitoid reproduction is usually fatal to the host. Aphids and their parasitoids offer a particularly interesting arena to study host-parasitoid coevolution because their defenses are largely driven by a suite of heritable bacterial endosymbionts (Oliver et al. 2010; Vorburger 2014). Symbiont roles have been most thoroughly investigated in the pea aphid, *Acyrthosiphon pisum*. In addition to infection with the obligate, nutrient providing *Buchnera aphidicola*, seven facultative endosymbionts commonly occur in natural pea aphid populations (Ferrari et al. 2012; Russell et al. 2013; Oliver et al. 2014). All seven symbionts are known or suspected to provide protective services against specialized enemies (Oliver et al. 2014; Heyworth and Ferrari 2015; but see: Doremus and Oliver 2017), including parasitoid wasps (Oliver et al. 2003; Vorburger et al. 2010) and entomopathogenic fungi (Scarborough et al. 2005; Łukasik et al. 2013b). However, infections

52 can be costly in other contexts (Oliver et al. 2008; Vorburger and Gouskov 2011). The best
53 studied defensive symbiont of aphids, *Hamiltonella defensa* (Moran et al. 2005b) occurs in ca.
54 40% of aphid species examined (Henry et al. 2015) and provides significant protection against
55 parasitoids in most aphids (Oliver et al. 2014; Vorburger 2014), although exceptions do occur
56 (Łukasik et al. 2013a). The protective phenotype of *H. defensa* has been linked to infection with
57 the temperate bacteriophage APSE, which possesses strain-specific infective "cassettes" encoding
58 different putative toxins hypothesized to hinder parasitoid development (Moran et al. 2005a;
59 Degnan and Moran 2008; Oliver et al. 2009; Martinez et al. 2014b). While experimental studies
60 clearly show that phage-containing *H. defensa* provide protection against parasitoids, clonal aphid
61 genotypes free of endosymbionts can also vary in susceptibility to parasitoids, and virulence
62 factors encoded on the *H. defensa* chromosome may also contribute to defense (Sandrock et al.
63 2010).

64 Extensive natural variation occurs in aphid-wasp interactions, including large variation in
65 susceptibility to parasitism owing to aphid-encoded and *H. defensa*-based defenses (e.g. Henter
66 and Via 1995; von Burg et al. 2008; Sandrock et al. 2010; Martinez et al. 2014a) as well as in
67 wasp infectivity (e.g. Henter 1995; Schmid et al. 2012). Interactions between the black bean aphid
68 *Aphis fabae* and its parasitoid wasp *Lysiphlebus fabarum*, the focal organisms of this study, are
69 highly specific with significant genotype-by-genotype interactions occurring between parasitoid
70 genotypes and symbiont strains (Rouchet and Vorburger 2012; Schmid et al. 2012). This
71 specificity suggests that endosymbionts could mediate host-parasitoid coevolution by inducing
72 negative frequency-dependence (Kwiatkowski et al. 2012).

73 Parasitoids likely counter endosymbiont-mediated defenses both behaviorally and
74 chemically. When faced with hosts harboring *H. defensa*, *L. fabarum* show greater success when
75 attacking younger aphids, in which endosymbiont load may be lower (Schmid et al. 2012). Other

parasitoids may be able to detect the presence of *H. defensa* in aphids and modify their behavior to either avoiding protected aphids (Łukasik et al. 2013a) or to deposit more eggs in aphids harboring *H. defensa* for increased chances of success (superparasitism; Oliver et al. 2012). Generally, the offensive arsenal of parasitoids comes from several sources and acts at different time points: venom injected with the egg, special cells excreted from the egg extra-embryonic membrane called teratocytes, and toxins secreted by the larvae (Burke and Strand 2014). Comparative studies suggest that these components are rapidly evolving (Colinet et al. 2014; Poirié et al. 2014).

Here, we investigate adaptation of the parasitoid wasp *L. fabarum* to symbiont-conferred resistance in the host aphid *A. fabae*. Previous experimental evolution rapidly (<10 generations) produced *L. fabarum* lineages with increased abilities to infect *H. defensa*-protected aphids, suggesting standing genetic variation in the ability of natural populations to overcome symbiont-mediated protection (Rouchet and Vorburger 2014). To further examine parasitoid counter-adaptation, we have repeated these experimental evolution assays (Rouchet and Vorburger 2014) using different field-collected wasps. We have used behavioral assays and phenotypic measures to compare the resulting lines, and used transcriptomic sequencing to examine the genetic basis of variable infectivity. To better examine these parasitoid features in the context of their host, we have also characterized the APSE toxins produced by the two *H. defensa* strains employed in our experiment.

Methods

Insects

The host aphid, *A. fabae* (Hemiptera; Aphididae) is a widely distributed agricultural pest in Europe, and is particularly damaging to broad beans (*Vica faba*) and sugar beets (*Beta vulgaris*) (Blackman and Eastop 2006). We used three sublines of a single clone of *A. fabae* (A06-407)

collected in July 2006 on *Chenopodium album* in St. Margrethen (Switzerland) (Vorburger et al. 2009). In addition to the naturally uninfected subline (A06-407), we used two symbiont-protected sublines (A06-407^{H402} and A06-407^{H76}), created by microinjection of *H. defensa* > 100 generations before use in the present experiment. The two symbiont strains were originally obtained from *A. fabae* clones collected in St. Margrethen, Switzerland (H402), and La Grande Motte, France (H76); these sites are broadly sympatric with but not from the same sites as the parasitoids used in this study. The two strains of *H. defensa* are clearly distinct based on sequences of two housekeeping genes and provide different average levels of protection to multiple lines of the parasitoid *L. fabarum* (Cayetano et al. 2015). The widely distributed parasitoid *L. fabarum* (Hymenoptera: Braconidae: Aphidiinae) is the most abundant and impactful parasitoid of *A. fabae* the field (Rothacher et al. 2016). Adult females inject eggs into the aphid hemocoel, where larval development proceeds within the living aphid until wasp pupation. At this point the aphid is killed and wasp metamorphoses within the "mummified' remnants of the aphid exoskeleton, from which a single adult emerges.

The base population for experimental evolution was founded from nine *L. fabarum* collections made in June and September 2012 at six locales across Switzerland (Table S1). They were reared separately at large population sizes for 24-30 generations, on a different unprotected aphid clone from that used in experiments. Prior to experimentation, wasps from all stocks were pooled (40 females and 10-35 males from each line) into a large polyester mesh cage (47.5 cm x 47.5 cm x 47.5 cm, BugDorm 44545F, MegaView Science, Taichung, Taiwan) and allowed to interbreed for two generations to establish a common, genetically variable stock. This interbreeding prior to experimentation aimed to reduce linkage disequilibrium and increase the power of selecting for the most beneficial alleles (Schlötterer et al. 2015).

Characterizing phage-encoded toxins in aphid sublines possessing H. defensa

Previous work in pea aphids has demonstrated that toxin-encoding APSE phages are likely responsible for causing mortality to developing wasps (Moran et al. 2005a; Degnan and Moran 2008; Oliver et al. 2009). Therefore, we sought to characterize the primary APSE toxins associated with each *H. defensa* strain, since they potentially present distinct targets for wasp counter-responses. We Sanger sequenced the putative primary toxin for *H. defensa* strains H402 and H76 by primer walking across the portion of the APSE cassette region containing and identifying distinct APSE strains. Please see Table S3 for primers and reaction condition.

Experimental evolution

We reared our base population of parasitoids in three different host-aphid sublines. Experimental evolution methods closely followed those of Rouchet & Vorburger (2014), but we emphasize that we have started with a different stock of wild-collected parasitoids. Briefly, wasps were moved to three different host aphid treatments (i.e. evolution treatments): *H. defensa*-free (clone A06-407), hereafter called "H- treatment", and the two different *H. defensa*-infected sublines (clones A06-407^{402} and A06-407^{76}), hereafter called "H402 treatment" and "H76 treatment", respectively (Figure 1a). Each evolution treatment was replicated four times, all housed in independent polyester mesh cages (24.5 cm x 24.5 cm x 24.5 cm, BugDorm-4020F), placed haphazardly (and re-arranged weekly) on an illuminated bench in a climate controlled room (23 ˚C) with a 16:8 h Light:Dark cycle. Each cage contained three, three-week old broad bean plants colonized by the respective aphid sublines.

For the first generation of experimental selection, each cage was stocked with 32 female and 20 male parasitoids from the mixed, sexual stock population described above. Infectivity of the starting population was also measured on this day (initial population, described below, Figure

148 1b). Following this, evolution lines were maintained by transferring 50 individual wasps to fresh
149 cages containing new aphids of the appropriate sublines (two week cycle). Providing new aphids
150 each parasitoid generation effectively arrests aphids as a static evolutionary target and allows us to
151 observe adaptation in parasitoids only. Successful parasitism on aphids protected by *H. defensa*
152 was very poor in the first generations and we frequently obtained fewer than 50 wasps to transfer.
153 To ameliorate the bottleneck this strong selection imposes and help avoid potential extinctions
154 (Rouchet and Vorburger 2014), we supplemented with individuals from the unselected base
155 population for the first 1-4 generations (depending on the line) to obtain the same inoculum size
156 for all lines (Table S2). After four generations, all lines produced sufficient offspring to maintain
157 them by transferring 50 individuals every generation until our final measurements at generation
158 21.

159 Infectivity was estimated at three points in the course of the experiment by testing
160 parasitism success of singly isolated female wasps. These tests were made in the single starting
161 pool (Initial Population), and then in each of the 12 separately evolved populations after 10 and 21
162 generations of experimental evolution. For this, single parasitoid females were placed in caged
163 colonies of 48-72 h old aphid nymphs (mean colony size 20 ± 5.9 SD, range 3 – 36) and allowed
164 to oviposit for 12 h. The proportion of mummified (i.e. successfully parasitized) aphid hosts was
165 counted 11 days later as described by Henter & Via (1995). Counting was done blindly with
166 respect to host and parasitoid identities. Initial measures from the starting population tested ten
167 replicates on each aphid subline. At generations 10 and 21, five replicates were tested for each of
168 the four replicate lines from all three treatments, again on all aphid sublines (180 in total). All
169 infectivity tests were carried out in complete randomized blocks, i.e. one replicate per group
170 handled and placed together in randomized order.

171 Statistical analysis of the outcome of each infectivity test was performed in R version 3.1.2
172 (R-Core-Team 2012). Severe overdispersion in these proportional success/failure data meant that
173 it could not be analyzed using generalized linear models with binomial errors. Therefore, the
174 proportion of aphids mummified was arcsine square-root transformed and analyzed with linear
175 mixed models (LMM) using lmer in the lme4 package (Bates et al. 2015) and the lmerTest library
176 (Kuznetsova et al. 2013) for significance tests. The proportion of aphids mummified by the
177 original stock population was compared among the three potential aphid hosts (H-, H402 and
178 H76). After 10 and 21 generations of evolution, we tested for the effects of aphid subline (H-, H76
179 or H402, fixed effect), experimental evolution treatment (wasps evolved on H-, H76 or H402,
180 fixed effect), and evolution line nested within evolution treatment (4 replicate lines per evolution
181 treatment, random effect), as well as their interactions. This full model was also compared to one
182 which was restricted to wasps from evolution treatments H76 and H402 and the two *H. defensa*-
183 infected aphid sublines, in which the evolution treatment × aphid subline interaction is directly
184 interpretable as a genotype × genotype interaction between parasitoids and host endosymbionts,
185 which would reflect specificity in parasitoid counter-adaptation.
186
187 *Correlated responses after experimental evolution*
188 To examine physical trade-offs that might be associated with evolved infectivity on symbiont-
189 protected aphids, we measured wasp wet mass, development time, egg load, and egg length at
190 generation 16. To separate short-term, plastic responses to the presence of *H. defensa* from fixed,
191 likely genetically determined differences among evolution treatments, each wasp line was reared
192 on the aphid subline on which they had evolved and on unprotected (H-) aphids (Figure 2).
193 Parasitoid females from all lineages were individually caged on plants containing small colonies
194 of three-day old aphids and allowed to oviposit for 8 h. Three replicate colonies per evolution line

were used to infect H- hosts and six replicates to infect H76 and H402 hosts (due to lower parasitism success on aphids possessing *H. defensa*). When mummies started forming, colonies were checked daily to record when wasps hatched (development time in days). All wasps were weighed on a Mettler MX5 microbalance (Mettler Toledo GmbH, Greifenssee, Switzerland) and female wasps were frozen in insect Ringer's solution for later ovary dissection (preliminary tests showed that freezing did not alter egg size). To determine egg load, ovaries were removed from the females and gently compressed under a cover slip on a microscope slide until they burst and released the eggs, which were then counted. To estimate egg size, eggs were photographed at 100x or 400x magnification, and the length of the ten largest eggs was measured for each individual using the Zen 2012 software (blue edition, Carl Zeiss Microscopy). Parasitoid life-history traits were analyzed with LMMs, including evolution line (nested within treatment) and replicate (nested within evolution line) as random effects to account for non-independence. In tests that included both males and females (development time and wet weight), sex was also included as a fixed factor.

Parasitoid behavior

We hypothesized that parasitoids could adapt to the presence of *H. defensa* in their hosts by preferentially attacking younger, less strongly protected host stages (Schmid et al. 2012), or by increasing superparasitism in *H. defensa*-protected aphids (Oliver et al. 2012). Therefore, we tested host stage preference of female wasps after 18 generations of experimental evolution and preferential oviposition in protected and unprotected aphids after 20 generations.

For the first test we offered single female wasps individual aphids of each developmental stage (nymphs 1-4 and adult) on a leaf disc placed on agar in a petri dish (5 replicates per line for a total of 60). Each wasp line was offered only aphids of the subline they evolved on. Wasps were

219 removed from the dishes after 5 h and the dishes immediately frozen. Oviposition was quantified
220 by dissecting aphids under a microscope and counting the parasitoid eggs in each individual. In
221 some replicates, the adult aphid gave birth to additional 1st instar nymphs during the 5 h exposure
222 period. In these cases we dissected all 1st instar nymphs and analyzed the average number of eggs
223 in 1st instars because it was not possible to distinguish the newborns from the youngest nymph
224 originally present in the dish. The number of eggs deposited per aphid was analyzed with a LMM,
225 because using averages for the 1st instar nymphs precluded the use of a GLMM with Poisson
226 errors. We tested for the effects of evolution treatment, host stage, and their interaction, while
227 accounting for evolution line within treatment and replicate as random effects. For comparison, we
228 also ran a Poisson GLMM after excluding the 1st instar nymphs from the dataset.
229 For the second test of oviposition preference, we offered individual wasps six *H. defensa*-
230 free and *H. defensa*-infected four-day old aphids, again on leaf discs in petri dishes for 5 h. Four
231 replicates were carried out per line for a total of 48. Wasps from lines evolving on aphids with *H.*
232 *defensa* were offered aphids from their "home" aphid subline and H- aphids. Wasps from lines
233 evolving on *H. defensa*-free aphids were offered H- and H76 in one half of the replicates and H-
234 and H402 in the other half. To later distinguish the two types of aphids, we marked the protected
235 aphids in half of the replicates and the unprotected in the other half by clipping a small part of the
236 last antennal segment. Prior to their use, wasps were allowed to forage on plants infested with both
237 types of aphids to gain experience for 24 h. Oviposition was scored by dissection as before, and
238 the number of eggs deposited per aphid was analyzed using a GLMM with Poisson errors, testing
239 for the effects of evolution treatment, host type (*H. defensa*-free or –infected) and their interaction
240 with likelihood ratio tests, dropping the interaction from the full model and each main effect
241 individually from a main effects model. Evolution line within treatment and replicate were
242 accounted for as random effects. Wasps that failed to oviposit were excluded from both analyses.

243

244 *Gene expression comparisons among treatments*

245 To compare gene expression among wasps from the different evolution treatments, we carried out
246 transcriptome sequencing (RNA-seq). Observations in the pea aphid, *A. pisum*, suggest that larval
247 mortality induced by *H. defensa* varies depending upon their associated APSE, and can occur both
248 early and late in development (Henter and Via 1995; Martinez et al. 2014b). We therefore chose to
249 sequence adult female wasps, as in many cases successful parasitism would require very early
250 parasitoid counter-defenses, likely derived from maternal factors including venom. As with the
251 correlated phenotypic measures described above, we sampled wasps that had hatched from both
252 their "home" aphid environment (H- , H402 or H76 possessing aphids) and from symbiont-free
253 (H-) aphids; only wasps from the H402 and H76 treatments were reared in two possible
254 environments, as the H- treatment parasitoids were unable to successfully infect either of the
255 protected aphid lines. With this, we aimed to avoid detecting expression patterns that are solely in
256 response to immediate exposure to the toxic environment created by *H. defensa* (i.e. developing in
257 its presence). To minimize variation among libraries, we used only 12-24 h old virgin females, all
258 directly frozen (-80 ˚C) at approximately 06:00 h. For each of the 12 lines, we sequenced three
259 biological replicates, i.e. cDNA libraries from three different pools of wasps (mean 6.3 wasps per
260 library, 60 cDNA libraries in total).

261 Total RNA extractions from whole wasps were performed using 1 ml of Trizol reagent
262 (Invitrogen, Carlsbad, CA, cat #15596) according to the manufacturer's instructions, with the
263 following modifications: ground samples were incubated overnight at room temperature in Trizol,
264 and ethanol precipitations were incubated overnight at 4 ˚C. Products of Trizol extraction were
265 subsequently spin column purified using the RNeasy mini kit (Qiagen, Venlo, Netherlands, cat #
266 74104), purified from contaminating DNA (RNAse free DNase kit, Qiagen), verified on an

Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and quantified with a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). All cDNA libraries were prepared using the Illumina TruSeq RNA kit (v2, Cat # RS-122-2001) with an input of 150-500ng total RNA; libraries were prepared according to manufacturer's instructions but for the substitution of ProtoScript M-MuLV reverse transcriptase (New England Biolabs, Ipswich, MA, USA, Cat # E6300S). In all cases, mRNA was poly-A-selected and the PCR amplification step was performed for 12 cycles. Library quantity was determined using both Qubit measures and the KAPA Library Quantification Kit for Illumina (Kapa Biosystems, Wilminton MA, USA); quality was verified using a Bioanalyzer. Quantified libraries were normalized, randomly pooled into 14-15 libraries per lane, and all pools were sequenced twice in 100-cycle single-end sequencing runs on the Illumina HiSeq 2500 v3 chemistry at the Functional Genomics Center Zürich.

RNA-seq data handling and transcriptome assembly

Prior to *de novo* assembly, raw Illumina reads were filtered to retain only very high quality sequences in several steps. Using PRINSEQ lite v0.20.4 (Schmieder and Edwards 2011) low quality (Q<28) sequences were trimmed and all reads containing an "N" were discarded. Illumina adaptor trimming with Cutadapt v1.4.1(Martin 2011) was then followed by an additional implementation of PRINSEQ-lite to trim poly A/T stretches from both sequence ends and discard sequences shorted than 50 base pair (bp). These cleaned reads were *de novo* assembled with Trinity v2014.07.17 (Grabherr et al. 2011) using the default parameters, plus the optional implementation of "Pasafly" to reduce overly abundant isoforms in favor of full length transcripts. To further remove lowly supported transcripts, we utilized two scripts from the Trinity package ("align_and_estimate_abundance.pl" and "filter_fasta_by_rsem_values.pl") to quantify transcripts and remove all contigs supported by fewer than two transcripts per million reads (tmp<2). To

maximize read depth in the differential expression (DE) analysis, a second set of reads was generated with the same data and cleaning steps, except that we relaxed the quality cutoff to retain Q>20. Lastly, we manually removed ribosomal sequences from the assembly, as poly-A-selection during library prep removed the majority of these reads and we assume those that remain are unequal across samples. We screened the resulting transcripts for the core BUSCO gene set from arthropods (Simão et al. 2015).

Annotation of de novo *assembly*

All transcripts were identified by a blastx (Camacho et al. 2009) search against the NCBI non-redundant (*nr*) database (e-values< 1e-10, db version December 2015). To further identify potential genes involved in wasp venom, we matched contigs to two additional sources of information. First, candidate toxin genes from many taxa (6,058 protein sequences) were obtained from the UniProt Animal Toxin Annotation Program database (UATdb, Jungo et al. 2012) and were matched to our transcripts using tblastn (e-values < 1e-10, De Wit et al. 2012). Second, we identified further potential toxin-associated transcripts by comparing the blastx annotations against the *nr* database to known parasitoid venoms recently collected from other parasitoid species, adapted primarily from three sources: Colinet et al. (2013b), Poirié et al. (2014), and Burke and Strand (2014).

Differential gene expression analysis

Read counts were generated by mapping to the *de novo* transcriptome using Bowtie2 (Langmead and Salzberg 2012) with the "sensitive" settings. Unambiguously mapped reads were extracted using the eXpress package (Roberts and Pachter 2013), and these counts were analyzed for differential expression using the DESeq2 (Love et al. 2014) package in R. DESeq2 analyzes

315 transcriptomic data using normalized read counts, and compares logarithmic fold change between
316 treatments. Using empirical Bayes shrinkage, gene-wise dispersion is shrunk towards the function
317 of average expression strength across all genes. Each gene is then fitted with a negative binomial
318 GLM, from which fold change is calculated. *P-values* are generated with the Wald test (Love et al.
319 2014). This approach is advantageous for data such as this, where sample sizes are relatively
320 limited and variation among biological replicates can be high. Prior to both analyses, we
321 visualized the data for batch effects (e.g. grouping by lanes in the Illumina flow cell) or outliers
322 using a principal component analysis (PCA) of rlog transformed data, performed in DESeq2 (Love
323 et al. 2014).

324 We analyzed differential expression with three separate models and subsets of the data
325 (Analyses 1, 2, and 3. Figure 2). This was necessary because not all wasp lineages are able to
326 reproduce in all aphid sublines, so that the data does not encompass all possible evolution lineage-
327 aphid host combinations. Analysis 1 compared wasps from all three treatments in a common
328 environment. Here, we fit a GLM to counts from H-, H76 and H402 reared only on unprotected
329 (H-) aphids for one generation, taking replicate line within treatment into account. Within this, we
330 made the three possible pairwise contrasts among lineages.

331 Analysis 2 compared the two lineages reared in the presence of *H. defensa*, and included
332 individuals reared both on their respective aphid sublines (H402 or H76) and on unprotected (H-)
333 aphids. For this subset of the data, several comparisons were made. First, a GLM was fit that
334 tested for the effects of evolution treatment (H402 vs. H76), aphid environment (H- vs *H. defensa*-
335 infected) and their interaction, taking replicate line within treatment into account. Within this, we
336 tested for genes whose expression might be specific to the evolution treatment × aphid
337 environment interaction using a Likelihood ratio test to compare the original GLM to a second

model lacking the interaction term. In Analysis 2, we have the most power to compare expression between the two lineages reared in the presence of *H. defensa*.

Lastly, Analysis 3 further examined the Analysis 2 data in two subsets to individually examine wasp cohorts from treatment H76 or H402, and compared expression in the presence or absence of *H. defensa* (Figure 2). This comparison specifically tests for the influence of the aphid host environment. In all analyses, differentially expressed (DE) genes were deemed significant after correction for multiple testing using the procedure of Benjamini and Hochberg (1995) with a false discovery rate (FDR) of 0.05. Within significantly DE genes, we tested for an over-representation of putative toxins using a Fisher's Exact Test, implemented in R.

Results

APSE-Toxin characterization in H. defensa

The APSE cassette associated with *H. defensa* strain H76 encodes a 1587 amino acid YD-repeat protein (NCBI GenBank: KU175898) that is ca. 90% similar to the YDp encoded by APSE3 in *A. pisum* (NCBI GenBank: ACJ10121.1), but 96 AA shorter due to an early stop codon. *H. defensa* strain H402 is infected with an APSE cassette encoding a 293 amino acid homolog of cytolethal distending toxin (CdtB, NCBI GenBank: KU175897) 96% similar to that of APSE6 in *Chaitophorus* aphids (NCBI GenBank: ACJ10134.1).

Evolution of infectivity

The initial *L. fabarum* population struggled to parasitize aphids possessing *H. defensa*: mean infectivity was 56% on unprotected aphids and no parasitism was observed on the two protected aphid sublines H402 and H76 (Figure 1b). It is important to note that observing no parasitism in

these assays, which contained a limited number of wasps and aphids, does not mean that the parasitoids were completely unable to infect *H. defensa*-protected aphids. In our rearing cages, which contained thousands of available aphid hosts, we did observe parasitoid reproduction in the H76 and H402 treatments. However, their parasitism success was very low initially, hence the need to supplement from the unselected base population for 1-4 generations (21-40 individuals), after which all evolution lines were self-sustaining (Table S2).

At generation 10, a response was clearly visible (Figure 1b). While all three lineages retained similar abilities to parasitize the unprotected hosts (40-50%), wasps from the H- treatment still had very low or no parasitism success on aphids harboring *H. defensa* strains H402 (3.5%) and H76 (0%). In contrast, wasps that had evolved on *H. defensa*-protected aphids were able to partially overcome the resistance conferred by their host's specific *H. defensa* strain, although parasitism remained lower than on unprotected aphids (20% and 7.5% for the H402 and H76 treatment on their respective aphid hosts). Despite the increased infectivity on the *H. defensa*-protected aphids they evolved with, parasitoids did not show increased infectivity on aphids harboring the other strain of *H. defensa* (Fig. 1b). These results were reflected in significant effects of aphid subline, evolution treatment and a significant aphid subline × evolution treatment interaction on the proportion of aphids parasitized at generation 10 (Table 1). This interaction was mainly caused by the specific adaptation of parasitoids to the symbiont strains they evolved with, as the interaction was highly significant when the analysis was restricted to the H402 and H76 treatments tested on both protected sublines (Table 1).

At generation 21 the results of the infectivity assay were very similar to those at generation 10 (Figure 1b), although the overall percentages of mummification were somewhat lower, including on unprotected aphids (28.9% - 40.6%). There was again a significant interaction between aphid subline and evolution treatment (Table 1), reflecting that adaptation to the presence

386 of *H. defensa* was highly specific, which was further supported by the very strong interaction in
387 the restricted analysis (Table 1). In all cases, we did not detect significant differences among
388 replicate lines within treatment (Random effects, Table 1).
389
390 *Correlated phenotypic responses*
391 Parasitoid development time, wet weight, egg load, and egg size were measured after 16
392 generations of experimental evolution (mean values in Table S4). When parasitoids from all
393 treatments were reared in the same environment, that is *H. defensa*-free aphids, there was no
394 significant variation in any of these traits (Table S5, Figure S1). Hence there was no evidence for
395 evolved responses in these life-history traits. However, when wasp lines were reared in the aphids
396 they evolved on, we observed reduced performance (longer development time and lower egg load)
397 in wasps developing in the *H. defensa*-protected H402 and H76 lines (Figure S1, Table S6),
398 indicating that host aphid environment can affect these traits.
399
400 *Parasitoid behavior*
401 The host stage preference experiment provided no evidence for evolved behavioral differences
402 among evolution treatments. The mean number of eggs laid per aphid (Figure 3) was not
403 significantly different among wasp evolution lineages, (LMM, $F_{2, 46} = 1.111$, $P = 0.338$), but there
404 was significant variation among host stages, with the highest numbers of eggs deposited on
405 average in first instar nymphs and the lowest numbers in adults ($F_{4, 184} = 8.377$, $P < 0.001$), albeit
406 no indication that the bias towards younger host stages differed among evolution treatments
407 (treatment × host stage interaction, $F_{8, 184} = 1.305$, $P = 0.243$). When 1st instar nymphs were
408 excluded and the remaining host life stages analyzed with a Poisson GLMM, the variation among
409 host stages, which was mainly driven by higher numbers of eggs in 1st instars (Fig. 3), was no

longer significant (LR $\chi^2 = 4.771$, df = 3, $P = 0.189$). The other results remained similar, i.e. no significant differences among evolution treatments (LR $\chi^2 = 4.155$, df = 2, $P = 0.125$) and no significant treatment × host stage interaction (LR $\chi^2 = 7.851$, df = 6, $P = 0.249$), also suggesting no evolved differences in host stage preference.

In the host discrimination experiment (Figure 4), wasps from all treatments deposited slightly more eggs in aphid nymphs without *H. defensa*, but this effect was not significant (LR $\chi^2 = 0.595$, df = 1, $P = 0.440$). The mean number of eggs deposited per aphid differed significantly among evolution treatments (LR $\chi^2 = 6.422$, df = 2, $P = 0.040$), with wasps from the H- treatment laying more eggs than wasps from the treatments with *H. defensa*-infected aphids (Fig. 4), but there was no significant evolution treatment × host type interaction (LR $\chi^2 = 0.396$, df = 2, $P = 0.820$), i.e. no evidence for evolved differences in host discrimination.

Transcriptome assembly and annotation

In total, we obtained >830 million 100 bp reads across 59 samples (1 library failed from treatment H402, replicate line 4). After filtering for low quality and contaminated sequences, >410 million sequences were used for the *de novo* transcriptome assembly. The resulting 79,553 contigs were reduced to 69,230 contigs after removal of lowly supported contigs and contaminating ribosomal 18S/28S sequences. Blast annotation to the *nr* database assigned putative identities to 30,347 of the contigs, and the BUSCO analysis found 83% of the 2,675 core arthropod genes. This indicates that we have constructed much of the transcriptome for this tissue, sex, and age of wasp (Simão et al. 2015).

Further annotation to match to putative venom toxins identified 1,650 transcripts (Table 2). Blast matches to the UATdb identified 503 transcripts matching to venom and toxins spanning

433 from Hymenoptera to spiders and snakes, over 300 of which were not identified in the matches to
434 proteins identified from the parasitoid literature (Colinet et al. 2013b; Burke and Strand 2014;
435 Poirié et al. 2014). Matches to these previously identified parasitoid candidates yielded 1,399
436 proteins, 1,147 of which did not overlap with the UATdb identifications. It should be noted that
437 these matches are from our whole-body transcriptome and include some genes associated with
438 many functions (e.g. chitinase), so further examination of these should use tissue-specific
439 expression. These annotations were broadly classified in Table 2, and full annotation information
440 is in Supplemental Data 1.
441
442 *Differential gene expression patterns among lineages*
443 For differential expression analysis, the relaxed quality filter (Q>20) yielded >667 million reads,
444 612 million of which were successfully mapped to the transcriptome (1.5- 25 million reads per
445 library, mean 10 million). As a visual inspection of the data, we used PCA to check for batch
446 effects or strong effects of replicate line within treatment. This identified one library as a large
447 outlier (from treatment H402, replicate line 2); this sample was removed as a potentially "bad"
448 library (Gierliński et al. 2015) from all analyses (PCA after sample removal, Figure S2)
449 Analysis 1 compared 34 individual libraries across the three treatments reared for one
450 generation on unprotected aphids using three pairwise contrasts of expression profiles (H76/H-,
451 H402/H- and H76/H402, Table 3, Figure 5). Gene expression in either H76 or H402, relative to H-
452 revealed that most genes showing significant differential expression (DE) did so only in wasps
453 from one treatment (251 uniquely DE genes in H76 and 599 in H402, Figure 5). Nonetheless, we
454 identified 99 genes that were DE in both comparisons, and an additional four candidates that were
455 DE in both, yet in opposite directions; all four were downregulated in H402 and upregulated in
456 H76, relative to H-. With the exception of these last four, all categories contained genes encoding

457 putative venom components (up to 22 candidates), and these were significantly enriched in both
458 H76 and H-, relative to H402 (Table 3).

459 Analysis 2 compared gene expression among 46 libraries from lineages H76 and H402,
460 reared both in the presence and absence of *H. defensa* (Figure 2). The likelihood ratio test
461 indicated that our full model best fit the data; five transcripts were significantly affected by the
462 addition of the evolution treatment × aphid environment interaction (FDR<0.05). These five
463 transcripts all appear to be more highly expressed in H402 wasps when reared on unprotected
464 aphids, and more lowly expressed in H76 wasps reared in the same conditions (Supplemental Data
465 3), unfortunately none were identifiable by blast. The results of this full model allowed the most
466 complete comparison of lineages H76 and H402, and yielded more DE genes than any other
467 comparison (1,949: Table 3) as well as the highest fold changes between treatments (Log_2FC=
468 5.65). Within this, we identified up to 60 annotated venom components that were more highly
469 expressed in the H76 lineage, but only 15 that were more highly expressed in the H402 lineage.
470 The larger number of significant genes may be in part due to the greater number of replicates we
471 have in this analysis, but the largest observed fold change among DE genes is also more than
472 twice that found in Analysis 1.

473 To further explore the effect of aphid host environment, Analysis 3 separately compared
474 expression in the presence and absence of *H. defensa* in wasps from the H402 or H76 evolution
475 treatments. In the H402 lineages, 21 DE genes were detected, with much smaller fold changes
476 among treatments (<1.0) and a single putative toxin in each group. For the same comparison in
477 H76, no DE genes were detected (Table 3, Supplemental Data 4).

478

479 *Viral gene expression*

We observe several DE transcripts that are likely viral in origin. The most striking of these comes from an RNA virus (gene cluster c32773), contains five transcripts (2,557-6,058bp), and matches well to the complete polyprotein of the *Dinocampus coccinellae* paralysis virus (DcPV) identified from a parasitoid targeting ladybugs (50-70% nucleotide identity). Four transcripts matching this are highly DE in Analysis 2 (FDR 2.58e-14 -5.47e-6, $\log_2$(fc) 3.2 -4.9, all annotated in Supplemental Data 3). Three of these are also highly DE in Analysis 1 comparisons involving H402 (Supplemental Data 2). In general, expression of all of these genes is highest in both the H- and H76 treatment cages relative to treatment H402, mirroring venom gene enrichment.

We also find transcripts with homology to polydnaviruses. These DNA viruses are known from other parasitoids, where they are integrated in the wasp's genome and expressed as part of the offense machinery, suppressing the host's immune defenses upon injection alongside the parasitoid egg (Burke and Strand 2012; Strand and Burke 2012; Burke et al. 2014). We find individual transcripts matching to putative Bracoviruses isolated from either *Chelonus inanitus* or *Cotesia congregate* (Supplemental Data 1). Two of these were significantly DE in our data: transcripts matching to a hypothetical cystein protease (Calpain-B) and a hypothetical transmembrane channel-like protein (7 TMC7). Additionally, we find approximately 100 additional transcripts that are of putative viruses of any type, though not all highly expressed. Some likely include viral infections, e.g. transcripts closely matching the Wuhan Louse Fly Virus 2 (Li et al. 2015) and Ngewontan virus (Vasilakis et al. 2013). One putative infection is DE, matching to the *Acheta domesticus* mini ambidensovirus, which, in Analysis 1 was more highly expressed in H76 relative to H-, and had between 4,900 and 14,900 normalized read counts.

Discussion

503 Using experimental evolution we have demonstrated rapid and specific adaptation of the parasitoid
504 wasp *L. fabarum* to symbiont-protected hosts. Most likely this experiment has selected for pre-
505 existing genetic variation in our wild-caught founding populations given that: (1) different
506 infective phenotypes arose rapidly (< 10 generations), (2) these results are very close to previous
507 experimental evolution in this system that started with different founding populations (Rouchet
508 and Vorburger 2014), and (3) there was little difference in infectivity among the replicate lines
509 reared within each treatment. These results show that a high prevalence (here 100%) of a
510 particular strain of defensive symbiont favors specific genotypes of the parasitoid, and thus
511 supports an important assumption of frequency-dependent selection mediated by symbionts
512 (Kwiatkowski et al. 2012). Within communities, the feedback between parasitoid populations with
513 varying infective abilities and available symbiont-protected aphids could lead to rapid turnover in
514 their respective genetic composition, and thus lead to changes over time.
515 The primary evidence for specific adaptation by parasitoids to symbiont-conferred
516 resistance lies in the significant evolution treatment × aphid subline interactions at both
517 generations 10 and 21. This shows that parasitoids reared on aphids with a particular strain of *H.*
518 *defensa* were able to partially overcome the protection conferred by this strain, while parasitoids
519 reared in the other two treatments remained very unsuccessful. These differentially infective
520 phenotypes also align nicely with our finding that the two *H. defensa* strains used each carry a
521 distinct APSE infective cassettes, which house different primary toxins. The H76-associated
522 APSE encodes a putative YDp toxin most similar to that found in APSE3 of *H. defensa* infecting
523 pea aphids. In pea aphids, this APSE3-*H. defensa* combination confers high levels of protection
524 against the wasp *Aphidius ervi*, and loss of APSE3 results in complete loss of the protective
525 phenotype (Oliver et al. 2009). The H402-associated APSE encodes a CdtB toxin most similar to
526 one found in APSE6 in *Chaitophorus* aphids. The protective phenotype in *Chaitophorus* is not

527 known, but we do know that APSE2 *H. defensa* carrying a related CdtB in pea aphids confers
528 partial protection from the parasitoid *Aphidius ervi* (Oliver et al. 2005). The amount of symbiont-
529 provided protection can also depend on the species of parasitoid (McLean and Godfray 2015), and
530 in pea aphids, *H. defensa* strains with either CdtB and YDp putative toxins provide no protection
531 against another braconid parasitoid, *Praon pequodorum* (Martinez et al. 2016). The two *H.*
532 *defensa* strains presented to wasps here possess distinct APSE haplotypes and virulence cassettes,
533 and thus likely represent different challenges to the parasitoid, which require specific counter-
534 adaptations. Because we have utilized a single aphid clone in our experiments, the specific
535 infective phenotypes evolved by wasps reared in the presence of *H. defensa* reflect adaptation to
536 the symbionts, and it is possible that they at least partially result from adaptation to the APSE-type
537 and toxins they produce, although the parasitoids could have adapted to multiple symbiont-
538 encoded traits.

539 Specific parasitoid adaptation to the two defensive symbiont strains was also reflected in
540 patterns of gene expression. When reared in a common (*H. defensa*-free) aphid host (Analysis 1),
541 most instances of DE among comparisons were associated with a single treatment (i.e. up or down
542 regulation in H76 or H402 and not both; Fig. 5), suggesting considerable variability in standing
543 gene expression. This variability was much more pronounced than expression differences of
544 individual lines (e.g. H76) in the immediate presence or absence of *H. defensa* (Analysis 3). This
545 consistency of gene expression contrasts with observations from host-specialized species of
546 *Rhagoletis* flies, where transcripts showing evolved differences among species were much more
547 plastic in response to the fruits that they exploit (Ragland et al. 2015). It is also possible, however,
548 that the single generation of rearing on H- aphids could not sufficiently erase epigenetic signatures
549 of developing in a *H. defensa*-possessing host, and such transgenerational priming is known from
550 a number of insects (Vilcinskas 2016). While epigenetics could contribute to this system, we do

not believe these are entirely driving these patterns; genotype-by-genotype interactions have bene previously observed between *L. fabarum* and *H. defensa* (Rouchet and Vorburger 2012; Cayetano and Vorburger 2013), and under experimental conditions aphid hosts have been shown to increase particular parasitoid genotypes (Käch et al.). Further investigation of this should look at infectivity after multiple generations of rearing in symbiont-free hosts as well as genetic variation between these lineages.

Interestingly, putative toxin genes were overrepresented among the differentially expressed genes in each wasp lineage. The *de novo* transcriptome we assembled contains many genes tentatively identified to produce toxins that may be components of wasp venom (Table 2). Further characterization of these putative venom proteins is warranted through tissue-specific analysis, as has been done in *Microplitis demolitor* (Burke and Strand 2014), *Aphidius ervi* (Colinet et al. 2014), and many others (Colinet et al. 2013b; Poirié et al. 2014). Yet it is reasonable to hypothesize that some of the DE transcripts identified as putative toxins likely impact the ability of *L. fabarum* to exploit aphid hosts protected by *H. defensa*. Many of these were highly expressed, including the Metalloproteinases, which have been identified across a number of parasitoid species and bear a similarity to highly toxic proteins found in diverse animal groups (de Graaf et al. 2010; Colinet et al. 2013a; Poirié et al. 2014). Metalloproteinases may impact host development (Price et al. 2009) or modulate host immunity (Asgari and Rivers 2011; Moreau and Asgari 2015), and different forms of these proteins may have activity at different stages (e.g. venom vs. teratocyte) of the life cycle in the parasitoid *M. demolitor* (Burke and Strand 2014). Also interesting are acetylcholinesterases /carboxylesterases, which belong to a group of serine esterases characterized in both insects and vertebrates and known to perform diverse functions, including resistance to insecticides (Newcomb et al. 1997). In the parasitic wasp *Dinocamus coccinella*, carboxylesterases are prominent proteins found in the teratocytes, indicating they may

575 be important for parasitoid development (Gopalapillai et al. 2005). This finding is particularly
576 interesting in the present case because detoxification of symbiont-produced defensive compounds
577 may by one possible route by which parasitoids could adapt to hosts possessing *H. defensa*.
578 In addition to the putative toxins described above, we find evidence of DE in transcripts
579 that appear to be viral in origin. The most compelling of these is a set of transcripts that bear
580 strong similarity to the entire polyprotein of the *Dinocampus coccinellae* paralysis virus (DcPV).
581 This RNA virus has been implicated as a key part of infectivity in the parasitoid *Dinocampus*
582 *coccinellae*, where it appears to modify behavior of its ladybug host through replication in host
583 cerebral ganglia (Dheilly et al. 2015). The similarity of these two viruses certainly merits further
584 investigation of viral presence, expression, and of aphid behavior. We also find expressed genes
585 bearing homology to Bracoviruses, which are not previously reported from the braconid subfamily
586 Aphidiinae (Herniou et al. 2013). Polydnavirus genomes consist of two functional components, a
587 suite of nudivirus-like genes involved in viral replication, and proviral segments, often consisting
588 of insect-associated genes, some of which are virulence factors expressed in hosts that contribute
589 to wasp development (Burke and Strand 2012; Strand and Burke 2012; Burke et al. 2014). Hence,
590 genes with homology to bracoviruses are not necessarily indicative of BV presence and further
591 work should examine the *L. fabarum* genome for these functional components. We should also
592 consider that any viruses with varying prevalence between lineages may be infection, including
593 those that may be asymptomatic (Reineke and Asgari 2005; Oliveira et al. 2010).
594 The life-history traits we compared among evolution lineages largely reflect that *H.*
595 *defensa* creates a challenging environment for wasp growth. In the presence of *H. defensa*,
596 development time was generally longer, wasps tended to be smaller (wet weight), and egg load
597 was significantly reduced. However, with just one generation of rearing on aphids lacking *H.*
598 *defensa*, these traits recovered to equal those of the control lines (Figure S1). Overall, this suggests

599 that the reduced performance in some life-history traits of wasps evolving with *H. defensa*-
600 protected hosts is largely a plastic response to larval development in an unfavorable environment,
601 rather than a correlated evolutionary response to adaptation to *H. defensa*. This is in contrast with
602 the apparently fixed expression profiles we found, and suggest further that gene expression at
603 different developmental stages could reveal different patterns.

604 There were also no clear behavioral differences among female wasps from the three
605 evolution treatments. Ovipositing in younger aphids has been shown to increase parasitoid success
606 particularly in symbiont-protected aphids (Schmid et al. 2012), so a reasonable strategy would be
607 for parasitoids to target younger aphids when they harbor *H. defensa*. However, we see no
608 evidence for that here. Females from all treatments laid the most eggs in first instar nymphs and
609 this bias towards young hosts was not stronger in females from the H76 and H402 treatments. This
610 indicates that parasitoids were adapted to the presence of *H. defensa* in their hosts not by a
611 behavioral change towards attacking more susceptible host stages, but presumably by
612 physiological adaptations improving the survival of the wasps' eggs or larvae. We also did not
613 observe any evidence suggesting that *L. fabarum* would either preferentially attack or avoid aphids
614 possessing *H. defensa*, nor any evolved differences in host discrimination among treatments.
615 Previous work on the parasitoid *A. ervi* suggested that parasitoids can use "superparasitism" to
616 overcome defenses conferred by *H. defensa*, i.e. by injecting two or more eggs instead of one
617 (Oliver et al. 2012). In opposition to this, tests using the grain aphid, *Sitobian avenae*, found that
618 both *A. ervi* and *Ephedrus plagiator* actively avoided laying eggs in individuals protected by *H.*
619 *defensa* (Łukasik et al. 2013a). Our finding of no detectable discrimination does not clarify this
620 issue but rather suggests that parasitoid behavior is diverse, with attack strategies varying among
621 species. For *L. fabarum* used here, the provisional conclusion is that female wasps can either not
622 detect *H. defensa* in their hosts or have not evolved any behavioral response to its presence. The

623 latter would be explicable if parasitoids are host-limited rather than egg-limited, such that it would
624 not be beneficial to economize on eggs even when hosts are highly resistant, but we do not know
625 whether this is the case in *L. fabarum*.

626 Overall, our results suggest that wild parasitoid populations contain the genetic variation
627 required for adaptation to different strains of defensive symbionts in their hosts, and that this
628 variation may be maintained by coevolutionary processes that act on relatively short time scales.
629 Although parasitoid adaptation was ultimately fast, it did not take off easily. All eight populations
630 reared on protected aphids required supplementation of individuals from the unselected stock
631 population in the very first generations, without which they might have gone extinct. This implies
632 that we did not initially capture enough successful genotypes in our starting populations and that
633 the responsible alleles are relatively rare. Small and/or genetically depauperate populations would
634 thus have difficulty establishing themselves in a well-defended host population in the wild. Once
635 established, however, parasitoids readily maintained themselves in the *H. defensa*-protected host
636 populations.

637 Work on field populations of the pea aphid (*Acyrthosiphon pisum*) has shown that
638 endosymbiont frequencies fluctuate on the scale of months, and that this is associated with a rise
639 and fall in parasitoid-induced mortality (Smith et al. 2015). Our results suggest that this is likely to
640 be accompanied by changes in parasitoid infective abilities, and associated gene expression
641 profiles, on a similarly short time scale. There will also be variation above the population level in
642 natural environments. Work in other parasitoid systems has demonstrated landscape level variation
643 exists on small spatial scales, and that parasitoids are more likely to go locally extinct than their
644 hosts (Cronin and Reeve 2014). This may also apply to aphid parasitoids, as their hosts occur in
645 patches of suitable host plants across urban, agricultural, and natural landscapes (Nyabuga et al.
646 2010), and these are unlikely to be synchronized across the landscape. Thus there will be

landscape-level variation in the genetic composition of hosts and their symbionts, creating a selection mosaic that will in turn affect the genetic composition of parasitoid populations.

In summary, following experimental evolution of a parasitoid wasp we have found transcriptomic changes associated with increased infective abilities among lineages, yet few behavioral or phenotypic differences. These likely represent some of the diversity found in wild populations, which also exhibit variation in the ability to parasitize symbiont-protected hosts (e.g. Vorburger and Rouchet 2016). The overrepresentation of putative venom components in our DE genes suggests that they may play a role in *L. fabarum* counter-adaptation. In the wild, an increase in host-possessed protective endosymbionts may reduce parasitism, but will also select for particular genotypes of parasitoids, which in turn may increase and select for alternative defensive symbionts (Kwiatkowski et al. 2012). Often acting on short temporal scales, these eco-evolutionary dynamics (Fussmann et al. 2007; Turcotte et al. 2011) can directly impact the prevalent parasitoid genotypes in a given population and maintain a genetically diverse parasitoid population over time and space.

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Table 1: Results of linear mixed effects models of the proportion of aphids mummified by parasitoids after 0, 10 and 21 generations of experimental evolution.

Effect	Full analysis			Wasps evolved and tested on protected aphids		
	ndf, ddf (for Fixed Effects)	*F* (for Fixed Effects)/ LR χ^2_1 (for Random Effects)	*P*	ndf, ddf	*F*/ LR χ^2_1	*P*
Generation 0 (initial stock population)						
Aphid subline	2, 27	24.302	<0.001			
Block	Random effect	0	1.00			
Generation 10						
Aphid subline	2, 171	69.914	<0.001	1, 76	4.020	0.049
Evolution Treatment	2, 171	3.133	0.046	1, 76	4.020	0.049
Aphid subline x Evolution treatment	4, 171	3.389	0.011	1, 76	28.882	<0.001
Evolution line (treatment)	Random effects	0	1.000		0	1.000
Evolution line x aphid subline		0	1.000		0	1.000
Block		0	1.000		0	1.000
Generation 21						
Aphid subline	2, 148.7	53.097	<0.001	1, 10.55	5.108	0.046
Evolution Treatment	2, 7.6	1.959	0.206	1, 10.53	5.356	0.042
Aphid subline x Evolution treatment	4, 148.7	3.492	0.009	1, 10,55	22.755	<0.001
Evolution line (treatment)	Random effects	0.031	0.90		0	1.000
Evolution line x aphid subline		0	1.00		0.098	0.800
Block		0	1.00		0.828	0.754

ndf: numerator degrees freedom, ddf: denominator degrees of freedom. Proportion mummified was arcsine square-root transformed before analysis. *P* values of random effects are based on likelihood ratio tests, *P* values of fixed effects on type III *F* tests with Satterthwaite's approximation carried out by the lmerTest library in R (Kuznetsova et al. 2013).

Table 2: Putative toxins matching to *L. fabarum* transcripts identified by from the UniProt Animal Toxin Annotation database sequences (e-value > 1e-10, UATdb) and matches in the *nr* blast annotation to candidates sourced from the literature. Transcripts that were significantly differentially expressed (DE) among evolution lineages are highlighted in grey.

Putative toxin / venom components of *L. fabarum*	**Species**	***n transcripts***	***n* DE**
Acid phosphatase, including Venom acid phosphatase [6, 9, 3, 18]	Lb, Lh, Nv, Pt	26	3
Actin [4, 21]	Ae, Pp	80	1
Alkaline phosphatase [21]	Pp	12	
Alpha and delta -latrocrustotoxin /Lt1a [17]	O	74	2
Alpha-N-acetyl glucosaminidase [19, 3]	Ci, Lb	3	
Angiotensin-converting enzyme [19]	Ci	14	1
Antigen 5-like protein [6]	Nv	3	1
Arginine kinase [21]	Pp	2	
Arylsulfatase B [6]	Nv	6	
Aspartylglucosaminidase [11, 9, 3]	At, Lh	1	
ATP synthase [20]	Pp	46	3
Beta-1,3-glucan recognition/binding protein [6]	Nv	2	1
Beta-fibrinogenase [17]	O	2	2
C1q-like venom protein [12]	Nv, G1	1	
Calglandulin[17]	O	16	1
Calreticulin [1, 7, 9,3, 5, 6, 21]	Cr, Hd, Lb, Lh, Ma, Mh, Nv, Pp	3	
Carboxylesterase-6 / Acetylcholinesterase / Esterase/ Lipase / Lysosomal acid [19, 7, 9, 3, 2, 6, 13, 4, 17]	Ap, Ci, Hd, Lb, Lh, Md, Nv, Ph, O	313	19
Chemosensory protein-like protein [19]	Ci	5	
Chitin binding protein (we find chitin binding peritrophin) [19, 6]	Ci, Nv	2	
Chitinase [10, 19, 9, 3, 6]	Cc, Ci, Lb, Nv	29	
Cystein-rich (venom) protein [7, 9, 3, 5, 2, 6, 17]	Hd, Lb, Lh, Ma, Md, Mh, Nv, O	19	2
Dipeptidyl peptidase [17, 6, 7]	Nv, Hd, Ap, Vv, O	30	3
Elongation factors [4, 9, 3]	Ae, Lh	65	3
Endonucleases [6]	Nv	49	
Endoplasmin [4]	Ae	4	
Fibronectin domain-containing protein [9, 3]	Lb	10	
Gamma-glutamyl transpeptidase [8, 6]	Ae, Nv	15	2
Glucose dehydrogenase [6]	Nv	33	3
Glutaminyl-peptide cyclotransferase [17]	O	4	
Heat shock protein [20]	Pp	54	12
Hyaluronidase [17]	Ap, Vv, O	3	
Icarapin [17]	Ap	1	
Imaginal disc Growth Factors-like [19, 2]	Ci, Md	2	
Immunoglobulin-like proteins [6]	Nv	28	1
Inosine-uridine preferring nucleoside hydrolase [6, 20, 9, 3]	Nv, Pp, Lb, Lh	2	
Laccase / Copper-containing oxidases [13, 15]	Ph	24	5
Lectin / esp. C-type lectin [19, 7, 6, 17]	Ci, Hd, Nv, O	13	
Leucine-rich repeat domain containing protein [4]	Ae	152	4
Low-density lipoprotein receptor [6]	Nv	60	5
Metalloproteinase / Neprilysin / Neprilysin-1 [4, 19, 16, 7, 9, 3, 2, 5, 13, 17]	Ae, Ci, Ep, Hd, Lb, Md, Mh, Ph, O	187	12
Multiple inositol polyphosphate phosphatase-like protein [6]	Nv	11	
Odorant binding proteins [19, 6]	Ci, Nv	25	4
Peroxiredoxin [17]	O	8	2
Phenoloxidase [13]	Ph	5	
Phospholipase [18, 17]	Pt, Ap, Vv, O	39	2

Plancitoxin-1 [2, 17]	Md, O	4	
Plasminogen [17]	O	3	
Polynucleotide kinase/phosphatase [2]	Md	4	1
Protease inhibitor, esp. Kazal-type serine protease inhibitor, serpin [7, 9, 3, 2, 6, 17]	Hd, Lb, Lh, Md, Nv, O	42	4
Reticulocalbin-2 [17]	O	1	1
Ryncolin [17]	O	2	
Sarco/endoplasmic reticulum calcium ATPase [12]	G1	6	
Serine proteases and homologs / Thrombin-like [4, 19, 1, 7, 9, 3, 2, 6, 14, 21, 17]	Ae, Ci, Cr, Hd, Lh, Md, Nv, Ph, Pp, Ap, Bi, O	106	9
Spermine oxidase [9, 3]	Lh	10	
Superoxide dismutase [9, 3]	Lb	7	
Sushi/SCR/CCP [9, 3]	Lb, Lh	9	
Trehalase [15, 17]	Ph	7	
Tropomyosin 4 [22]	Ae, Pp	19	4
Tumor protein * [17]	O	2	
Tyrosine 3- /tryptophan 5- monooxygenase/hydroxylase [16, 9, 3]	Ep, Lh, Lb	11	1
U21-ctenitoxin-Pn1a [17]	O	2	
Venom /factor /allergen/ protein* [17]	O	34	6
Venom phosphodiesterase [17]	O	2	
Venom prothrombin / Coagulation factor [17]	O	21	1

Species codes: Ae, *Aphidius ervi*. At, *Asobara tabida*. Ap, *Apis mellifera*. Bi, *Bombus iitus*. Cc, *Chelonus sp*. near *curvimaculatus*. Ci, *Chelonus inanitus*. Cr, *Cotesia rubecula*. Ep, *Eulophus pennicornis*. G1, *Ganaspis sp*. 1. Hd, *Hyposoter didymator*. Lb, *Leptopilina boulardi*. Lh, *Leptopilina heterotoma*. Ma, *Microctonus aethiopoides*. Md, *Microplitis demolitor*. Mh, *Microctonus hyperodae*. Nv, *Nasonia vitripennis*. Ph, *Pimpla hypochondriaca*. Pt, *Pipla turionellae*. Pp, *Pteromalus puparum*. Vv, *Vespula vulgaris*. O, Other (non-hymenoptera).

n is the matching number of transcripts (i.e. individual genes or multiple splice variants, includes putative blast matches). n sig indicates the number of these that were DE in one or more analysis (Table 3).

Full list of matches to the UATdb are listed in Supplemental Data 1.

Candidate adapted from literature: (Asgari et al. 2003), 2. (Burke and Strand 2014)[+], 3. (Colinet et al. 2013a) [+], 4. (Colinet et al. 2014) 5. (Crawford et al. 2008), 6. (de Graaf et al. 2010), 7. (Dorémus et al. 2013), 8. (Falabella et al. 2007), 9. (Goecks et al. 2013), 10. (Krishnan et al. 1994), 11. (Moreau et al. 2004), 12. (Mortimer et al. 2013), 13. (Parkinson et al. 2001), 14. (Parkinson et al. 2002), 15. (Parkinson et al. 2003), 16. (Price et al. 2009), 17. (UATdb, Jungo and Bairoch 2005), 18. (Uçkan et al. 2006), 19. (Vincent et al. 2010), 20. (Zhu et al. 2010b), 21.(Zhu et al. 2010a), 22. (Poirié et al. 2014) [+]

[+] Primary source of this adapted table.

*because these are general names, only the blast matches to that UATdb were retained for this category

Table 3: Summary of tests for differential gene expression among parasitoid wasps from three experimental evolution treatments (significant: FDR<0.05). Within each comparison, the number of significantly differentially expressed (DE) genes are listed alongside the lineage in which their expression was increased. Enrichment of putative toxins from two sources, and their combination, in the differentially expressed transcripts was tested with Fisher's exact tests.

Comparison	Greater expression in:	*n* sig. DE genes	Largest fold change (Log_2)	Sig. DE UATdb genes	Sig. DE genes annotated from literature	Sig. DE putative toxins, both lists
Analysis 1: H402, H76 and H- wasps, reared only in H- aphid environment						
H402 vs. H-	H402	291	2.35	3	2	4
	H-	411	1.13	12***	19***	22***
H76 vs. H-	H76	157	1.23	1	3	3
	H-	197	1.09	3	5	5
H402 vs. H76	H402	225	0.75	4˚	4	5
	H76	234	1.25	4˚	10**	11**
Analysis 2: H402 vs H76 lineages, both aphid environments						
H402 vs. H76	H402	978	5.65	3	15	16
	H76	971	5.30	24***	51***	60***
Analysis 3: H+ vs H- aphids, separately within lineage						
H402	H402,H402	11	0.73	0	1	1
	H402,H-	12	0.41	0	1	1
H76	H76,H76	0				
	H76,H-	0				

UATdb**,** Uniprot Animal Toxin database. Table 2, lists a larger set of putative venom toxins. Significant enrichment of toxin genes based on Fishers exact test: ˚p< 0.1, **p< 0.05, ***p< 0.01.

SUPPLEMENTAL TABLES

Table S1: Summary of collection locales where accession of *L. fabarum* were collected (numbers in brackets) to established the mixed starting population for experimental evolution. All locales are in Switzerland. In each case approximately 15- 40+ individuals collected in the field were pooled to establish a sexually reproducing culture in the lab. These were maintained separately until pooling in on 17 August 2013 to establish a mixed, genetically diverse population. After two generations of mixed rearing, this large population was used to found the 12 experimental evolution lines. All wasps were collected as mummies of the ivy aphid, *A.hederae* found on ivy (*Hedera helix*).

Collection region	**Date**	**Plant**	**Aphid host**
Regensberg (1)	17-June-2012	*Hedera helix*	*Aphis hederae*
Lausanne (2)	02-Sept-2012	*Hedera helix*	*Aphis hederae*
Fribourg (2)	12- Sept -2012	*Hedera helix*	*Aphis hederae*
Geneva (1)	16- Sept -2012	*Hedera helix*	*Aphis hederae*
Nyon (2)	19- Sept -2012	*Hedera helix*	*Aphis hederae*
Renens (1)	19- Sept -2012	*Hedera helix*	*Aphis hederae*

Table S2: Summary of wasp rearing at the beginning of experimental evolution. All lines were started by adding 32 female and 20 male wasps to cages with plants and aphids. Thereafter, lines were maintained by transferring 50 offspring to a new cage every generation. Additional wasps were added to cages in initial generations when < 50 offspring emerged. These supplemental individuals came from the unselected original source population and served to reduce genetic bottlenecking and to prevent extinctions. In all cases, no further supplementation was necessary after Generation 4, and 50 individuals were used establish the next generation of each line.

Wasp supplemented to			*Supplementation from original source population*				
Experimental evolution treatment	**Replicate line**	Date:	2, 3 & 4 October 2013	16 & 17 October 2013	30 & 31 October 2013	13&14 November 2013	27 November 2013
		End of:	Generation 1	Generation 2	Generation 3	Generation 4	Generation 5
H402	1		40 (30f +10m)	26 (21f +5m)	40 (unsexed)	30 (unsexed)	0
H402	2		26 (20f +6m)	21 (20f +1m)	40 (unsexed)	30 (unsexed)	0
H402	3		24 (20f +4m)	0	0	0	0
H402	4		35 (30f +5m)	0	0	0	0
H76	1		36 (30f +6m)	0	0	0	0
H76	2		30 (24f +6m)	25 (18f +7m)	0	0	0
H76	3		30 (23f +7m)	0	0	0	0
H76	4		42 (29f +13m)	0	0	0	0
H-	1		0	0	0	0	0
H-	2		0	0	0	0	0
H-	3		0	0	0	0	0
H-	4		0	0	0	0	0

Table S3: Primers used to characterize primary phage toxins associated with *H. defensa* strains H76 and H402. **YDp**; reaction mixtures were amplified with an initial denaturation of 3 min at 94°C, followed by 30 cycles of denaturation at 94°C for 1min, annealing at 50°C for 1 min then amplification at 72°C for 2.20 min followed by final extension at 72°C for 10 min. CdtB**;** reaction mixtures were amplified with an initial denaturation of 3 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s for first cycle and gradually reducing to 55°C, then amplification at 72°C for 2 min followed by final extension at 72°C for 10 min.

YDp amplification strain H76	
PCR primer name sequence 5' to 3'	Additional Internal primer for sequencing
B6F: GGTTGGTGTTTTACGGTGGT B6R: TTGAACGTTAAACTTGCCATAA	
B7F: ATTTCGTCCCATATGCGTTT B7R: GCAAAGAATATGCCCATCGT	
B8F: AGCTGGGACTCGCAGTTTT B10R: CCGGTGACGCTAATGGAA	B8F_76: AGTACGGCTCTACCGAGCAA B8R: TTCGGACCACGTCTCTTTTT B10F: GTCTGAAACCTCTGCCCAAG
B11f: GTCGGGTATTAACCCAAGCA B11R: GGGAAGCCAATAGACCGTTT	
B12F: AGTTTTGGGCACGAAATCAC B12R: ACTGCCGTAAGGTGACCAGA	
CdtB amplification in strain H402	
P5_F: CGATAGCGGCAGGTTTTAAG Holin-R: TTTTTCACCCGAAAACGGTA	CDT: ATACGCAAGGCGCTAGATG

Table S4: Summary of life history measures (mean values ± 1 standard deviation), made after 16 generations of experimental evolution. All data is plotted in Figure S1.

Evolution Line	Aphid Host	Replicate Cage	Parasitoid Wet Mass (m & f)	Development Time (days)	Egg Load (n eggs)	Egg Length (μm)
H-	H-	1	0.160±0.03	13.00±0.76	230.9±79.9	109.8±5.6
		2	0.136±0.03	13.14±1.17	167.5±72.4	103.7±8.6
		3	0.167±0.04	12.05±0.22	229.2±87.4	111.8±9.6
		4	0.116±0.03	12.05±0.22	124.5±52.6	107.1±4.0
H402	H-	1	0.226±0.05	12.57±0.53	205.8±46.6	113.9±8.1
		2	0.177±0.07	12.72±0.96	188.6±39.1	113.8±4.4
		3	0.120±0.02	12.63±1.01	173.4±46.4	104.0±5.2
		4	0.169±0.04	12.79±0.62	225.0±107.5	111.8±5.9
H76	H-	1	0.142±0.01	12.60±1.07	176.7±77.2	119.4±4.2
		2	0.180±0.05	12.21±0.63	155.7±112.8	107.1±8.3
		3	0.140±0.05	13.00±0.78	147.0±71.5	111.0±11.2
		4	0.167±0.04	12.73±0.96	235.8±74.1	116.4±4.8
H402	H402	1	0.108±0.03	13.00±1.07	164.9±57.6	109.9±4.8
		2	0.138±0.02	13.29±1.53	157.0±83.1	110.8±4.1
		3	0.131±0.05	13.89±1.13	176.3±94.2	114.6±13.2
		4	0.128±0.02	13.60±0.89	190.0±70.7	109.2
H76	H76	1	0.105±0.02	14.14±0.90	107.0	112.3
		2	0.108±0.03	13.08±1.44	130.3±37.3	99.5±10.6
		3	0.162±0.03	12.92±1.11	106.0±45.9	117.5±7.5
		4	0.115±0.02	13.33±0.52	141.1±59.9	105.4±5.5

Table S5. Results of linear mixed models for parasitoid life history traits after 16 generations of experimental evolution, as compared in Analysis 1 (All evolution lineages reared on unprotected aphids, Figure 2).

Source, Analysis 1	Ndf for Fixed Effects	Ddf for Fixed Effects	F for Fixed Effects/ LR X^2_1 for Random Effects	*P*
Wet Weight				
Evolution Treatment	2	24.19	0.292	0.750
Sex	1	155.4	0.535	0.466
Evolution Treatment x Sex	2	155.1	2.823	0.063
Cage	*Random effects*		0	1.000
Replicate			35.10	<0.001
Development Time				
Evolution Treatment	2	23.69	0.989	0.906
Sex	1	166.67	1.063	0.304
Evolution Treatment x Sex	2	166.46	0.140	0.869
Cage	*Random effects*		0	1.000
Replicate			44.30	<0.001
Egg Load				
Evolution Treatment	2	8.25	0.192	0.828
Cage	*Random effects*		0.137	0.700
Replicate			0	1.000
Egg Size				
Evolution Treatment	2	10.16	0.952	0.418
Cage	*Random effects*		0.749	0.390
Replicate			3.549	0.060

Table S6. Results of linear mixed models for parasitoid life history traits in all three evolution treatments after 16 generations of experimental evolution, compared only on the host aphid upon which they have been reared (i.e. H- treatment reared on unprotected aphids, H76 treatment reared only on H76 possessing aphids, H402 treatment reared only on H402 possessing aphids).

Source, Analysis on rearing aphids only	Ndf for Fixed Effects	Ddf for Fixed Effects	F for Fixed Effects/LR X^2_1 for Random Effects	*P*
Wet Weight				
Evolution treatment	2	10.7	1.810	0.211
Sex	1	105.6	0.981	0.324
Evolution treatment x Sex	2	105.6	1.226	0.298
Cage	*Random effects*		2.615	0.100
Replicate			0.809	0.400
Development Time				
Evolution treatment	2	31.3	3.428	0.045
Sex	1	121.7	1.230	0.270
Evolution treatment x Sex	2	121.4	1.487	0.230
Cage	*Random effects*		<0.001	1.000
Replicate			5.66	0.020
Egg size				
Evolution treatment	2	8.1	0.500	0.624
Cage	*Random effects*		0.141	0.700
Replicate			2.176	1.000
Egg load				
Evolution treatment	2	6.4	5.420	0.042
Cage	*Random effects*		0	1.000
Replicate			0	1.000

Figures

Figure 1:

a) Experimental evolution design: founding wasps were collected at six locales and allowed to interbreed for two generations prior to experiment. They were then divided and reared in one of three aphid "environments": two possessing protective *H. defensa* (treatments H402 and H76), one without protective endosymbionts (treatment H-).

b) Mean wasp infectivity, measured as proportion of aphids successfully mummified in a standard assay, and calculated across the four replicate lines within each treatment; error bars indicate one standard error. Tests were made at three time points: in the initial population, prior to division into three aphid environments, and after 10 and 21 generations of experimental evolution. Positions marked "x" indicate instances in which no mummies were observed across all tests.

Figure 2:

Sampling design for RNA-seq after 11 generations of experimental evolution. Female wasps were reared on both their "home" aphid subline, or on unprotected (H-) aphids for one generation prior to collection. Gene expression was compared in three separate analyses of differential gene expression. (a) Analysis 1 used only parasitoid wasps sampled after one generation rearing unprotected (H-) aphids. Expression here was compared with three pairwise contrasts among lineages. (b) Analysis 2 compared only the two treatments evolved in the presence of *H. defensa* (H402 and H76), including wasps reared both on their "home" aphid subline and on H- for one

generation. Expression here was compared (1) between lineages H76 and H402 or (2) between aphids possessing and lacking *H. defensa*. (c) Analysis 3 separately compared wasps from either the H402 or H76 treatments, and compared expression in wasps reared in aphids with or without their respective *H. defensa* strains.

Figure 3:

Host stage preference does not differ among treatments after 18 generations of experimental evolution. Bars depict the mean number of eggs (±1 standard error) laid by female parasitoids from each experimental evolution treatment in five developmental stages of host aphids. Wasps from all treatments were offered aphids of the subline they evolved on.

Figure 4:

No evidence for host discrimination after 20 generations of experimental evolution. Bars depict the mean number of eggs deposited per aphid when parasitoids were given a choice of aphids with and without *H. defensa*. Wasps from the H76 and H402 treatments were offered unprotected (H-) and protected aphids from the subline they evolved with, wasps from the H- treatment were offered unprotected aphids and protected aphids from either subline in half of the tests.

Figure 5:

Significantly differentially expressed (DE) genes are largely lineage-specific, Analysis 1. Heatmap depicting $\log_2$(fold change) of all significantly expressed genes (FDR<0.05) in either the H402 or H76 lineages relative to H-. Analysis 1 only included individuals collected after

rearing for one generation in a common (H-) environment. Number of putative toxin products are from both sources used in this paper: UATdb and the list in Table 2.

Supplemental Figures

Supplemental Figure 1

Summary of data collected for four phenotypes measured from evolution treatments: (a) Wet weight, (b) Egg load, (c) Days to develop, and (d) Egg length. Wasps from the H76 and H402 treatments were measured both on their home aphid environment (protected by *H. defensa*) and after one generation rearing on H- aphids. The respective evolution treatment and host aphid (for 1 generation prior) are marked with colored bars below all graphs: H402 (pink), H76 (orange), or H- (green).

Supplemental Figure 2

a) Sampling design for RNA-seq after 11 generations of experimental evolution. Female wasps were reared on both their "home" aphid subline, or on unprotected (H-) aphids for one generation prior to collection.
b) Analysis 1, PCA plot of all gene expression. This comparison used only parasitoid wasps sampled after one generation rearing unprotected (H-) aphids. Three biological replicates were sequenced for each of the four replicate lines per evolution treatment. PCA plots

summarize global gene expression differences among lineages, and are based on rlog transformations of expression count data, calculated by DESeq2.

c) Analysis 2, PCA plot of all gene expression. Here, only the two treatments evolved in the presence of *H. defensa* were compared (H402 and H76), including wasps reared both on their "home" aphid subline and on H- for one generation. Graph generated as in part (b).

Figure 1

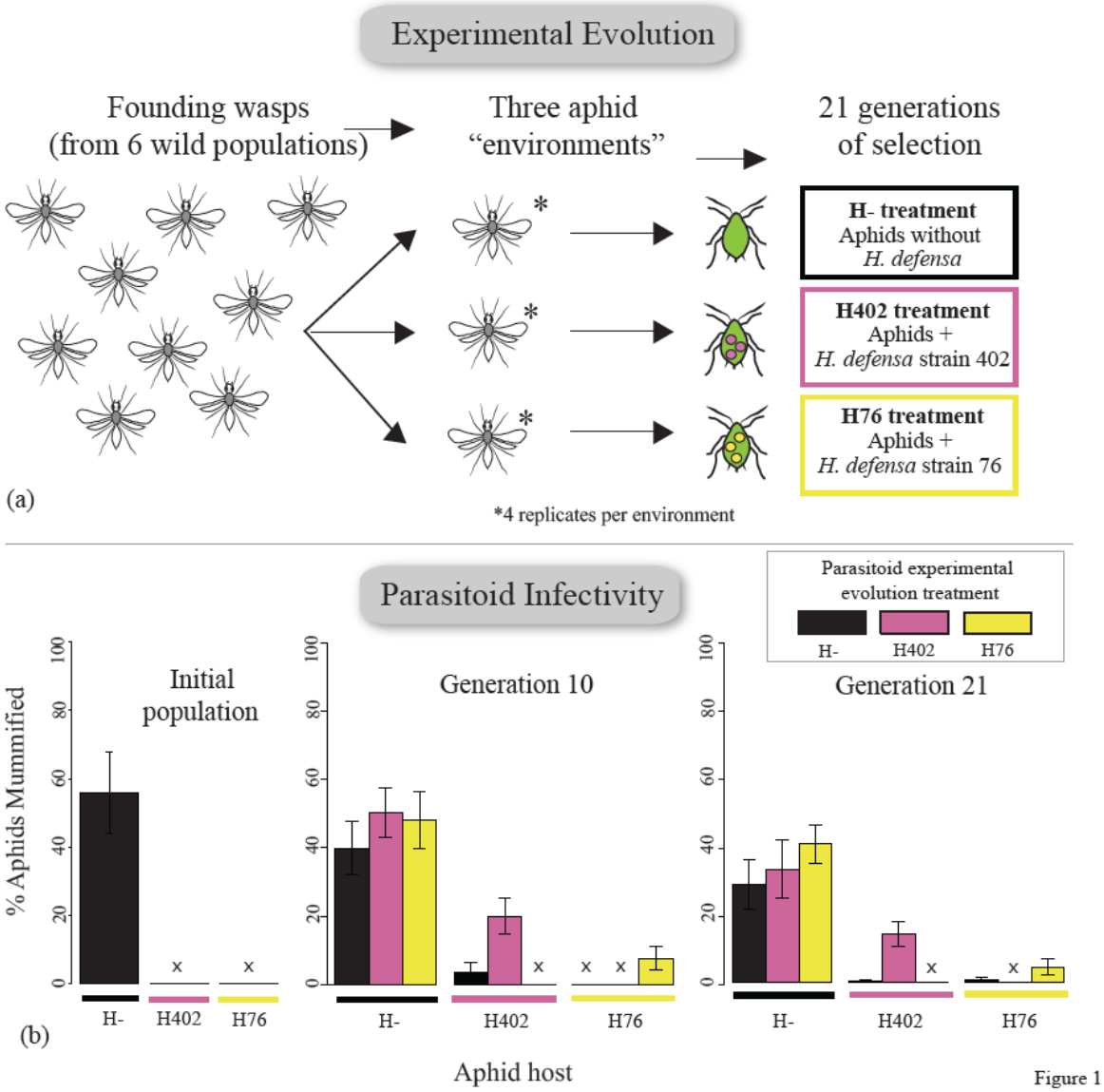

Figure 1

1145 **Figure 2**

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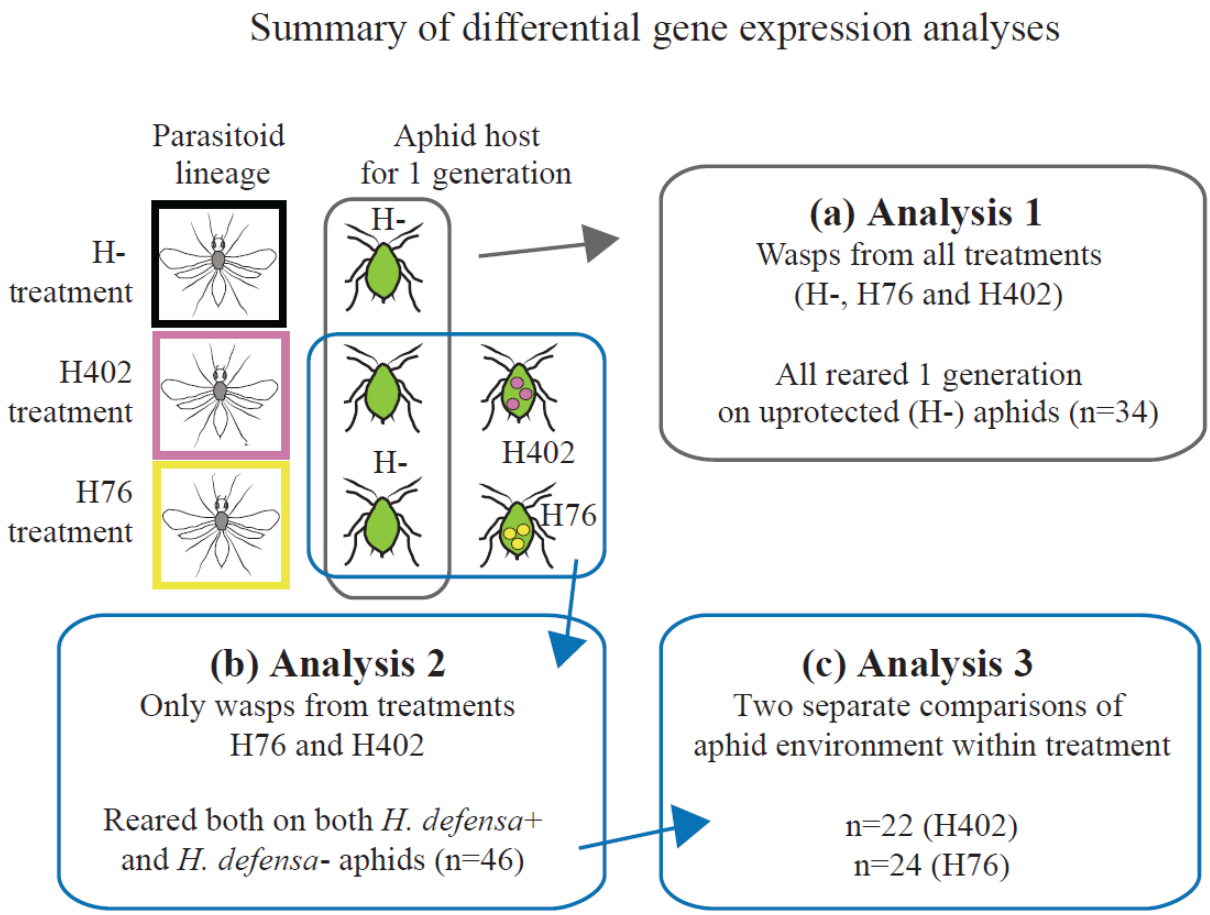

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1149 **Figure 3**

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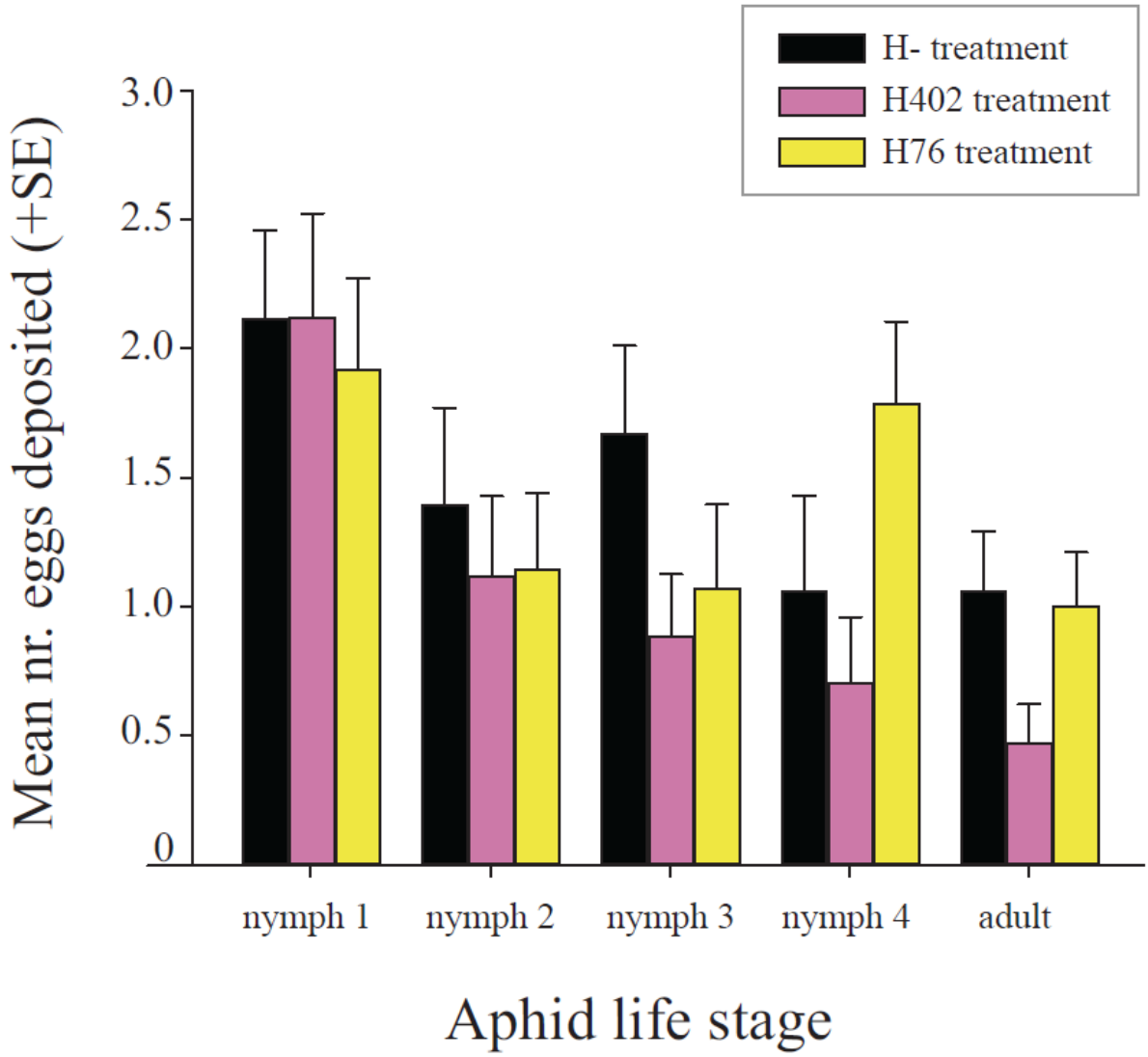

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1153 **Figure 4**

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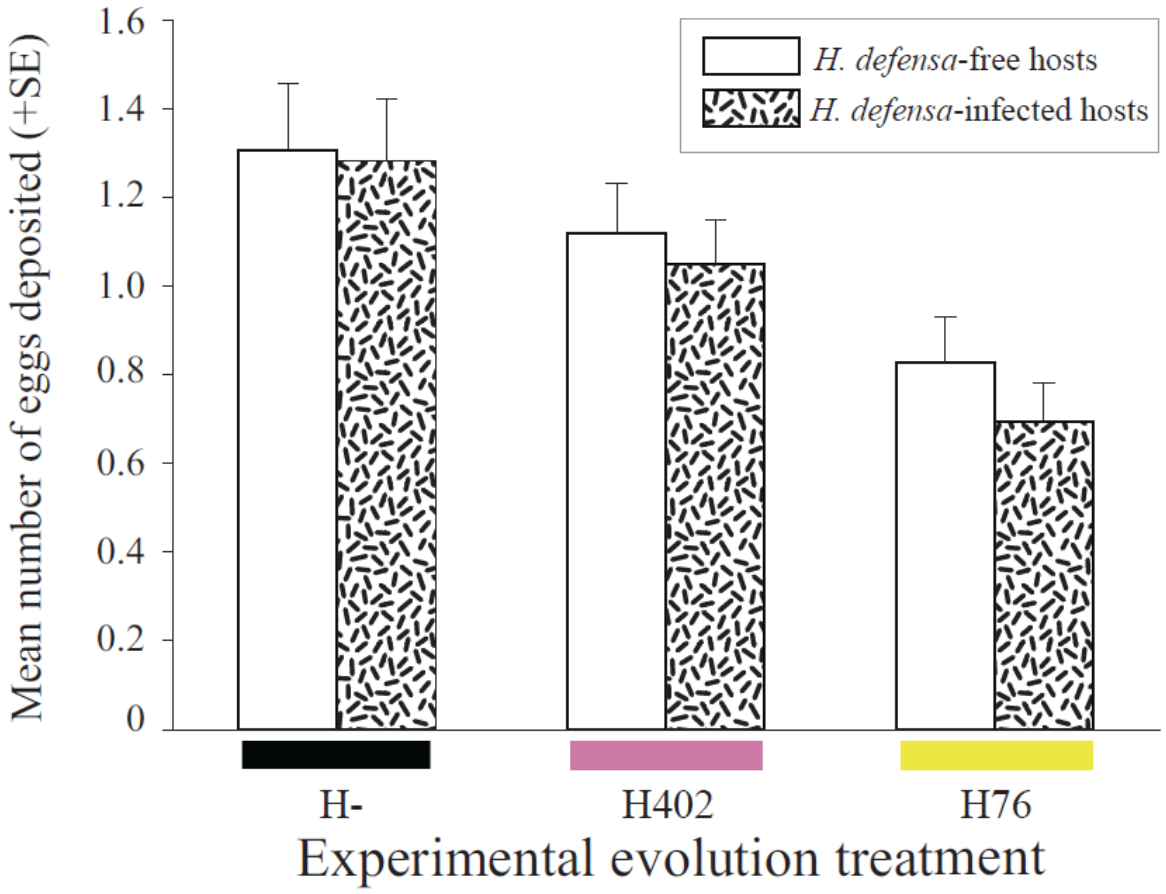

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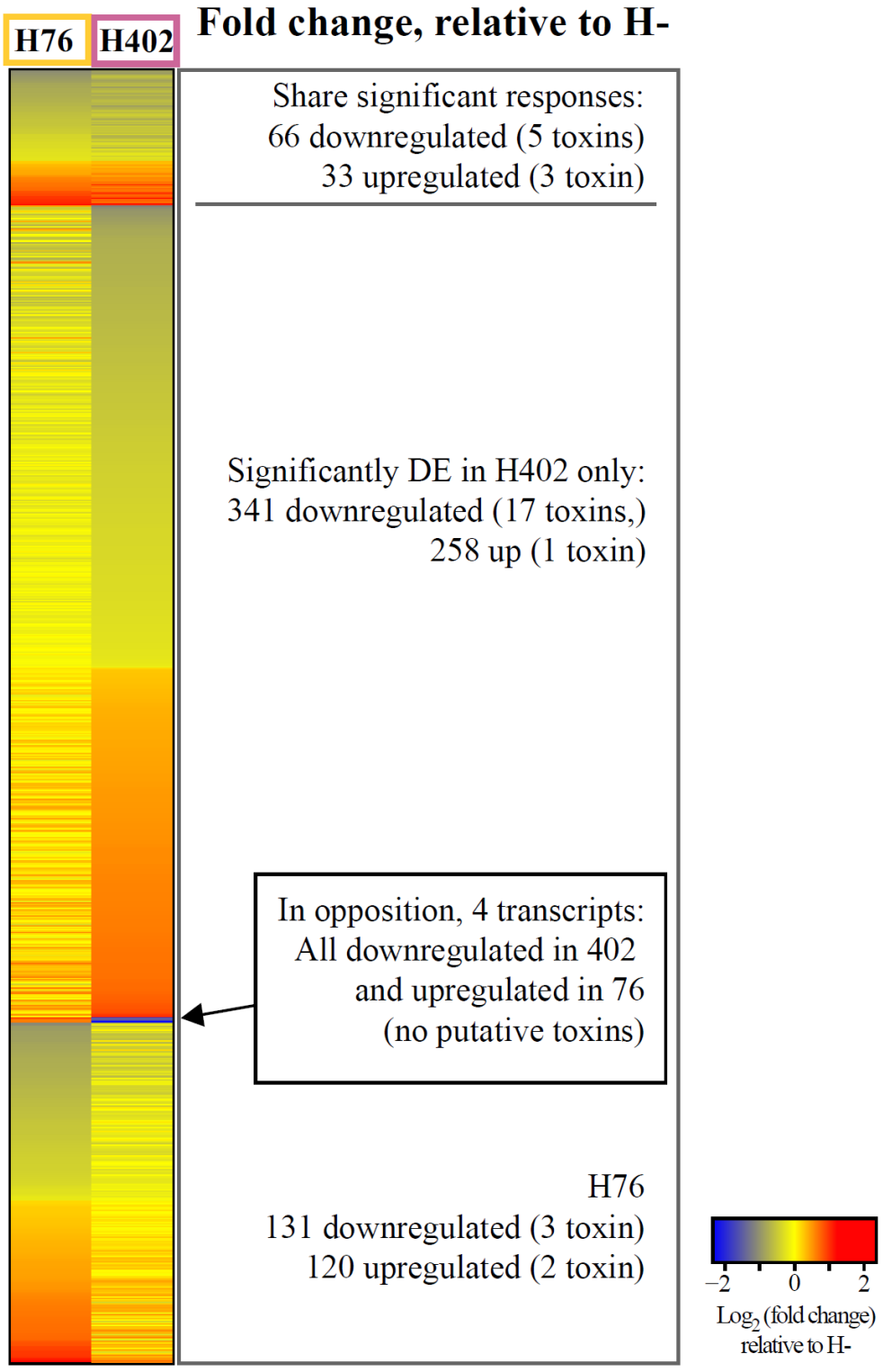
1157 **Figure 5**



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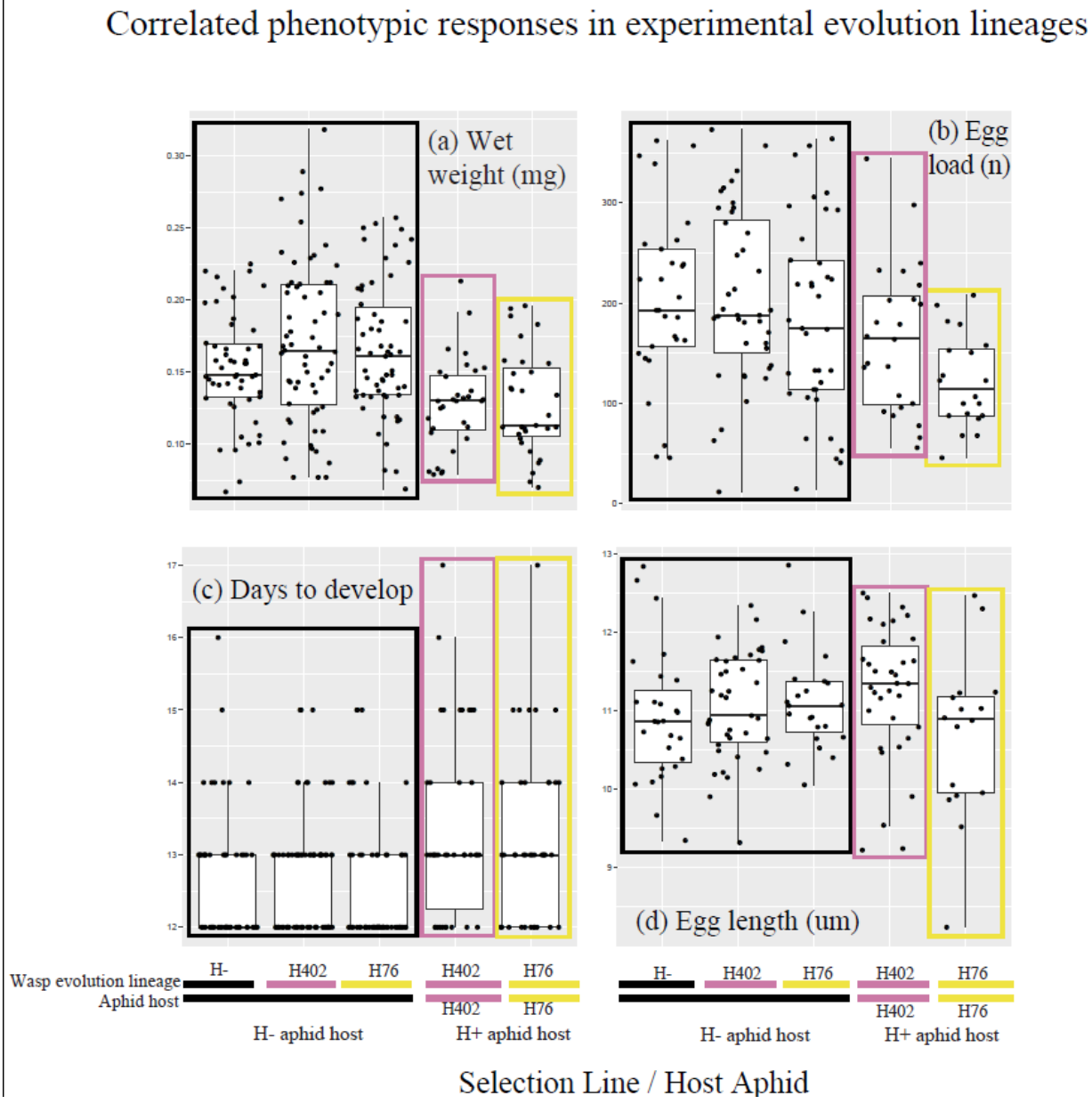
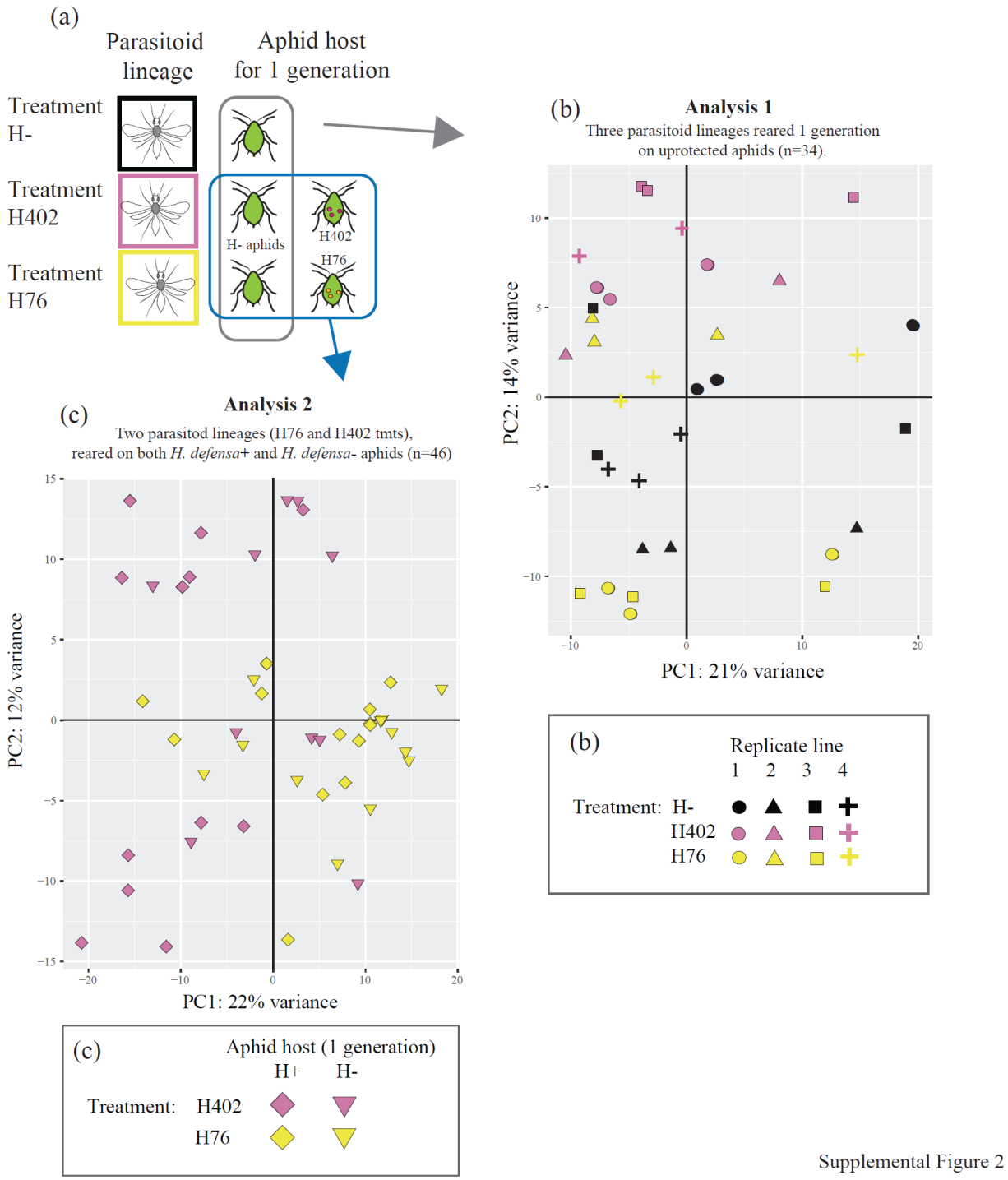
Supplementary Figure 1



Supplementary Figure 2



Supplemental Figure 2

Supplementary Data Files

Supplementary Data 1

Full list of transcripts included in study, their best blast hit, and blast match to the UATdb of putative venom and toxins.

Supplementary Data 2

Full results of Differential Expression Analysis 1: the three pairwise contrasts between the three lineages, all reared on H- aphids. Includes their best blast matches and putative venom annotations.

Supplementary Data 3

Full results of Differential Expression Analysis 2: comparing lineages H76 and H402, reared on both protected and un-protected aphids. This full analysis includes the main comparison between lineages H76 and H402, plus the results of the Likelihood ratio test, which examined the effect of the lineage x aphid environment interaction term.

Supplementary Data 4

Full results of Differential Expression Analysis 3: two separate comparisons (H76 or H402 lineages), comparing wasps reared for one generation on aphid protected by *H. defensa* (H+, i.e. H402 or H76) or not (H-). These two pairwise expression analyses include their best blast matches and putative venom annotations.