Prior adaptation of parasitoids improves biological control of symbiont-protected pests

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Abstract
There is increasing demand for sustainable pest management to reduce harmful effects of pesticides on the environment and human health. For pest aphids, biological control with parasitoid wasps provides a welcome alternative, particularly in greenhouses. However, aphids are frequently infected with the heritable bacterial endosymbiont Hamiltonella defensa, which increases resistance to parasitoids and thereby hampers biological control. Using the black bean aphid (Aphis fabae) and its main parasitoid Lysiphlebus fabarum, we tested whether prior adaptation of parasitoids can improve the control of symbiont-protected pests. We had parasitoid lines adapted to two different strains of H. defensa by experimental evolution, as well as parasitoids evolved on H. defensa-free aphids. We compared their ability to control caged aphid populations comprising 60% unprotected and 40% H. defensa-protected aphids, with both H. defensa strains present in the populations. Parasitoids that were not adapted to H. defensa had virtually no effect on aphid population dynamics compared to parasitoid-free controls, but one of the adapted lines and a mixture of both adapted lines controlled aphids successfully, strongly benefitting plant growth. Selection by parasitoids altered aphid population composition in a very specific manner. Aphid populations became dominated by H. defensa-protected aphids in the presence of parasitoids, and each adapted parasitoid line selected for the H. defensa strain it was not adapted to. This study shows, for the first time, that prior adaptation of parasitoids improves biological control of symbiont-protected pests, but the high specificity of parasitoid counter-resistance may represent a challenge for its implementation.

KEYWORDS
aphids, biological control, defensive symbiosis, parasitoid, resistance evolution

1 | INTRODUCTION
Agricultural intensification responds to the need of feeding a growing human population (Godfray et al., 2010). Intensive farming requires crop protection to limit yield losses to pest species. Chemical control with pesticides can be very effective, but it has devastating effects on biodiversity (Beketov, Kefford, Schafer, & Liess, 2013; Geiger et al., 2010) and it entails risks to human health (Alavanja, Hoppin, & Kamel, 2004; Kim, Kabir, & Jahan, 2017; Schwarzenbach, Egli, Hofstetter, Gunten, & Wehrli, 2010). Furthermore, rapid evolution of pest resistance limits the operational lifespan of pesticides (Hawkins, Bass, Dixon, & Neve, 2019).
These concerns led to an increasing demand for sustainable alternatives, such as biological control with natural enemies (Heimpel & Mills, 2017).

Just like the application of insecticides, the introduction or the mass release of a natural enemy may impose strong selection on insect pests. Under the well-supported assumption that insect populations harbor ample heritable variation for their susceptibility to natural enemies (Dubuffet et al., 2007; Henter & Via, 1995; Kraaijeveld & Godfray, 1997; Sandrock, Gouskov, & Vorburger, 2010), resistance to biocontrol agents is expected to evolve. However, Holt and Hochberg (1997) argued convincingly that the evolution of resistance to biological control is much less frequently observed than resistance to chemical pesticides. Possible reasons include temporally variable selection in the face of trade-offs with resistance, weak selection as a consequence of spatial structuring, and importantly, coevolutionary dynamics (Holt & Hochberg, 1997). Parasitoids and predators exhibit genetic variation as well and are thus able to evolve counter-resistance (Cavigliasso et al., 2019; Kraaijeveld, Hutcheson, Limentani, & Godfray, 2001), highlighting the opportunity for genetic improvement of biocontrol agents (Kruitwagen, Beukeboom, & Wertheim, 2018; Lommen, de Jong, & Pannebakker, 2017). Nevertheless, biological control is not immune to resistance evolution, as shown, for example, by the increasing resistance of Argentine stem weevils to the introduced parasitoid Microctonus hyperodae in New Zealand (Tomasetto, Tylianakis, Reale, W writen, & Goldson, 2017).

Aphids are among the most important agricultural pests worldwide (Dedryver, Le Ralec, & Fabre, 2010). While chemical control of aphids still predominates in open fields, biological control of aphids has been adopted widely in greenhouse production, where the confined space facilitates the deployment of natural enemies (van Lenteren, 2012). An important component of the biocontrol arsenal against aphids is parasitoid wasps (Boivin, Hance, & Brodeur, 2012). Aphids may possess a particularly effective and intriguing defense in the form of heritable bacterial endosymbionts that have evolved the ability to protect their hosts against parasitoids (Oliver, Smith, & Russell, 2014; Vorburger, 2014). The best-studied defensive symbiont of aphids is Hamiltonella defensa (Moran, Russell, Russell, Koga, & Fukatsu, 2005), which belongs to the Enterobacteriaceae and confers strong resistance to parasitoid wasps by killing the developing wasp larvae (Oliver, Russell, Moran, & Hunter, 2003; Schmid, Sieber, Zimmermann, & Vorburger, 2012). The ability to protect against parasitoids requires the presence of a toxin-encoding bacteriophage called Acrithosiphon pism secondary endosymbiont (APSE) in H. defensa's genome (Brandt, Chevignon, Oliver, & Strand, 2017; Oliver, Degnan, Hunter, & Moran, 2009). The strength of protection provided by H. defensa is variable (Oliver, Moran, & Hunter, 2005) and exhibits considerable specificity, such that different strains are unequally effective against different parasitoid species (Asplen et al., 2014; Cayetano & Vorburger, 2015) or even different genotypes of the same species (Cayetano & Vorburger, 2013; Schmid et al., 2012). This variation may be related to the fact that different strains of H. defensa often carry variants of APSE that encode different primary toxins (Degnan & Moran, 2008; Moran, Degnan, Degnan, Santos, Dunbar, & Ochman, 2005).

Already when H. defensa-conferring resistance to parasitoids was discovered, it was hypothesized that it may be responsible for observed failures of parasitoids to limit aphid abundance on crops (Gillespie, Quiring, Footit, Foster, & Acheampong, 2009; Oliver et al., 2005). When tested in a laboratory setting, the introduction of parasitoids indeed resulted in a rapid increase of aphids harboring H. defensa, such that the increasingly resistant populations escaped control by parasitoids (Käch, Mathé-Hubert, Dennis, & Vorburger, 2018). Defensive symbionts like H. defensa thus represent a challenge for biological control of pest aphids (Vorburger, 2018).

Unlike pesticides, biological control agents may evolve counter-resistance, given genetic variation, including the resistance conferred by heritable endosymbionts. This was demonstrated by applying experimental evolution to Aphidius ervi, the main parasitoid of the pea aphid (A. pism) (Dion, Zété, Simon, & Outreman, 2011), and to Lysiphebus fabarum, the main parasitoid of the black bean aphid (Aphis fabae) (Dennis, Patel, Oliver, & Vorburger, 2017; Rouchet & Vorburger, 2014). Both aphid species are important agricultural pests (Blackman & Eastop, 2017). Parasitoids adapted rapidly and showed a significantly improved ability to parasitize H. defensa-protected aphids after only 4–10 generations of selection. In the case of L. fabarum, counter-resistance was specific to the H. defensa strains carried by the aphids on which the wasps were evolved (Dennis et al., 2017; Rouchet & Vorburger, 2014). Such specificity is also a characteristic of natural populations of L. fabarum. A large collection of field-collected lines varied widely in the ability to parasitize aphids infected with different strains of H. defensa (Vorburger & Rouchet, 2016).

These findings suggest that prior adaptation of parasitoids to aphids carrying protective symbionts could be a viable strategy to improve biological control of pest aphids in which such symbionts occur. We tested this hypothesis by deploying experimentally evolved lines of parasitoids from previous work (Dennis et al., 2017) in caged populations of black bean aphids. We found that prior adaptation to H. defensa can indeed allow parasitoids to control aphid populations that would otherwise escape control due to the rapid evolution of symbiont-conferring resistance. This result represents a proof of principle for the genetic improvement of biocontrol agents by experimental evolution.

2 | MATERIALS AND METHODS

2.1 | Insect lines

As hosts, we used one H. defensa-free and two H. defensa-infected clonal lines of A. fabae with the same genetic background. The common genetic background was a single clone of A. fabae referred to as A06–607. It was collected in July 2006 from Chenopodium album in St. Margrethen (Switzerland) and does not contain any known facultative endosymbionts of aphids (Vorburger, Sandrock, Gouskov,
H. defensa

The two H. defensa-positive lines were generated by microinjection of H. defensa-containing hemolymph from two different donor clones (A06-76 and A06-402) into clone A06-407, which resulted in stable, heritable infections with H. defensa (Rouchet & Vorburger, 2014). The infected lines are designated as A06-407H76 and A06-407H402, where H76 and H402 refer to the two strains of H. defensa. These strains are clearly distinct based on sequences of two housekeeping genes, and they contain different variants of the APSE phage encoding different toxins (Dennis et al., 2017).

As parasitoids, we used three sexual stocks of L. fabarum that have a common origin but differ in their history of laboratory adaptation to aphid hosts. These populations are derived from an experimental evolution study described in Dennis et al. (2017). Briefly, four replicate populations of L. fabarum were reared on each of the three aphid lines described above: A06-407, A06-407H76, and A06-407H402, which resulted in significant and specific counteradaptation of parasitoids to H. defensa-conferring resistance. Parasitoids reared on each of the H. defensa-infected lines evolved increased infectivity (ability to overcome host resistance and successfully parasitize hosts) on aphids possessing their but not the other strain of H. defensa, whereas aphids reared on H. defensa-free aphids remained poorly infective on either of the H. defensa-protected lines (Dennis et al., 2017). Parasitoid adaptation to H. defensa-protected aphids did not entail any obvious correlated responses in terms of parasitoid life-history traits or a reduced ability to parasitize unprotected aphids. The experiment was terminated after 24 generations by merging the four replicate populations of the same treatment into a single population. The three evolved populations are still maintained on their respective hosts in our laboratory. By the start of the present experiment, they had been reared on these aphid lines for 120 generations, and they had maintained the described pattern of specific adaptation (data not shown).

2.2 Experimental procedures

Aphid populations were reared in 25 polyester insect cages with dimension 32.5 × 32.5 × 32.5 cm (BugDorm-4F3030; MegaView Science). Cages initially contained four potted broad bean plants (Vicia faba, aged 14 days) and were placed on a single shelf in a climatized room set at 22°C with a 16-hr photoperiod. Under these conditions, A. fabae reproduces by viviparous parthenogenesis exclusively. Aphid populations were started by adding 15 adult females of line A06-407 and 5 adult females each of A06-407H76 and A06-407H402. Forty percent infection with H. defensa corresponds closely to the prevalence of this symbiont in the natural populations we study (Vorburger et al., 2009). The addition of aphids marked day 0 of the experiment. Four days later, two additional plants were added to the cages and cages were assigned randomly to one of five treatments (5 replicates each): control (no parasitoids); H- (parasitoids evolved on H. defensa-free aphids); H76 (parasitoids evolved on H76-infected aphids); H402 (parasitoids evolved on H402-infected aphids); and H76 + H402 (mixture of parasitoids evolved on H76- and on H402-infected aphids). Treatments were applied on day 7 of the experiment by adding 22 female and 12 male wasps of the respective lines of L. fabarum to each cage (11 and 6 each for the mixed treatment). On the same day, aphid density was estimated for the first time; thereafter, aphid and parasitoid density was estimated twice per week. On each occasion, we removed the two oldest plants from the cages and replaced them with two new plants. One of the removed plants was selected randomly for counting, the other was cut and returned to the cage so that aphids could migrate over to live plants and parasitoid mummies on the plants could hatch inside the cage. The retained plant was sealed in a plastic bag and frozen to arrest aphid reproduction, before all aphids and parasitoid mummies were counted. After day 18 of the experiment, plants started to show stunted growth because of aphid infestations; hence, we began to quantify plant size. For this, the plants were disassembled into stalks and leaves, which were spread out and photographed on a white background with size reference. The area of all plant material in the photographs was estimated with ImageJ v. 1.52 (Schneider, Rasband, & Eliceiri, 2012), from which we calculated the variable “plant surface” = 4 × total stalk area + 1 × total leaf area, since aphids feed on the underside of leaves and on the stalks, which have a quadratic cross section in V. faba. The last count took place 67 days after the addition of the aphids to the cages. At this point, we also took a haphazard sample of 24 aphids per cage to quantify their population composition at the end of the experiment, and we determined the total fresh weight of all plants in the cages (aboveground parts) as a measure of plant condition.

2.3 Final composition of aphid populations

The DNA of aphids collected at the end of the experiment was extracted using the “salting out” protocol described in (Sunnucks and Hales, 1996). We tested each individual for infection with H. defensa by diagnostic PCR, amplifying part of the 16S ribosomal RNA gene with symbiont-specific primers (Ferrari, West, Via, & Godfray, 2012). We also ran a diagnostic PCR for the obligate endosymbiont of aphids, Buchnera aphidicola, as a control to verify the presence of amplifiable symbiont DNA in the extractions. For all H. defensa-positive individuals, we identified the H. defensa strain by amplifying and Sanger sequencing a fragment of the bacterial housekeeping gene MurE (murE) as in Cayetano, Rothacher, Simon, and Vorberger (2015). It turned out that the diagnostic PCR for H. defensa was somewhat more sensitive than that for B. aphidicola, because for a few DNA extractions from very small aphids, we obtained a clear amplification product for H. defensa but not for B. aphidicola. To avoid any bias, the proportion of H. defensa-positive and H. defensa-negative aphids per cage was estimated only from samples that tested positive for B. aphidicola, but the relative frequencies of infection with H76 or H402 among the H. defensa-positive aphids were estimated from all samples for which a murE sequence could be obtained.
2.4 Statistical analyses

Aphid density on plants was expressed as individuals per cm², that is, #aphids/plant surface area, and parasitoid density as the number of mummies per cm². To obtain comparable values from the early counts when plant growth was not visibly impacted by aphids and plant size not quantified, we assumed a plant surface of 149.8 cm², which is the average of healthy plants of the same age. Densities were analyzed with mixed linear models after square root transformation for aphid densities and log-transformation for parasitoid densities (log(#mummies + 1)/plant surface area) to improve normality of residuals and homogeneity of variances. We tested for the effects of treatment, time (day of count), and the treatment × time interaction. Cage was included as a random effect to account for the non-independence of successive counts from the same cage. For aphid and parasitoid densities, we ran global models with all treatments as well as models for all pairwise comparisons between treatments with sequential Bonferroni correction to account for multiple testing (Rice, 1989). Analyses were carried out in R v. 3.5.0 (R Core Team, 2017), using the lm4 library (Bates, Maechler, Bolker, & Walker, 2015) with the lmerTest library for significance tests of fixed and random effects (Kuznetsova, Brockhoff, & Christensen, 2015).

Plant fresh weights at the end of the experiment were compared among treatments with a one-way ANOVA, followed by pairwise comparisons using Tukey's HSD (Tukey, 1977). The final composition of aphid populations was analyzed with permutational MANOVA on the arcsine square root-transformed proportions of H. defensa-free, H76-infected, and H402-infected aphids, using the adonis function in the vegan library (Oksanen et al., 2019), followed by pairwise post hoc comparisons with the pairwise.perm.manova function in the RVAideMemoire package (Hervé, 2020). For all treatments, we also tested whether the total proportion of H. defensa-infected aphids at the end of the experiment differed from the initial proportion of 0.4, using one-sample t tests.

3 RESULTS

The treatments had a clear effect on the aphid population dynamics (LMM, treatment: F_{4,20} = 10.992, p < .001; time: F_{18,360} = 33.656, p < .001; treatment × time: F_{72,360} = 2.893, p < .001), and also, the parasitoid population dynamics differed significantly among the four treatments that contained wasps (treatment: F_{3,16} = 1.566, p = .237; time: F_{18,288} = 67.088, p < .001; treatment × time: F_{54,288} = 2.655, p < .001) (Figure 1). Specifically, when we introduced L. fabarium from a population that was evolved experimentally on H. defensa-free (H-) aphids, the parasitoids established successfully but had no detectable effect on aphid population density compared to parasitoid-free control cages (Figure 1a, b, Table 1). When we introduced parasitoids from a population that was evolved on H402-infected aphids (i.e., parasitoids adapted to aphids carrying H. defensa strain H402), parasitoids reached higher densities than the parasitoids adapted to H- aphids, but the effect on aphid populations remained weak, such that aphid densities, although somewhat lower, did not differ significantly from the control and the H- treatments (Figure 1c, Tables 1 and 2). However, parasitoids adapted to H76-infected aphids did have a significant effect on aphid population dynamics. They suppressed aphid population growth, resulting in significantly lower aphid densities at the end of the experiment (Figure 1d, Table 1). Virtually the same result was observed when we introduced a mixture of H76- and H402-adapted parasitoids (Figure 1e, Table 1).

The broad bean plants clearly benefitted from successful aphid control in that plant fresh weights at the end of the experiment were highest in the two treatments where parasitoids managed to suppress aphid populations (Figure 2).

The aphid population compositions differed significantly among treatments at the end of the experiment (permutational MANOVA, F_{4,20} = 20.30, p < .001) and reflected the specificity of the selection imposed by the differently adapted parasitoids (Figure 3). All treatments differed significantly from each other in pairwise post hoc comparisons except for control versus H- and H76 versus H76 + H402 (Figure 3). In the control cages without parasitoids, the proportion of H. defensa-infected aphids had declined somewhat by the end of the experiment, likely reflecting the known cost of harboring H. defensa in the absence of parasitoids (Oliver, Campos, Moran, & Hunter, 2008; Vorburger & Gouskov, 2011), although this decline was not significant (t_{4,20} = −1.746, p = .156). All other treatments showed a weak (H- treatment: t_{4,20} = 1.677, p = .169) or strong (H76, H402, H76 + H402: all p < .001) increase of aphids possessing H. defensa. When the parasitoids were adapted to H402-infected aphids, the H402 strain nearly disappeared from the cages and the H. defensa-positive aphids mostly carried H76, whereas the opposite was the case when H76-adapted parasitoids had been added to the cages. The proportions of the two H. defensa strains remained more balanced when the parasitoids comprised H76- and H402-adapted wasps (Figure 3).

4 DISCUSSION

There is an urgent need for more sustainable pest control to reduce the harmful side effects of conventional control with pesticides (Geiger et al., 2010; Kim et al., 2017). Biological control with parasitoids is a much-used alternative to control pest aphids, particularly in greenhouse crops (Boivin et al., 2012), but its success can be hampered by the rapid evolution of symbiont-conferred resistance (Käch et al., 2018). Here, we show that parasitoids with improved infectivity through prior adaptation to defensive symbionts present in an aphid pest can reduce aphid population densities in a situation where unselected parasitoids fail. Improving biological control agents through selective breeding is not a new approach (Kruitwagen et al., 2018; Lommen et al., 2017). It has been applied to life-history traits such as development time and sex ratio, to behavioral traits like host finding, and to increased tolerance of host defenses (reviewed in Lirakis & Magalhães, 2019). We show for the first time, though, that parasitoid adaptation to a defense encoded by a microbial symbiont rather than
the host itself does indeed improve control of a symbiont-protected pest. Aphid population suppression resulted in improved plant growth, which is the main goal of biological control.

A potential problem of this approach is the high specificity of resistance conferred by *H. defensa* and the counteradaptations of parasitoids, because multiple strains of this symbiont with different defensive properties may occur in the same aphid species (Cayetano et al., 2015; Henry et al., 2013; Oliver & Higashi, 2019). With the two *H. defensa* strains used here, there is virtually no cross-infectivity of the experimentally evolved parasitoids. Although they attack them normally, parasitoids are unable to develop in aphids infected with the alternative strain of *H. defensa* (Dennis et al., 2017). Cross-infectivity may evolve, however, when the selection regimes include more similar strains of *H. defensa* (Rouchet & Vorburger, 2014).

The specific counter-resistance of experimentally evolved parasitoids was also reflected in how the aphid populations responded to selection by parasitoids. When H402-adapted parasitoids were applied, aphid populations became dominated by H76-infected aphids, and when H76-adapted parasitoids were applied, almost only H402-infected aphids survived. In the first case, this rapid response to selection prevented an effective control of aphid populations (Figure 1c), but in the second case, the parasitoids were still able to suppress aphid densities (Figure 1d). This difference is interesting, albeit not entirely explicable by our current knowledge of the system. We can exclude that H76-adapted wasps also parasitized H402-infected aphids effectively, because tests immediately before and after the present cage experiment confirmed that there was no cross-infectivity, as reported in Dennis et al. (2017) (data not shown). However, when the presence of a susceptible host population

### Figure 1

Dynamics of aphid and parasitoid population densities in the five experimental treatments. Values depict means of five replicate cages ± 1 SE. Arrows indicate the introduction of parasitoids to the cages. Note that the y-axis is on a logarithmic scale.

### Table 1

Tests of the treatment effect and the treatment × time interaction on aphid densities for all pairwise comparisons between treatments.

| Comparison               | Treatment | Treatment × Time |
|--------------------------|-----------|------------------|
|                          | F<sub>1,8</sub> | p     | F<sub>18,144</sub> | p     |
| Control versus H-        | 0.395     | .547 | 1.110            | .349  |
| Control versus H402      | 3.028     | .120 | 1.422            | .129  |
| Control versus H76       | 14.995    | .005 | 3.415            | <.001 |
| Control versus H76 + H402| 44.350    | <.001| 5.566            | <.001 |
| H- versus H402           | 2.057     | .189 | 0.990            | .474  |
| H- versus H76            | 14.578    | .005 | 3.152            | <.001 |
| H- versus H76 + H402     | 64.898    | <.001| 5.723            | <.001 |
| H402 versus H76          | 4.126     | .077 | 2.289            | .004  |
| H402 versus H76 + H402   | 11.852    | .009 | 5.280            | <.001 |
| H76 versus H76 + H402    | 0.360     | .565 | 1.116            | .342  |

Note: p-Values in bold print are significant after sequential Bonferroni correction for a table-wide α = 0.05.
TABLE 2 Tests of the treatment effect and the treatment × time interaction on parasitoid densities for all pairwise comparisons between treatments that contained parasitoids

| Comparison          | Treatment | Treatment × Time |
|---------------------|-----------|------------------|
|                     | $F_{1,6}$ | $p$             |
|                     | $F_{1,144}$ | $p$               |
| H- versus H402      | 3.030     | 0.120            |
|                     | 4.187     | <.001            |
| H- versus H76       | 0.711     | 0.424            |
|                     | 4.661     | <.001            |
| H- versus H76 + H402| 0.895     | 0.372            |
|                     | 7.310     | <.001            |
| H402 versus H76     | 1.618     | 0.239            |
|                     | 0.549     | 0.929            |
| H402 versus H76 + H402| 2.517     | 0.151            |
|                     | 0.603     | 0.893            |
| H76 versus H76 + H402| 0.000     | 0.992            |
|                     | 0.739     | 0.366            |

Note: $p$-Values in bold print are significant after sequential Bonferroni correction for a table-wide $\alpha = 0.05$.

FIGURE 2 Total plant fresh weights per cage differed significantly among treatments at the end of the experiment (ANOVA, $F_{4,20} = 7.351, p < .001$). Treatments with different letters are significantly different in pairwise post hoc tests (Tukey’s HSD). Error bars depict 1 SE

FIGURE 3 The composition of aphid populations differed significantly among treatments at the end of the experiment (permutational MANOVA, $F_{4,20} = 20.30, p < .001$). Treatments with different letters are significantly different in pairwise post hoc tests. Error bars depict 1 SE. The dashed line indicates the proportion of H. defensa-free aphids at the beginning of the experiment.

Symbiont-conferred resistance to parasitoids is not restricted to aphids. Since the original discovery in pea aphids (Oliver et al., 2003), new cases keep being discovered (Hansen, Jeong, Paine, & Stouthamer, 2007; Xie, Butler, Sanchez, & Mateos, 2014; Xie, Vilchez, & Mateos, 2010). Protection by heritable endosymbionts...
may also extend to other parasites and pathogens of current or potential use in biological control. Examples include the protection by several endosymbiont species against entomopathogenic fungi in aphids (Lukasik, Asch, Guo, Ferrari, & Godfray, 2013; Scarborough, Ferrari, & Godfray, 2005), or the Wolbachia-mediated protection against viral pathogens in flies and mosquitoes (Glaser & Meola, 2010; Hedges, Brownlie, O'Neill, & Johnson, 2008; Teixeira, Ferreira, & Ashburner, 2008). Thus, we expect that future research will show that defensive symbionts can challenge the biological control of various arthropod pests. Just as in pesticides, strong selection by biological control agents will favor the evolution of resistance (Tomasetto et al., 2017). In pest populations where defensive symbionts occur, this will result in an elevated prevalence of these symbionts that may reduce biocontrol success (Käch et al., 2018; Oliver et al., 2008). Unlike pesticides, however, biological control agents possess genetic variation to evolve counter-resistance. This genetic variation can be managed and selected to improve the performance of natural enemies for biological control (Kruitwagen et al., 2018; Lommen et al., 2017). Here, we provided a proof of principle, in a laboratory setting, that experimental evolution is an effective means to improve the biocontrol capacity of parasitoid wasps toward symbiont-protected pests. The confined space and the simplified single-crop habitat typical of greenhouse cultures certainly bear similarity to a laboratory setting, but it remains to be demonstrated whether the approach can also be applied successfully at the larger scale of real biocontrol interventions.

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CONFLICT OF INTEREST
None declared.

DATA AVAILABILITY STATEMENT
Data are available at Dryad Digital Repository: https://doi.org/10.5061/dryad.t76hdw4.

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REFERENCES
Abram, P. K., Brodeur, J., Urbaneja, A., & Tena, A. (2019). Nonreproductive effects of insect parasitoids on their hosts. Annual Review of Entomology, 64(1), 259–276. https://doi.org/10.1146/annurev-ento-011118-111753
Alavanja, M. C. R., Hoppin, J. A., & Kamel, F. (2004). Health effects of chronic pesticide exposure: Cancer and neurotoxicity. Annual Review of Public Health, 25, 155–197. https://doi.org/10.1146/annurev.publheal.25.101802.123020
Asplen, M. K., Bano, N., Brady, C. M., Desneux, N., Hopper, K. R., Maloloues, C., ... Heimpel, G. E. (2014). Specialisation of bacterial endosymbionts that protect aphids from parasitoids. Ecological Entomology, 39(6), 736–739. https://doi.org/10.1111/een.12153
Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67(1), 1–48. https://doi.org/10.18637/jss.v067.i01
Beketov, M. A., Keeford, B. J., Schafer, R. B., & Liess, M. (2013). Pesticides reduce regional biodiversity of stream invertebrates. Proceedings of the National Academy of Sciences of the United States of America, 110(27), 11039–11043. https://doi.org/10.1073/pnas.1305618110
Blackman, R. L., & Eastop, V. F. (2017). Taxonomic issues. In H. F. Van Emden, & R. Harrington (Eds.), Aphids as crop pests (2nd ed., pp. 1–36). Wallingford: CAB International.
Boivin, G., Hance, T., & Brodeur, J. (2012). Aphid parasitoids in biological control. Canadian Journal of Plant Science, 92(1), 1–12. https://doi.org/10.4141/cjps2011-045
Brandt, J. W., Chevignon, G., Oliver, K. M., & Strand, M. R. (2017). Culture of an aphid heritable symbiont demonstrates its direct role in defence against parasitoids. Proceedings of the Royal Society B: Biological Sciences, 284(1866), 20171925. https://doi.org/10.1098/rspb.2017.1925
Cavigliasso, F., Mathe-Hubert, H., Kremmer, L., Rebuf, C., Gatti, J. L., Malausa, T., ... Poirie, M. (2019). Rapid and differential evolution of the venom composition of a parasitoid wasp depending on the host strain. Toxins, 11(11), 19. https://doi.org/10.3390/toxins11110629
Cayetano, L., Rothacher, L., Simon, J. C., & Vorburger, C. (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(1799), 20142333. https://doi.org/10.1098/rspb.2014.2333
Cayetano, L., & Vorburger, C. (2013). Genotype-by-genotype specificity remains robust to average temperature variation in an aphid/endosymbiont/parasitoid system. Journal of Evolutionary Biology, 26, 1603–1610. https://doi.org/10.1111/jeb.12154
Cayetano, L., & Vorburger, C. (2015). Symbiont-conferring protection against Hymenopteran parasitoids in aphids: How general is it? Ecological Entomology, 40(1), 85–93. https://doi.org/10.1111/een.12161
Chevignon, G., Boyd, B. M., Brandt, J. W., Oliver, K. M., & Strand, M. R. (2018). Culture-facilitated comparative genomics of the facultative symbiont Hamiltonella defensa. Genome Biology and Evolution, 10(3), 786–802. https://doi.org/10.1093/gbe/evy036
Dedryver, C. A., Le Ralec, A., & Fabre, F. (2010). The conflicting relationships between aphids and men: A review of aphid damage and control strategies. Comptes Rendus Biologies, 333(6–7), 539–553. https://doi.org/10.1016/j.crvi.2010.03.009
Degnan, P. H., & Moran, N. A. (2008). Diverse phage-encoded toxins in a protective insect endosymbiont. Applied and Environmental Microbiology, 74(21), 6782–6791. https://doi.org/10.1128/AEM.01285-08
Dennis, A. B., Patel, V., Oliver, K. M., & Vorburger, C. (2017). Parasitoid gene expression changes after adaptation to symbiont-protected hosts. Evolution, 71(11), 2599–2617. https://doi.org/10.1111/evo.13333
Dion, E., Zélé, F., Simon, J. C., & Outreman, Y. (2011). Rapid evolution of parasitoids when faced with the symbiont-mediated resistance of their hosts. Journal of Evolutionary Biology, 24(4), 741–750. https://doi.org/10.1111/j.1420-9101.2010.02207.x
Dubuffet, A., Dupas, S., Frey, F., Drezen, J. M., Poirié, M., & Carton, Y. (2007). Genetic interactions between the parasitoid wasp Leptopilina
Rice, W. R. (1989). Analyzing tables of statistical tests. Evolution, 43, 223–225. https://doi.org/10.2307/2409177

Rouchet, R., & Vorburger, C. (2014). Experimental evolution of parasitoid infectivity on symbiont-protected hosts leads to the emergence of genotype-specificity. Evolution, 68(6), 1607–1616. https://doi.org/10.1111/eva.12377

Sandrock, C., Gouskov, A., & Vorburger, C. (2010). Ample genetic variation but no evidence for genotype specificity in an all-parthenogenetic host-parasitoid interaction. Journal of Evolutionary Biology, 23(3), 578–585. https://doi.org/10.1111/j.1420-9101.2009.01925.x

Scarborough, C. L., Ferrari, J., & Godfray, H. C. J. (2005). Aphid protected from pathogen by endosymbiont. Science, 310(5755), 1781–1781. https://doi.org/10.1126/science.1120180

Schmid, M., Sieber, R., Zimmermann, Y. S., & Vorburger, C. (2012). Development, specificity and sublethal effects of symbiont-conferred resistance to parasitoids in aphids. Functional Ecology, 26(1), 207–215. https://doi.org/10.1111/j.1365-2435.2011.01904.x

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671–675. https://doi.org/10.1038/nmeth.2089

Schwarzenbach, R. P., Egli, T., Hofstetter, T. B., von Gunten, U., & Wehrli, B. (2010). Global water pollution and human health. Annual Review of Environment and Resources, 35, 109–136. https://doi.org/10.1146/annurev-environ-100809-125342

Sunnucks, P., & Hales, D. F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus Sitobion (Hemiptera: Aphididae). Molecular Biology and Evolution, 13(3), 510–524. https://doi.org/10.1093/oxfordjournals.molbev.a025612

Teixeira, L., Ferreira, A., & Ashburner, M. (2008). The bacterial symbiont Wolbachia induces resistance to RNA viral infections in Drosophila melanogaster. PLoS Biology, 6(12), 2753–2763. https://doi.org/10.1371/journal.pbio.1000002

Tomasetto, F., Tylianakis, J. M., Reale, M., Wratten, S., & Goldson, S. L. (2017). Intensified agriculture favors evolved resistance to biological control. Proceedings of the National Academy of Sciences of the United States of America, 114(15), 3885–3890. https://doi.org/10.1073/pnas.1618416114

Tukey, J. W. (1977). Exploratory data analysis. Reading MA: Addison-Wesley.

van Lenteren, J. C. (2012). Internet book of biological control (6th ed.). Wageningen, The Netherlands: International Organisation for Biological Control (IOBC). Retrieved from http://www.iobc-global.org/publications_iobc_internet_book_of_biological_control.html

Vorburger, C. (2014). The evolutionary ecology of symbiont-conferring resistance to parasitoids in aphids. Insect Science, 21, 251–264. https://doi.org/10.1111/1744-7917.12067

Vorburger, C. (2018). Symbiont-conferring resistance to parasitoids in aphids – challenges for biological control. Biological Control, 116, 17–26. https://doi.org/10.1016/j.biocontrol.2017.02.004

Vorburger, C., & Gouskov, A. (2011). Only helpful when required: A longevity cost of harbouring defensive symbionts. Journal of Evolutionary Biology, 24, 1611–1617. https://doi.org/10.1111/j.1420-9101.2011.02292.x

Vorburger, C., & Rouchet, R. (2016). Are aphid parasitoids locally adapted to the prevalence of defensive symbionts in their hosts? BMC Evolutionary Biology, 16, 271. https://doi.org/10.1186/s12862-016-0811-0

Vorburger, C., Sandrock, C., Gouskov, A., Castañeda, L. E., & Ferrari, J. (2009). Genotypic variation and the role of defensive endosymbionts in an all-parthenogenetic host-parasitoid interaction. Evolution, 63(6), 1439–1450. https://doi.org/10.1111/j.1558-5646.2009.00660.x

Xie, J., Butler, S., Sanchez, G., & Mateos, M. (2014). Male killing Spiroplasma protects Drosophila melanogaster against two parasitoid wasps. Heredity, 112, 399–408. https://doi.org/10.1038/hdy.2013.118

Xie, J. L., Vilchez, I., & Mateos, M. (2010). Spiroplasma bacteria enhance survival of Drosophila hydei attacked by the parasitic wasp Leptopilina heterotoma. PLoS ONE, 5(8), e12149. https://doi.org/10.1371/journal.pone.0012149

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