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Self-perpetuating ecological–evolutionary dynamics in an agricultural host–parasite system

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Ecological and evolutionary processes may become intertwined when they operate on similar time scales. Here we show ecological–evolutionary dynamics between parasitoids and aphids containing heritable symbionts that confer resistance against parasitism. In a large-scale field experiment, we manipulated the aphid's host plant to create ecological conditions that either favoured or disfavoured the parasitoid. The result was rapid evolutionary divergence of aphid resistance between treatment populations. Consistent with ecological–evolutionary dynamics, the resistant aphid populations then had reduced parasitism and increased population growth rates. We fit a model to quantify costs (reduced intrinsic rates of increase) and benefits of resistance. We also performed genetic assays on 5 years of field samples that showed persistent but highly variable frequencies of aphid clones containing protective symbionts; these patterns were consistent with simulations from the model. Our results show (1) rapid evolution that is intertwined with ecological dynamics and (2) variation in selection that prevents traits from becoming fixed, which together generate self-perpetuating ecological–evolutionary dynamics.

When ecological forces change rapidly, strong shifts in natural selection can lead to rapid evolution[1–3]. Evolution occurring on the same time scale as ecological changes in population abundances can entangle ecological and evolutionary processes. The resulting ecological–evolutionary (eco–evo) dynamics[4–6] occur as ecological changes cause natural selection to modify the phenotypes that influence how species respond to ecological changes. In turn, these evolutionary responses determine how ecological forces affect further natural selection on focal populations. Only a few studies demonstrate eco–evo dynamics in natural systems[7–11], with most examples of eco–evo dynamics coming from laboratory or mesocosm systems[12].

Yoshida et al.[13] gave a classic demonstration of eco–evo dynamics for consumer–resource interactions between algae and herbivorous rotifers in a laboratory experiment. In populations where algal genetic diversity allowed evolution in response to rotifers, the result was cycles with longer periods and the maintenance of genetic diversity. Such eco–evo dynamics go beyond the trivial expectation for evolution to generate directional selection. For example, insecticides might select for resistant individuals and cause a subsequent increase in an insect population; however, such one-way 'hard selection' (involving absolute fitness) to fixation does not involve repeated feedbacks between ecology and evolution and was well-known before more recent interest in eco–evo dynamics. We think that the concept of eco–evo dynamics should necessarily address the maintenance of genetic diversity that perpetuates evolutionary dynamics and the maintenance of fluctuating ecological forces that perpetuate ecological dynamics[7,14–19]. However, demonstrating such self-perpetuating eco–evo dynamics in natural systems is a challenge.

Our study integrated (1) a large-scale field experiment to demonstrate rapid evolution in a host–parasitoid system; (2) a model fit to the experimental data to quantify the strength of selection; and (3) comparison of the fitted model to field survey data to investigate the spatial and temporal fluctuations in selection that maintain genetic diversity and perpetuate eco–evo dynamics. We took advantage of the tight ecological coupling between populations of pea aphids, *Acyrthosiphon pisum*, and their specialist insect parasitoid, *Aphidius ervi*, in *Medicago sativa* (lucerne/alfalfa) fields. Pea aphids were accidentally introduced into North America in the 1800s and *A. ervi* was introduced as a biological control agent from Europe starting in the 1950s[20,21]. Some pea aphids contain the symbiont *Hamiltonella defensa*, which passes with high fidelity from parthenogenic mothers to clonal offspring[22]. *Hamiltonella* confers resistance against parasitism by *A. ervi*[23] and, therefore, we used it as a genetic marker to follow the evolution of resistance. Because generation times are short (aphids ~2 weeks and parasitoids ~3 weeks[24]) and the interaction strength between aphids and parasitoids is often strong[24,25], our experiments and field surveys span many generations, allowing ample time to observe eco–evo dynamics.

Results

Field experiment. The large-scale field experiment using 'hoop houses' ($7 \times 30\,m^2$ screened cages, Extended Data Fig. 1) was designed to capture the natural dynamics of pea aphids and *A. ervi*, while preventing migration so that evolutionary changes in response to experimental treatments could be quantified. We manipulated harvesting within hoop houses to experimentally alter the interaction strength between aphids and parasitoids. One set of hoop houses was harvested asynchronously in strips (the 'asynchronous' treatment), so that growing lucerne was always available to aphids, while the other set of hoop houses was harvested synchronously, which reduced all available host plants for the aphids simultaneously. We expected asynchronous harvesting to benefit parasitoids by ensuring a continuous supply of aphids[26], thereby increasing the strength of parasitism pressure on aphids. By maintaining these

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harvesting treatments over much of the summer growing season, we imposed a 'press' manipulation[27] of the strength of aphid–parasitoid interactions. These treatments are realistic in the context of the lucerne agricultural system in which fields are harvested every 4–6 weeks. The alternative approach to manipulating the strength of aphid–parasitoid interactions by changing aphid or parasitoid abundances directly would have confounded the ecological component of eco–evo dynamics by giving only a short-term 'pulsed' manipulation of the system.

We introduced equal numbers of susceptible and resistant *Hamiltonella*-containing aphid clones into the hoop houses to ensure genetic variation in resistance to parasitism (Extended Data Figs. 2 and 3). Variation in protection levels conferred by *Hamiltonella* are correlated with the type of bacteriophages that are incorporated into the bacterial symbiont[28]. Clones containing the bacteriophage APSE3 confer the highest levels of protection and the clones we introduced contained *Hamiltonella*–APSE3 to ensure high levels of resistance. Prior field-experiment assays showed effectiveness of these clones against parasitism (Supplementary Information). We implemented the harvesting treatments from mid-summer to autumn 2015 (roughly six aphid generations). Aphid abundances were sampled by visually inspecting 500 lucerne stems and parasitism was inferred from a parasitism index given by the number of *A. ervi* mummies relative to the sum of mummies and pea aphids. As expected for our experimental design, peak values of the parasitism index were higher in the asynchronous hoop houses (repeated-measures linear mixed model, $P=0.007$, Supplementary Information).

At the end of the growing season, but before sexual male and female aphids were produced, 62% of aphids in the asynchronous hoop houses (51/79 and 63/98) contained *Hamiltonella*–APSE3, but only 10% (6/60 and 7/73) did in synchronous hoop houses. Thus, higher parasitism rates corresponded to greater resistance (Fig. 1). Although there are only two replicates of each treatment (due to the size of the hoop houses), the statistical difference between synchronous and asynchronous hoop houses is highly significant (binomial generalized linear model, GLM, $P \ll 0.0001$) and we can think of no explanation for this difference other than selection. Supporting evidence comes from smaller ($2\times2\times2\,m^3$) 'cube cages', two of which were embedded in each of the four hoop houses. The cube cages allowed us to determine whether there were differences among hoop houses that might affect the relative abundance of *Hamiltonella*–APSE3 aphid clones other than the harvesting treatment. The cube cages were not harvested and excluded the higher parasitoid abundance in the asynchronous hoop houses. The proportion of *Hamiltonella*–APSE3 aphid clones in the cube cages did not differ between hoop house treatments (binomial GLM, $P=0.76$, Extended Data Fig. 4).

To disentangle the joint effects of ecological and evolutionary processes, we fit a stage-structured model to the hoop house data to determine whether selection for *Hamiltonella*–APSE3 infected clones could explain the observed dynamics. In the model, the strength of resistance in clones with *Hamiltonella*–APSE3 was measured by increased aphid survival after being attacked by parasitoids. The cost of resistance was modelled as a decrease in aphid fecundity and hence the parasitism-independent intrinsic rate of increase. All hoop houses and three types of data were simultaneously fit by the model: aphid abundance, observed parasitism index and change in *Hamiltonella*–APSE3 frequency. The model's state–space structure accounted for temporal autocorrelation and observation error. The model shows strong statistical support for the parasitoid attack rate being higher in the asynchronous hoop houses ($\chi^2_1=33.6$, $P<0.0001$; see also Supplementary Information). The estimate of resistance is 0.73 (the probability of surviving parasitism, compared to 0 for susceptible clones), which is consistent with laboratory experiments[29,30]. These protective benefits entail a cost: in the model estimates, there was a reduction of the aphid parasitism-independent intrinsic rate of increase from $1.26\,d^{-1}$ for susceptible clones to $1.21\,d^{-1}$ for resistant clones. The joint estimate of resistance and the cost of resistance is highly significant ($\chi^2_2=109.8$, $P<0.0001$, Extended Data Fig. 5). Thus, the observed ecological and evolutionary dynamics are consistent with selection for resistance and its associated costs.

After aphid egg deposition and parasitoid pupal diapause on lucerne plants in autumn 2015, we removed the hoop house screens to avoid snow damage and replaced them before aphids emerged in spring 2016; no other treatment occurred. The first aphids emerging in spring were less likely to harbour *Hamiltonella*–APSE3 than aphids sampled in the autumn (26% in spring versus 41% in autumn, $P=0.0013$), although harvesting treatments did not affect the loss of symbionts (Extended Data Fig. 6). This shows that the frequency of defensive symbionts decreased during the sexual generation and overwintering. There are three possible explanations for the reduction in frequency of *Hamiltonella*–APSE3 clones. First, clones containing symbionts could have lower mating success. Second, the transmission from mother to egg might be less than 100% or the symbionts might die within the eggs. Third, aphids carrying *Hamiltonella*–APSE3 might have lower egg production, or the eggs or nymphs carrying *Hamiltonella*–APSE3 might have lower survival. These are the only field data that we know on the change in pea aphid symbiont frequency over winter and we do not have enough information to determine the cause.

Our first-year experimental results show an effect of ecological forces (habitat manipulation) on evolutionary changes in the aphid population, thus demonstrating eco–to–evo dynamics (Fig. 1, left panels). Our second-year results show evo–to–eco dynamics, with the more resistant aphids in the asynchronous hoop houses showing lower parasitism and higher population growth rates (Fig. 1, right panels). Parasitism on the first aphid generation in the spring was higher in synchronous than asynchronous hoop houses, consistent with a lower proportion of clones containing *Hamiltonella*–APSE3 in the synchronous hoop houses (Fig. 1). While we cannot rule out the possibility that higher parasitoid numbers survived in the synchronous hoop houses over winter, samples taken in November did not indicate more diapausing parasitoids in the synchronous hoop houses. For hoop houses in both treatments, the model-estimated attack rates (red lines in the narrow panels) were low, suggesting either a low overall survival of diapausing parasitoids over winter or few aphids available for parasitism as parasitoids were emerging in early spring. Aphid populations increased faster in the asynchronous hoop houses than in the synchronous hoop houses, consistent with decreased aphid mortality due to parasitism in asynchronous hoop houses with a greater proportion of resistant aphids. Ultimately, aphid densities increased until a fungal-pathogen outbreak terminated the experiment.

Field survey of population dynamics. From 2011 to 2016, we surveyed pea aphid abundance and parasitism in five to ten agricultural fields per year that were used for lucerne production (total of 983 field-day samples; Fig. 2). Aphids were sampled using sweep nets and parasitism was determined by dissecting fourth-instar and adult aphids and recording the number of second- and third-instar parasitoid larvae. Observed aphid abundances ranged over three orders of magnitude and observed parasitism ranged from 0% to 84%, providing a broad scope for evolutionary changes and eco–evo dynamics.

Using the model that we fit to the hoop house experiment, we calculated the parasitoid attack rate that would favour resistance and compared this to observed parasitism rates (see Methods). While the strength of selection for resistance depends on the parasitoid attack rate, the observed level of parasitism depends on

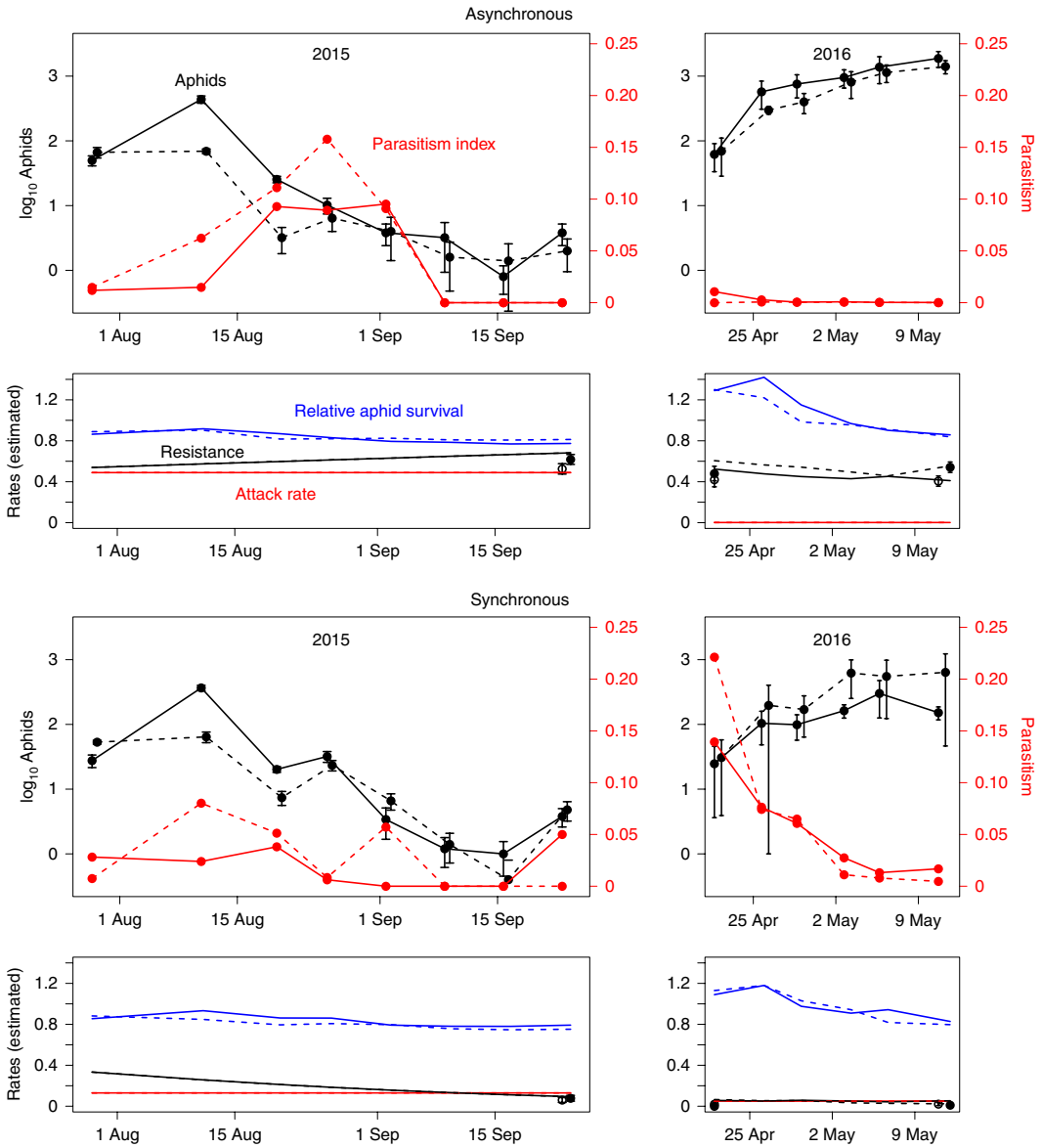

Fig. 1 | Field experiment showing eco-evo dynamics. Between mid-summer and autumn 2015 (panels on the left), the asynchronous harvesting treatment was applied in two hoop houses to maintain aphid habitat and increased parasitoid populations (top two panels) or synchronously to decrease parasitism pressure on aphids (bottom two panels). We counted aphids (solid and dashed black lines for the two hoop houses in each treatment) by visual inspection of 500 stems per cage; s.e.m. bars are given but in some cases are covered by the dots. Our index of parasitism (red lines) is the number of mummies as a proportion of the number of mummies and aphids. Peak parasitism rates (on 20 and 26 August and 2 September 2015) were higher in the asynchronous hoop houses ($P = 0.007$, Supplementary Information). The narrow panels give the estimated demographic rates (for the hoop houses in the panels above them) from the fitted model (Extended Data Fig. 8). The estimated daily parasitoid attack rates ($a(t)$ in equation (2)) is given in red and the density-independent relative aphid survival ($z(t)$ in equation (2)) is given in blue, with solid and dashed lines corresponding to the two replicates. Note that the relative aphid survival is scaled to the maximum estimated survival in 2015, so values greater than 1 in 2016 imply higher survival than the 2015 maximum. The estimated proportion of resistant clones is given by black lines and the black points with s.e.m. give the proportion of *Hamiltonella*–APSE3 clones from the genetic symbiont surveys.

both the attack rate and the level of resistance in the population because parasitoids are less likely to develop within resistant aphids. Although we can only observe parasitism, we can back-calculate the parasitoid attack rate for different levels of resistance. If the proportion of resistant aphids is 0.48 (the mean observed proportion of aphids carrying *Hamiltonella* with or without APSE3, 2012–2016, see later), observed parasitism greater than 21% favours resistance. This threshold is bounded by 13% and 30%, which correspond to extreme proportions of 0.88 and 0.02 resistant aphids we observed in the samples; the proportion of aphids attacked is the same in all three cases but the observed parasitism is lower when there are higher levels of resistance. For all three thresholds (based on mean and minimum or maximum observed resistance), the field data show periods of high and low parasitism (Fig. 2b) that either favour or disfavour resistance. This implies that there should be rapid increases and decreases in the proportion of resistance in the pea aphid population. Because parasitism can be very high, and because resistance is so effective (with an estimated 73% of attacked aphids surviving), this implies that there should be a large effect of evolution on the ecological dynamics of aphids.

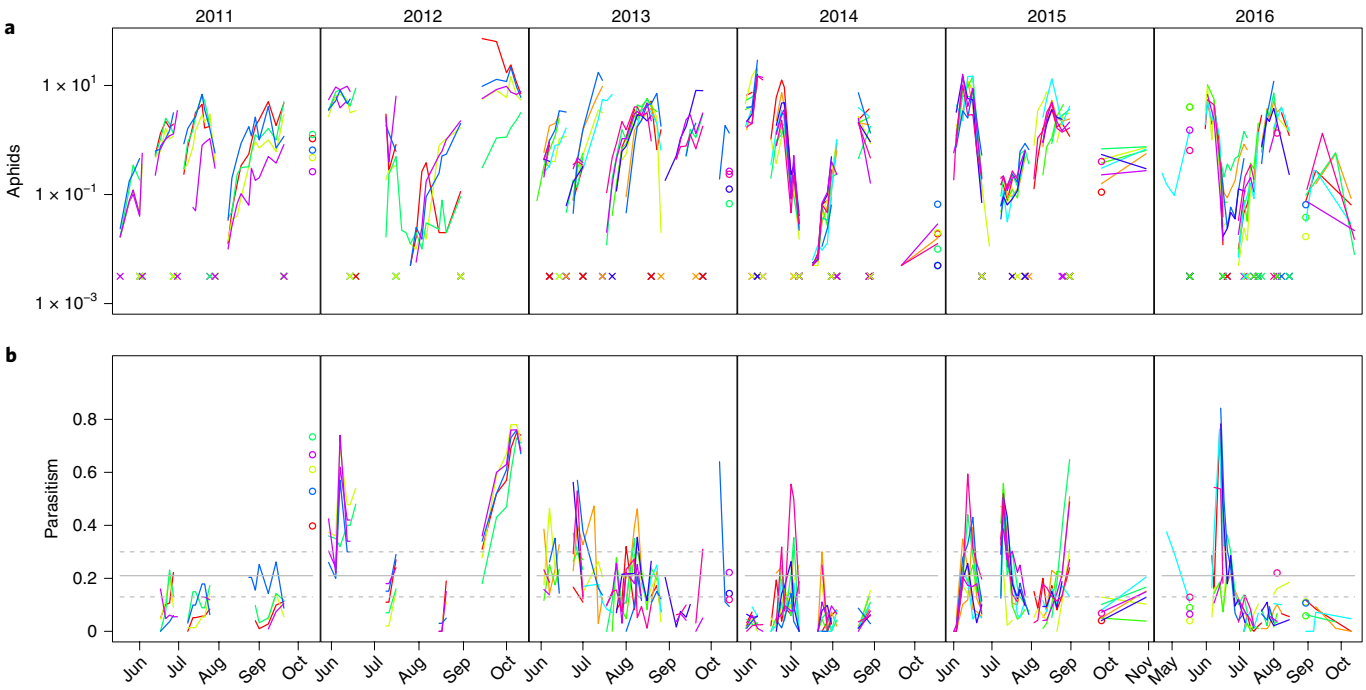

Fig. 2 | Dynamics of aphid abundance and parasitism from 2011 to 2016. a, Aphid abundances ($\log_{10}$ scale) during the growing season in five (2011–2012) and ten (2013–2016) fields per year. Harvesting is shown by crosses, periods without samples are shown as gaps and single samples per harvesting cycle are shown as circles. **b**, Proportion of fourth-instar and adult aphids containing parasitoid larvae; an average of 81 aphids were dissected at each sample. Dissections were not performed when densities were too low. The solid grey lines correspond to the level of parasitism above which selection favours resistant *Hamiltonella*–APSE3 clones assuming that the proportion of *Hamiltonella*-containing clones in the population is 48%, which is the average observed in genetic assays in the field (Fig. 3a); lower and upper dashed grey lines correspond to the maximum (0.88) and minimum (0.02) observed *Hamiltonella* frequencies (see Methods).

Resistance in the field. To assess evolution of resistance directly, we conducted field surveys for clones containing *Hamiltonella* from 2012 to 2016 in five to ten fields per year in early summer and again in autumn (but in 2012 only autumn). In these surveys, we could not initially distinguish between *Hamiltonella*–APSE3 and other *Hamiltonella* variants and therefore we aggregated all *Hamiltonella* variants (Fig. 3a). Clones containing *Hamiltonella* were found in every sample and there was remarkable variation both among fields sampled at the same time and within the same field sampled through time. This variation is much greater than expected if *Hamiltonella*-containing aphids were randomly (binomially) distributed among samples (variance inflation above binomial: $\sigma^2_\varepsilon = 0.37$, $\chi^2_1 = 88.6$, $P < 0.0001$).

The spatial and temporal variation in pea aphids containing *Hamiltonella* is consistent with strong selection for and against resistance. The known ecological effects of *Hamiltonella* are confined to protection against the dominant parasitoid at our study site, *A. ervi*. *Hamiltonella* provides little protection from the only other pea aphid–parasitoid at our field site, *Praon pequodorum*[31]. Therefore, variation in *A. ervi* attack rates is likely to be the main driver of variation in *Hamiltonella* frequencies. In addition, some variation in *Hamiltonella* frequencies could be due to association with other symbionts; for example, the X-type symbiont appears to reduce the fitness of pea aphid clones containing *Hamiltonella* and vertical transmission to parthenogenic offspring can be affected by symbiont co-infections[32,33]. *Hamiltonella* might also 'hitchhike' within clones that are being selected for traits that are unrelated to *Hamiltonella*[33,34]. To assess this hitchhiking effect, we analysed spatial and temporal variation in aphid colour morphs, green and red. This analysis uses the same aphids from the field survey (Fig. 3a). Of green and red aphids, 48% and 52% contained *Hamiltonella*, respectively, and hence colour was independent of whether clones contained *Hamiltonella*. Colour is likely to be under weak balancing selection[35] due to learned or innate colour preferences by parasitoids and some predators[36,37]. Nonetheless, we would expect selection for colour to be weak in comparison to selection for *Hamiltonella*-conferred resistance to parasitism. The spatial and temporal variation in colour was >0 ($\sigma^2_\varepsilon = 0.068$, $\chi^2_1 = 7.82$, $P = 0.005$), but much lower than for clones containing *Hamiltonella* ($\sigma^2_\varepsilon = 0.37$, bootstrap $P < 0.001$). This comparison implies that strong selection is responsible for the high variation in the frequency of aphid clones containing *Hamiltonella*.

To understand the field data in more detail, we extended the model we fit to the hoop house experiment for natural field conditions. The goal was not to fit the model to the field data because we lack knowledge about possible selection for or against resistance during periods of low aphid abundance (gaps in Fig. 2); we also have no estimates of immigration of *Hamiltonella*-containing clones, which would be particularly important during these low aphid-abundance gaps. Therefore, our goal instead was to simulate the model to ask whether it produces qualitatively similar patterns in the frequencies of resistant clones that we found from *Hamiltonella*-containing clones in the field (Fig. 3a). To extend the model to field conditions, we included the adult phase of the parasitoids so that they were dynamically coupled to the aphid dynamics. We also included dispersal of aphids and parasitoids among fields. To mimic agricultural management, we assumed that fields were harvested every 40 d, with equal numbers of fields harvested in each of the 40 d, so that harvesting was asynchronous. In the hoop house experiment, we used the harvesting treatment (asynchronous versus synchronous) to manipulate the interaction strength between aphids and parasitoids and expose eco–evo dynamics. For the simulation

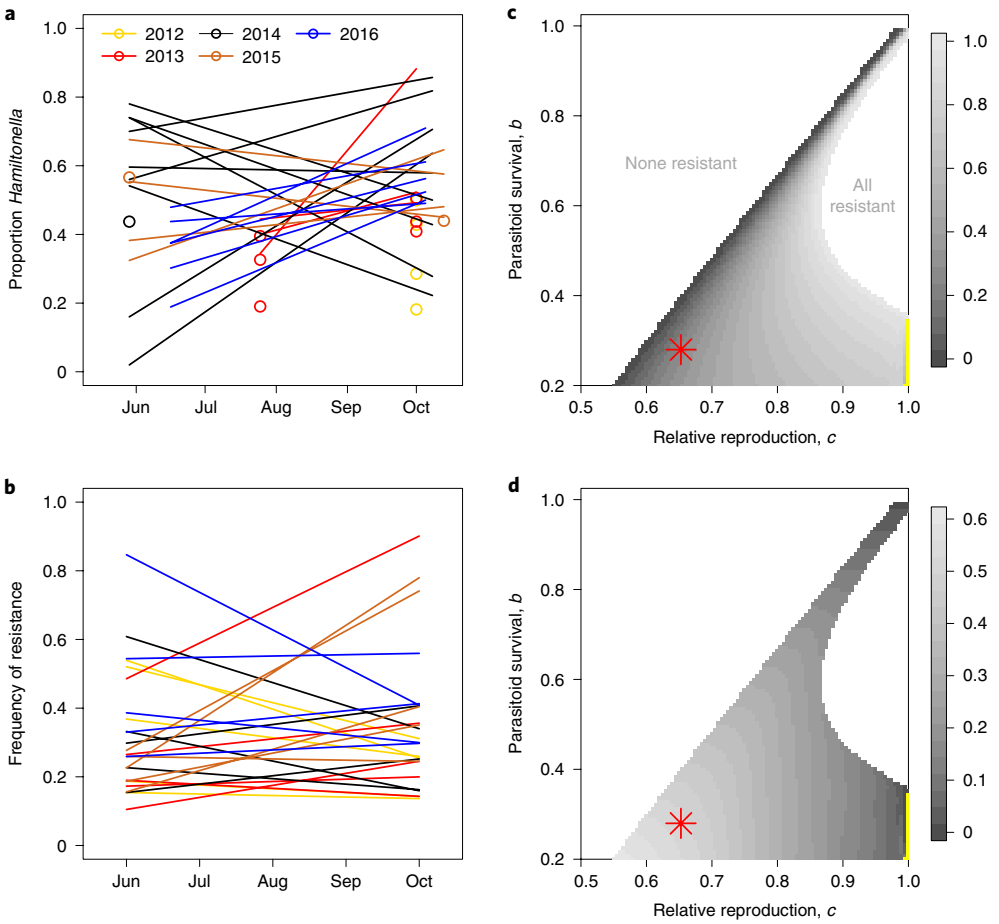

Fig. 3 | Field surveys of *Hamiltonella* and simulations of resistance. a, Proportion of pea aphid clones containing the *Hamiltonella* symbiont (both the APSE3 variant and other variants) in lucerne fields, 2012–2016. For fields sampled twice within a year, values are connected with a line. **b**, Example simulation for the frequency of resistant clones in five fields over 5 yr. Simulations use the parameter estimates from the hoop house experiment and are the same as in **c** and **d**. Consecutive simulated years are shown in different colours but these are not designed to correspond to years of the same colours in **a**; the simulations used parameter values from the model fit to the hoop house experiment but were not fit to the field survey data. **c**, Combination of values of costs and benefits of resistance for which regional balancing selection maintains both susceptible and resistant aphids in simulated populations. The model fit to the hoop house data was modified to include movement among fields and variation in the population growth rate of aphids among fields and through time; the magnitude of variation was selected to match that observed for aphids and parasitism in the field data (Fig. 2). The mean frequency of resistant clones was computed as the average from 40 simulated fields over 100 harvesting cycles. The red asterisk gives the estimates of relative mortality of resistant (*Hamiltonella*–APSE3) clones when parasitized (the benefit) and their relative population growth rate estimated from the hoop house experiment (the cost) (Fig. 1). The white region labelled 'none resistant' corresponds to parameter values in which the resistant clone goes to zero and the susceptible clone goes to zero in the region labelled 'all resistant'. When resistance is strong and costs of resistance are low, the resistant clones become sufficiently frequent to eliminate the parasitoid, in which case the resistant clones also go to zero (narrow yellow area at bottom right where *c* is close to 1). **d**, From the same simulations, the variation in the frequency of resistant clones (measured as the s.d. of the logit-transformed frequency) showing that high variation occurs when the benefits (reduced mortality, low *b*) and costs (reduced population growth rates, low *c*) are both large.

model, in contrast, any variation among fields will generate variation in selection pressures on aphids and we assumed asynchronous harvesting to mimic the regional variation in harvesting that occurs in agricultural systems.

The simulation model showed variation in resistant clones (Fig. 3b) of the same magnitude as the observed variation in *Hamiltonella*-containing clones (Fig. 3a). Despite this high variation, resistance neither vanished nor fixed in any field. This is because, in the absence of any environmentally driven variation in aphid or parasitoid demographic rates, the evolutionary dynamics of resistance at the regional scale show a stable equilibrium generated by the intertwining of ecological and evolutionary processes: when resistance increases, the parasitoid population declines, leading to selection against resistance and a return to equilibrium (Extended Data Fig. 7). At the scale of individual fields, however, the eco–evo dynamics cause high variation in the frequency of resistant clones.

The simulation model also shows that resistance could remain in the system over a broad range of benefits (reduced survival of parasitoid eggs) and costs (reduced parasitism-independent intrinsic rates of increase). The more effective the resistance (lower survival of parasitoid eggs), the more likely variation for resistance is maintained (Fig. 3c) and this does not require the benefits and costs of resistance to be closely balanced. The ease of picking parameters for the benefits and costs of resistance that give persistent resistance increases our confidence that eco–evo dynamics can be maintained in the pea aphid–*A. ervi* system. Finally, the highest spatiotemporal variation in resistance at the field scale occurs when the cost of resistance (reduced aphid reproduction, *c*) is relatively high; the cost

of resistance estimated by fitting the model to the hoop house data is high and is thus expected to generate high variation in resistance (Fig. 3d, red asterisks).

Discussion

Our large-scale hoop house experiment showed rapid evolution in pea aphids as the proportion of clones with *Hamiltonella*-conferred resistance increased when asynchronous harvesting favoured their parasitoid (Fig. 1). These evolutionary dynamics were followed by altered ecological dynamics in the next year, when populations of resistant aphids had low parasitism rates and high population growth rates. The experimental hoop houses isolated these populations, allowing us to see the eco–evo dynamics. Under open-field conditions, the eco–evo dynamics in many fields will be coupled through migration of both aphids and parasitoids, with variation in selection for and against resistance occurring at different times within the same field and at the same time in different fields (Fig. 2). This variation in selection is capable of driving high variation in the frequency of resistant aphid clones (Fig. 3a). Our model shows that the evolutionary variation we observed among fields is consistent with eco–evo dynamics; the model fit to the hoop house data shows high spatial and temporal variation among fields (Fig. 3b) and persistence of variation in resistance at the region scale (Fig. 3c,d).

Eco–evo dynamics such as those we have documented introduce new challenges for agriculture. Insect parasitoids are key in the biological control of agricultural pests and the supposed inability of pests to evolve resistance to biological control agents is often touted as an advantage over chemical insecticides. Our study joins Tomasetto et al.[38] as one of the few cases demonstrating the evolution of resistance of an agricultural pest to a parasitoid under field conditions[39,40]. If eco–evo dynamics such as we have shown in the pea aphid–*A. ervi* system are common in other pest systems, pest management strategies will need to consider evolutionary as well as ecological processes[41].

In classical biological control[42], an exotic pest is targeted by finding a control agent from the pest's established range. If the pest were like pea aphids, showing high variation in resistance in its established range and also high cost of resistance, then there is a practical lesson for classical biological control. A newly invading pest may lose all genetic variation for resistance if it is freed from its native parasitoids and the selection for resistance that they cause[43]. Therefore, an introduced control agent might be very effective initially. However, if new pest individuals that carried genetic variation for resistance were introduced, strong selection for resistance could diminish the initial effectiveness of biological control. Therefore, it would be a mistake to let down our guard against further introductions of that pest. Biosecurity programs aiming to limit new introductions of pests should also aim to limit recurrent introductions of new genetic variants of previously introduced pests that might carry resistance to biological control agents. In addition to reducing the chances of introducing resistance into the pest population, it might also be possible to maintain genetic variation in the control agent using augmentation biological control (continuous re-introductions of the control agent). This requires strains of control agents that can overcome pest resistance; while this does not appear to be the case for *A. ervi* and pea aphids, it might be a practical strategy in other systems[41,44,45].

Our results also give possible insight into conservation biological control, the use of management strategies that benefit control agents such as providing refuge habitat and food resources to parasitoids[46,47]. Evidence that providing habitat for biological control agents increases pest suppression is mixed[48] and our study suggests a possible explanation. Factors that benefit control agents will increase selection on pests for resistance, thereby seeming to counteract biological control conservation. Nonetheless, if resistance in the pests comes at a cost, as it does for pea aphids, then pest populations might decline even though there is little long-term change in the abundance of the control agent. This means that lack of increase in biological control agent populations, or even lack of increase in direct mortality they cause on the pest, might miss the impact that control agents have on pests due to increased cost of resistance through eco–evo dynamics. Eco–evo dynamics might make the success of conservation biological control programs go unnoticed.

The main lesson that we learned from our study was not that evolutionary processes occurred as rapidly as ecological processes; rapid evolution should be expected when selection is strong and genetic variation for resistance exists. Instead, the main lesson was that selection could generate large variation in resistance among fields (2–88% *Hamiltonella*-containing clones) while still maintaining variation in resistance at the regional scale. The maintenance of genetic variation is a key to understanding eco–evo dynamics[7,11].

Methods

***Hamiltonella* as a pea aphid trait.** *H. defensa* and other bacterial endosymbionts can be considered as part of the pea aphid extended genome, specifically as maternally inherited traits[34]. Vertical transfer from mother to parthenogenic offspring is very high[22]. In contrast, the rates of horizontal transfer among pea aphid clones and APSE among *Hamiltonella* strains, are likely to be very low. It is possible, however, to experimentally infect aphids with symbionts using syringes[49,50] and there is indirect phylogenetic evidence for horizontal transfer[22,51]. The most likely routes of horizontal transfer occur through parasitoid ovipositors (reported in black bean aphids, *Aphis fabae*[52]) and through host plants[49] (reported in wheat aphids, *Sitobion miscanthi*[53]). In pea aphids, however, horizontal transfer via these two routes is very unlikely to affect infection frequencies over ecological time scales. During sexual reproduction, there may be loss of symbionts from pea aphids during vertical transfer through the egg stage and also gain of symbionts through the male[54], although little is known under field conditions.

The mode of action of *Hamiltonella* to protect against parasitism is incompletely known. Soluble factors derived from APSE-infected *Hamiltonella* enter eggs and disable parasitoid development[55]. For APSE3, parasitoids never reach larval stages but, for some other phage variants (APSE2 and APSE8), larvae might survive to the first instar[56]. Because we assay parasitism by dissections recording only second- and third-instar parasitoid larvae, we do not detect unsuccessful parasitism due to *Hamiltonella*.

Molecular diagnostics for *H. defensa* and phage APSE. For both large-scale field experiment and long-term field data, we estimated frequencies of aphid infection with the protective symbiont *Hamiltonella*. We first conducted DNA extractions from single, whole aphids using E.Z.N.A Tissue DNA Kits (Omega Bio-Tek) following the manufacturer protocols. Then we performed diagnostic PCR with primers specific to *Hamiltonella*[57]. Samples testing positive for *Hamiltonella* were then tested with additional diagnostics to determine APSE phage type, which correlates strongly with the level of antiparasitoid protection provided[28]. To do this, we used PCR primers specific to the primary toxin associated with each APSE variant found in Wisconsin lucerne: APSE1 encoding a shiga toxin *stx* (stxF5′-GG TTAAAATGTATTCGCCACAAA, stxR5′- TGACGCGCTGCCCTTACTGT); APSE2 and APSE8 encoding distinct alleles of cytolethal distending toxin *cdtB*[2] (ref. [29] with primers from ref. [58]) and APSE3 encoding the YD-repeat protein (YDp-1F 5′CGGGCATACCTGTTTGGGCGT; YDp-1R 5′ CCCGGGAGCCTATC GCTCGT). For APSE2, APSE3 and APSE8, we conducted 35 cycles with a denaturing temperature of 95 °C (10 s), an annealing temperature of 58 °C (10 s) and extension at 72 °C (10 s), while for APSE1 we conducted 35 cycles with a denaturing temperature of 95 °C (1 min), an annealing temperature of 55 °C (1 min) and extension at 72 °C (2 min).

Hoop house experiment. The large-scale field experiment was conducted in four large hoop houses (7 × 30 m^2 field cage) in a lucerne field at the Arlington Agricultural Research Station. The hoop houses were covered with aphid-proof screening that was put into place on 24 June 2015. Lucerne was harvested from each hoop house on 25 June 2015 and plants were allowed to recover for 2 weeks before initiating the experiment. On 7 July 2015, we distributed evenly within each hoop house 2,000 aphids from four different aphid clones. Two clones (500 aphids each) contained *Hamiltonella*–APSE3, while the other two clones (500 aphids each) did not, and each pair of Ham+ and Ham– clones contained one red and one green clone. Although colour does not affect the expression of resistance, we used both red and green clones to guard against any other types of bias. Some species of ladybeetle predators show a colour preference[36] and *A. ervi* shows a learned preference for colour in which successful experience attacking, for example, green pea aphids increases the chance that they will then attack green relative to red clones[37]. After allowing the aphids to establish within hoop houses for 10 d, we stocked each hoop house with 200 *A. ervi* mummies (pupating parasitoids

within the exoskeleton of the aphid). Within each hoop house we established two $2\times2\times2\,m^3$ cube cages to serve as unharvested controls and evaluate differences among the hoop houses in factors other than harvesting.

The hoop houses were assigned to either synchronous or asynchronous harvest treatments in 2015. The two synchronous hoop houses were harvested on 11 August and 9 September using a walk-behind sickle mower to cut lucerne and rakes to remove the plants and attached insects. The two asynchronous hoop houses were first harvested on 27 July with the same technique and equipment but only half of the hoop house was harvested and the second half was harvested at the same time as the synchronous hoop houses (11 August); harvesting of half the hoop houses was repeated on 25 August and 9 September. The 7-m wide cages were divided into four strips lengthwise, with alternating strips harvested at the same time so that there were always two non-contiguous strips left unharvested (each $1.75\times30\,m^2$). We conducted aphid and parasitism surveys each week by counting the number of pea aphids and mummies visible on 500 lucerne stems per high tunnel.

On 8 November 2015, the hoop houses were sampled for aphids (sexuals at this time in the season) and mummies. The covers were removed from the hoop houses on 4 December so that snow accumulation would not damage them. After snowfall ceased and before aphids hatched from eggs in spring 2016, the screen covers were reinstalled on each hoop house (15 March). Surveys of aphids and parasitism resumed on 22 April and continued every 4–6 d until 11 May. An outbreak of an entomopathic fungus caused the aphid populations to crash and we terminated the experiment.

To obtain aphid samples for genetic analysis, following the counts of aphids and mummies on 23 September 2015, we used sweep nets to sample 100 adult aphids per hoop house; single individuals were taken in a grid with 2 m between sweeps to obtain a random sample of aphid clones. We performed similar samples on 22 April and 11 May 2016 but collected only 50 aphids. For the analyses, we computed proportions of resistant aphids after removing clones from the sample that contained symbionts other than *Hamiltonella*–APSE3 because the clones that we introduced into the hoop houses either had only *Hamiltonella*–APSE3 or were free of symbionts; therefore, clones containing non-*Hamiltonella*–APSE3 symbionts were present before the hoop houses were initially closed.

Two $2\times2\times2\,m^3$ unharvested cube cages were placed in each hoop house as controls to determine whether factors other than harvesting treatment and associated parasitism could explain differences in the selection for resistance seen among hoop houses. In repeated-measures linear mixed models on $\log(x+x_{min})$ transformed data, there was no statistical difference in aphid abundances between control cubes in hoop houses with different harvesting treatments ($P=0.38$) and the parasitism index was higher in control cages within hoop houses in the synchronous harvesting treatment ($P=0.046$); this is the opposite direction from what was outside the control cages in the synchronous hoop houses. Genetic sampling of the cube cages on 23 September 2015 (Extended Data Fig. 4) showed no significant difference in the number of *Hamiltonella*–APSE3 aphids relative to uninfected aphids according to harvesting treatment in the surrounding hoop house ($P=0.76$); the trend was for greater resistance in the cube cages that experienced greater parasitism (in the hoop houses that were synchronously harvested), as would be expected due to selection for resistance.

Field survey data. We conducted field surveys to measure pea aphid abundances and parasitism by *A. ervi* over most of the growing season from 2012 to 2016. Samples were taken over the summer from five to ten fields per year.

Samples of pea aphids consisted of sweep-netting fields in five haphazardly selected locations. The number of sweeps per location was determined by the aphid abundance and varied between three and 100. Samples were generally taken twice per week when weather permitted. After harvesting, the next sample was delayed by 1–3 weeks, depending on the rate of lucerne regrowth, because lucerne <10 cm cannot be sweep-netted effectively. All adult and fourth-instar aphids were collected and returned to the laboratory, where up to 100 per field were dissected to determine parasitism. Only parasitoid larvae in the second or higher instars were scored because eggs and first-instar larvae are too small for easy detection.

Genetic samples were collected by sweeping at 50 locations per field and selecting a single adult aphid at each location. Locations were spaced at least 5 m apart to increase the representation of separate clones within the samples. Samples were taken generally before the first harvest of the summer and after the last harvest, when aphid densities allowed a complete collection of 50 aphids.

Model of resistance fitted to hoop house experiment data. The statistical model was designed to estimate differences between aphid clones with and without the *Hamiltonella*–APSE3 in two key characteristics: their susceptibility to parasitism by *A. ervi* and their parasitism-independent intrinsic rates of increase. The hoop house experiment was conducted under uncontrolled, temporally varying abiotic conditions, extending from 7 July 2015 through 11 May 2016, and we would expect seasonal changes in the abundances of predators of aphids and parasitoids, such as by ground beetles within the hoop houses. Therefore, the modelling approach had to allow for changes in both intrinsic rates of increase and parasitoid attack rates through time. Rather than try to incorporate seasonality using 'hard' assumptions for these changing rates, we allowed estimates of these rates to change through time as inferred from the data. The model included fixed parameters to estimate the differences in parasitism-independent intrinsic rates of increase and susceptibility between aphid clones, assuming that clones within the same hoop houses experienced parallel changes in their intrinsic rates of increase and parasitoid attack rates.

We used a stage-structured model, because (1) pea aphid and parasitoid populations change rapidly, (2) the observed stage of the parasitoid (mummies) measured in the experiment appears with a delay following parasitism and (3) second- and third-instar aphids are most susceptible to parasitism[59]. The population growth of aphids is described by the Leslie matrix for the five aphid instars (stages):

$$L(c)=\begin{pmatrix}0.5 & 0 & 0 & 0 & 2.55c\\ 0.5 & 0.5 & 0 & 0 & 0\\ 0 & 0.5 & 0.5 & 0 & 0\\ 0 & 0 & 0.5 & 0.5 & 0\\ 0 & 0 & 0 & 0.5 & 0.8\end{pmatrix}$$

The time spent by aphids in each of the first four instars is on average 2 d and adults live on average 5 d (ref. [24]). The daily fecundity is $2.55c$ where c is a fixed constant equal to 1 for susceptible aphids and estimated in the model fitting for the fecundity of resistant relative to non-resistant aphid clones. The relative parasitoid attack rate on the five instars is $w=(0.12, 0.27, 0.39, 0.16, 0.06)$ (ref. [59]). The attacks on aphids are assumed to follow a negative binomial distribution, such that proportion of aphids in instar i escaping attack per day is $A_i=(1+a(t)w_i/k)^{-k}$, where $k=0.347$ is the aggregation parameter and $a(t)$ is the overall attack rate that will depend, in part, on the female adult parasitoid abundance[59]. We let $a(t)$ depend on time t to account for changes in the parasitoid attack rate over the course of the experiment; changes in $a(t)$ through time are determined by the data in the model-fitting process, as described below. To model resistance of aphids to parasitism, the estimated parameter b is the survival of parasitoids in resistant aphids; $b=1$ for susceptible aphids. Thus, for a susceptible aphid line, the proportion of aphids in instar i successfully parasitized per day is $1-A_i$, while the proportion of resistant aphids successfully parasitized is $b(1-A_i)$. To keep track of parasitoid larval development, we use a five-stage Leslie matrix, L_y, for eggs, three developmental instars and pupae (mummies); specifically:

$$L_y=\begin{pmatrix}0.5 & 0 & 0 & 0 & 0\\ 0.5 & 0.5 & 0 & 0 & 0\\ 0 & 0.5 & 0.5 & 0 & 0\\ 0 & 0 & 0.5 & 0.667 & 0\\ 0 & 0 & 0 & 0.333 & 0.95\end{pmatrix}$$

The time for parasitoid larvae to kill the aphid and form a mummy is on average 8 d and the average total generation time from oviposition to adult is 18 d (ref. [24]). The survival of successfully parasitized aphids is the same as unparasitized aphids until the parasitoid kills the aphid and a mummy is formed.

The model that describes the dynamics is:

$$\begin{aligned}\mathbf{X}_s(t)&=z(t)\mathbf{A}\,L(1)\mathbf{X}_s(t-1)+\varepsilon_s(t)\\ \mathbf{X}_r(t)&=z(t)(1-b(1-\mathbf{A}))L(c)\mathbf{X}_r(t-1)+\varepsilon_r(t)\\ \mathbf{Y}(t)&=z(t)L_y\,\mathbf{Y}(t-1)\\ \mathbf{Y}_1(t)&=z(t)(1-\mathbf{A})L(1)\mathbf{X}_s(t-1)+b(1-\mathbf{A})L(c)\mathbf{X}_r(t-1)\end{aligned}\quad(1)$$

where $\mathbf{X}_s(t)$ and $\mathbf{X}_r(t)$ are 5×1 vectors containing the abundances of aphid instars of the susceptible and resistant clones, respectively, at time t, $\mathbf{Y}(t)$ is the 5×1 vector containing parasitoid larvae in their five-stage classes and $\mathbf{Y}_1(t)$ is the first element of $\mathbf{Y}(t)$ which corresponds to eggs in attacked aphids. The horizontal 1×5 vector $\mathbf{A}$ contains the stage-specific terms A_i. The term $z(t)$ accounts for changes in survival and/or development time of aphids and parasitoids at time t; changes in $z(t)$ are determined from the data during the model fitting. The environmental error terms $\varepsilon_s(t)$ and $\varepsilon_r(t)$ are assumed to affect only the reproduction process (formation of first-instar aphids); all stages, and also the parasitized aphids, experience variance that is propagated from the first instars. We modelled process error in this way, because it generates covariance in the error variance for the different instars without explicitly having to incorporate parameters for covariance terms, which are difficult to estimate in the model fitting. Finally, because the model is iterated on a natural (rather than logarithmic) scale of abundances, the process errors $\varepsilon_s(t)$ and $\varepsilon_r(t)$ have variances $\sigma^2_e\,\mathbf{X}^2_{s,1}(t)$ and $\sigma^2_e\,\mathbf{X}^2_{r,1}(t)$ that are proportional to the squared abundances of first-instar susceptible and resistant aphids, respectively, where σ^2_e is a parameter scaling the overall variances.

The attack rate $a(t)$ and relative survival/development time $z(t)$ are themselves treated as state variables:

$$\begin{aligned}a(t)&=a(t-1)+\varepsilon_a(t)\\ z(t)&=z(t-1)+\varepsilon_z(t)\end{aligned}\quad(2)$$

The errors $\varepsilon_a(t)$ and $\varepsilon_z(t)$ are assumed to be normally distributed with variances σ^2_a and σ^2_z. The initial values of $a(t)$ and $z(t)$, denoted a_0 and z_0, were estimated in

the model fitting[60]. To allow for differences in the parasitoid attack rate between harvesting treatments, we included the parameter Δ_a to give the difference in attack rates between synchronously and asynchronously harvested hoop houses: thus, $a(t)$ in the equations above is replaced by $\Delta_a a(t)$ for the asynchronously harvested hoop houses.

We fit the model first to the data from all hoop houses in 2015 to estimate the benefits (the probability that a parasitoid larva is killed within an aphid host and hence the aphid survives, $1-b$) and costs (the proportional reduction in fecundity, $1-c$) of resistance. The model was fit by maximum likelihood in state–space form using a Kalman filter[61]. The measurement equations for the state–space model are:

$$\begin{aligned}&\text{aphids}(\tau) = \sum_{i=1}^{5}\mathbf{X}_{\text{s},i}(\tau) + \sum_{i=1}^{5}\mathbf{X}_{\text{r},i}(\tau) + \sum_{i=1}^{4}\mathbf{Y}_i(\tau) + \alpha_a(\tau)\\&\text{mummies}(\tau) = \mathbf{Y}_5(\tau) + \alpha_m(\tau)\\&\text{proportion resistant}(T) = \text{Binomial}\left(\sum_{i=1}^{5}\mathbf{X}_{\text{s},i}(T), \sum_{i=1}^{5}\mathbf{X}_{\text{r},i}(T)\right)\end{aligned}$$

The number of observed aphids at sample τ is the sum of all aphids regardless of their stage or resistance, or whether they are parasitized. The measurement error uncertainty, $\alpha_a(\tau)$, is normally distributed with s.e.m. equal to the observed s.e.m. from the data (since five replicate measurements were taken at each sample). The measurement equation for mummies was similar. For genetic samples to determine the proportion of aphids that were resistant and susceptible (with and without *Hamiltonella*–APSE3), we assumed that aphids were randomly sampled at sample T, leading to a binomial measurement distribution.

Model simulations. To understand the dynamics and persistence of both resistant and non-resistant aphids in field populations, we simulated the model fit to the hoop house experiment modified to include an adult parasitoid stage and aphid and parasitoid dispersal. Mummies from the process equations (equation (1)) develop into parasitoid adults and the parasitoid attack rate a is assumed to be proportional to the density of adult parasitoids. In the stage-structured process equations, the duration of individuals within each stage follows a geometric distribution which has a long tail; some individuals can live a very long time. Therefore, to match the roughly 20-d generation time of the parasitoid, the transition rate from mummy to adult was decreased from 0.95 in the fitted model to 0.85 in the simulation model and adult survival was set to 0.5. Because we explicitly modelled parasitoids, we fixed the attack rate $a(t) = 2.25$, a value that gives a mean parasitism rate in the model equal to that in the survey data. A 'carrying capacity' was given to the aphids by multiplying the aphid density-independent per capita population growth rate by $\exp(-S(t)/K)$, where $S(t)$ is the sum of aphid abundances among all instars and clones at time t; K is only a scaling term and the dynamics are independent of the assigned value[24]. To incorporate the effects of periodic harvesting, 40 fields were modelled, each harvested every 40 d in a staggered pattern. Harvesting was assumed to kill 99% of aphids (including parasitized aphids) and all mummies[62]. Emigration of adult aphids and parasitoids from fields were assumed to be 0.01 and 0.05 d^{-1}, with dispersing individuals evenly redistributed among all 40 fields. The dispersal rate for aphids was based on estimates of the proportion of aphid adults that possess wings and therefore can engage in long-range dispersal, and the dispersal rate for parasitoid adults was based on observations[63]. Finally, log-normal random variation with a s.d. of 0.25 was added to aphid survival to account for variation among fields.

For Fig. 3, we simulated 4,000 d (100 harvesting cycles) without seasonality and computed the mean frequency of resistance (Fig. 3c) and the s.d. of the logit-transformed frequency of resistance (Fig. 3d) while varying the costs and benefits of resistance (parameters b and c). To compare with the field survey data, we selected five fields in the simulations that were harvested on consecutive simulated days because the survey fields were often harvested within a few days of each other; Fig. 3b gives the frequency of resistance in these five fields in the last five 'years' of the simulation, where 'year' is taken as 120 d (corresponding to the growing season). The simulations with the selected parameters give a reasonable caricature of the field survey data. Specifically, the s.d. in log aphid abundance and logit-transformed parasitism were 0.78 and 0.62, compared to corresponding values from the survey data of 0.71 and 0.60.

We acknowledge that many biological features of the natural system are not included in the simulation model, such as seasonality and variation in the abundances of other predators. Nonetheless, the qualitative results that (1) persistence of resistant and susceptible aphids occurs over a wider range of values of costs and benefits when these costs and benefits are higher (Fig. 3b) and that (2) variation in the frequency of resistance at the field scale increases with increasing costs and benefits (Fig. 3d) were found for every set of parameter values that we evaluated, such as the aggregation of parasitoid attacks on aphids, k, and the dispersal of aphids and parasitoids among fields.

Estimating thresholds for selection for resistance. The field surveys provide measurements of the abundance of pea aphids and the percentage parasitism obtained through dissection. We approximated the threshold of parasitism above which selection favours resistance from the model fit the data from the hoop house experiment. Specifically, we computed the per capita population growth rates of susceptible and resistant aphid clones, r_s and r_r, given the parasitoid attack rate a. We treated these per capita population growth rates as the short-term population-level fitness. Therefore, if $r_s > r_r$, resistance is selected against, and if $r_s < r_r$, resistance is favoured. It is not possible, however, to estimate the attack rate a from only the percentage parasitism in field. This is because the observed percentage parasitism depends not only on the attack rate a but also the level of resistance in the population: if resistance is high, then there must be a high attack rate a to give the same level of parasitism that would be observed in a susceptible population with a low value of a. Therefore, we calculated the attack rate, and the values of r_s and r_r, conditional on the actual (and unknown) relative abundances of susceptible and resistant clones, denoted Q. By selecting different values of Q, we then bound the possible thresholds of observed parasitism which favour resistance.

To compute r_s and r_r for a given value of Q, we used equation (1) to calculate the stable-stage distributions of both unparasitized and parasitized aphids. The per capita population growth rates of the susceptible and resistant clones, r_s and r_r, are given by the leading eigenvalues from the matrices $A\,L(1)$ and $(1-b(1-A))\,L(c)$ (ref. [64]), giving values of r_s and r_r. We then combined the matrix $A\,L(1)$ with L_y and computed the leading eigenvector to give the relative proportions of susceptible aphids that are parasitized at the stable age distribution given a value of a (ref. [64]). Similarly, matrices $(1-b(1-A))\,L(c)$ and L_y were combined to give the relative proportion of resistant aphids that are parasitized. From these, we calculated percentage parasitism for susceptible and resistant aphids, p_s and p_r, as the proportions of fourth-instar/adult aphids containing second-/third-instar parasitoid larvae to correspond to the observed dissection data. Thus, equation (1) gives values of p_s and p_r, and also values of r_s and r_r, for given values of the attack rate a. Although we cannot distinguish susceptible from resistant clones in the field survey data, if we assume that the relative abundance of susceptible and resistant clones is Q, then the observed percentage parasitism is $Qp_s + (1-Q)p_r$. We then work backwards: from the observed percentage parasitism and Q, we calculate relative values of p_s and p_r; from these we estimate the attack rate a and this gives the relative values of r_s and r_r. In Fig. 2b, the grey clones in the panels for parasitism are computed with $Q = 0.88$, 0.48 and 0.02, which give the maximum, mean and minimum values of Q observed in the field surveys (Fig. 3a).

Variation in the frequency of clones with *Hamiltonella* among fields. The genetic surveys of aphids containing *Hamiltonella* showed high variation among fields sampled at the same time (Fig. 3a). To quantify this variation, we fit a logit normal-binomial generalized linear mixed model:

$$\begin{aligned}&\text{logit}(p) = b_0 + \text{Sample}_i + \varepsilon\\&\mathbf{Y} = \text{Binomial}(N, p)\end{aligned}$$

where p is the probability of an aphid clone to contain *Hamiltonella*. To account for differences among fields sampled at different times, fields were grouped by the categorical fixed-effects variable Sample_i corresponding to each sample event; thus, Sample_i absorbs variation in the mean number of *Hamiltonella*-containing clones sampled at different times. The variance in ε gives the extent to which the variance among fields exceeds the variance that would be expected if *Hamiltonella*-containing clones were randomly distributed (according to a binomial distribution). The statistical test for greater-than-binomial variation is a likelihood ratio test between this model and the model in which the variance of ε is zero; this test was highly significant ($\sigma^2_\varepsilon = 0.37$, $\chi^2_1 = 88.6$, $P < 0.0001$).

As a comparison, we performed the same analysis comparing red and green aphid clones among fields using the same survey data. Aphid colour is a single-locus, biallelic character[65]. Previous work showed that colour is under stabilizing selection[35], although this stabilizing selection is weak in comparison to the selection quantified in the hoop house experiment. The variation in colour among fields was statistically significant but much lower than for *Hamiltonella*-containing clones ($\sigma^2_\varepsilon = 0.068$, $\chi^2_1 = 7.82$, $P = 0.005$). Furthermore, the bootstrapped difference between σ^2_ε for colour and *Hamiltonella*-containing clones was 0.36 ± 0.12 ($P = 0.0004$).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Experimental and observational data that support the findings of this study have been deposited in figshare.com at https://doi.org/10.6084/m9.figshare.11828865.v1.

Code availability

Codes used for analyses in this study have been deposited in figshare.com at https://doi.org/10.6084/m9.figshare.11828865.v1

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Author contributions

A.R.I., K.O., B.T.B., J.P.H. and V.C.R. were involved in the conceptualization of this project. A.R.I. and K.O. conducted the formal analysis. A.R.I., J.P.H., K.O. and V.C.R. obtained funding. B.T.B., R.M.P., K.L.K., A.R.I., J.P.H. and K.O. carried out investigations. A.R.I., B.T.B., R.M.P. and K.O. were responsible for the project adminstration. A.R.I. wrote the original draft and all authors were involved in editing and reviewing.

Competing interests

The authors declare no competing interests.

Additional information

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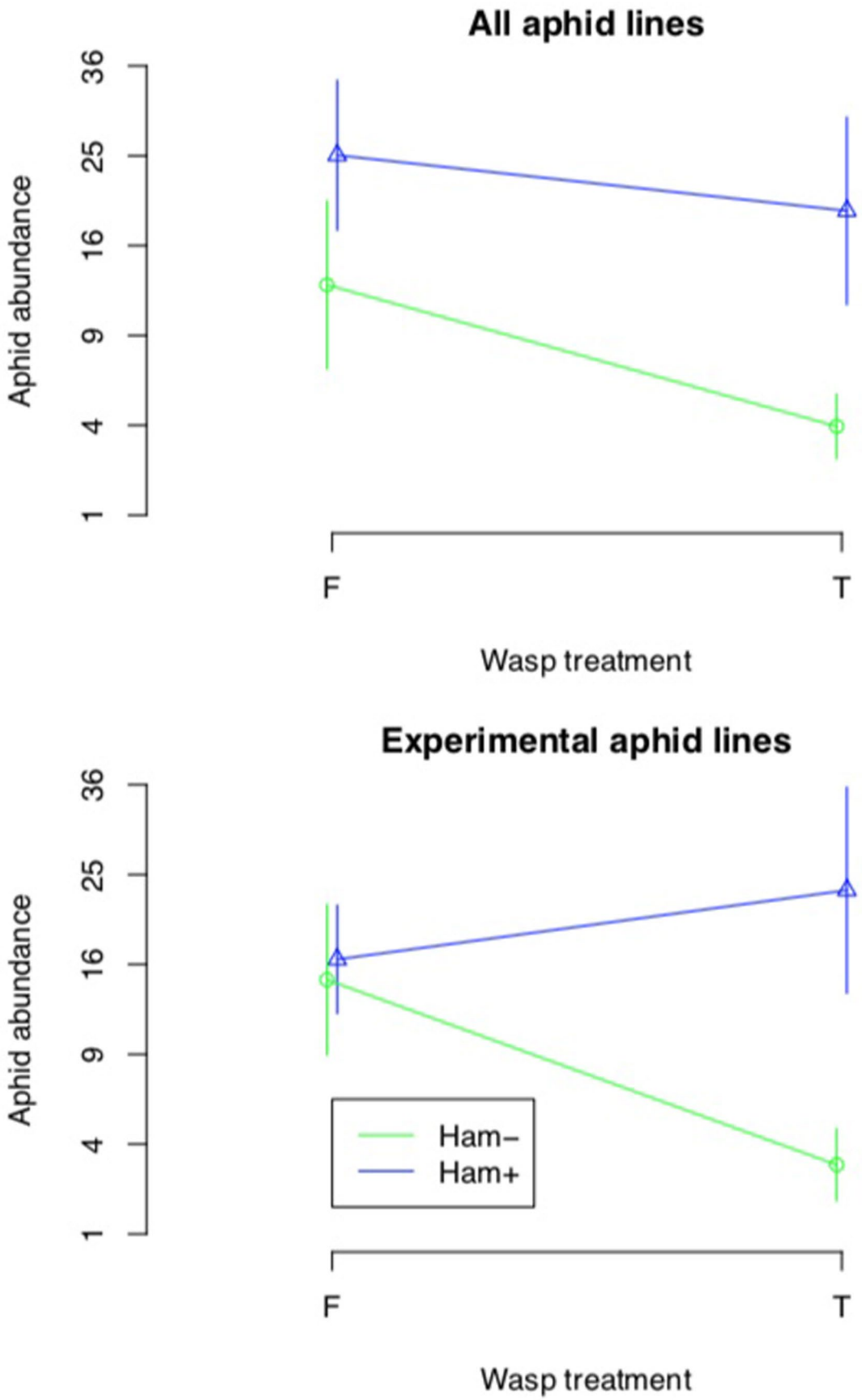
Extended Data Fig. 1 | Field experiment hoop houses. a, Photograph of a hoop house experimental screen cage with lucerne harvested asynchronously in four strips (photo credit A. R. Ives). **b**, Google Earth satellite image of the four hoop houses in September, 2010. (A different experiment was being performed that compared treatments within and outside the hoop houses).

Random effects	Variance
focal line	0.54
competing line	0.42
observation	0.78

Fixed effects	Estimate	z	Pr(>|z|)
intercept	2.52		
Ham+	0.29	0.45	0.65
parasitoid+	-1.33	-3.11	0.00185
Ham:parasitoid	1.43	2.45	0.0144

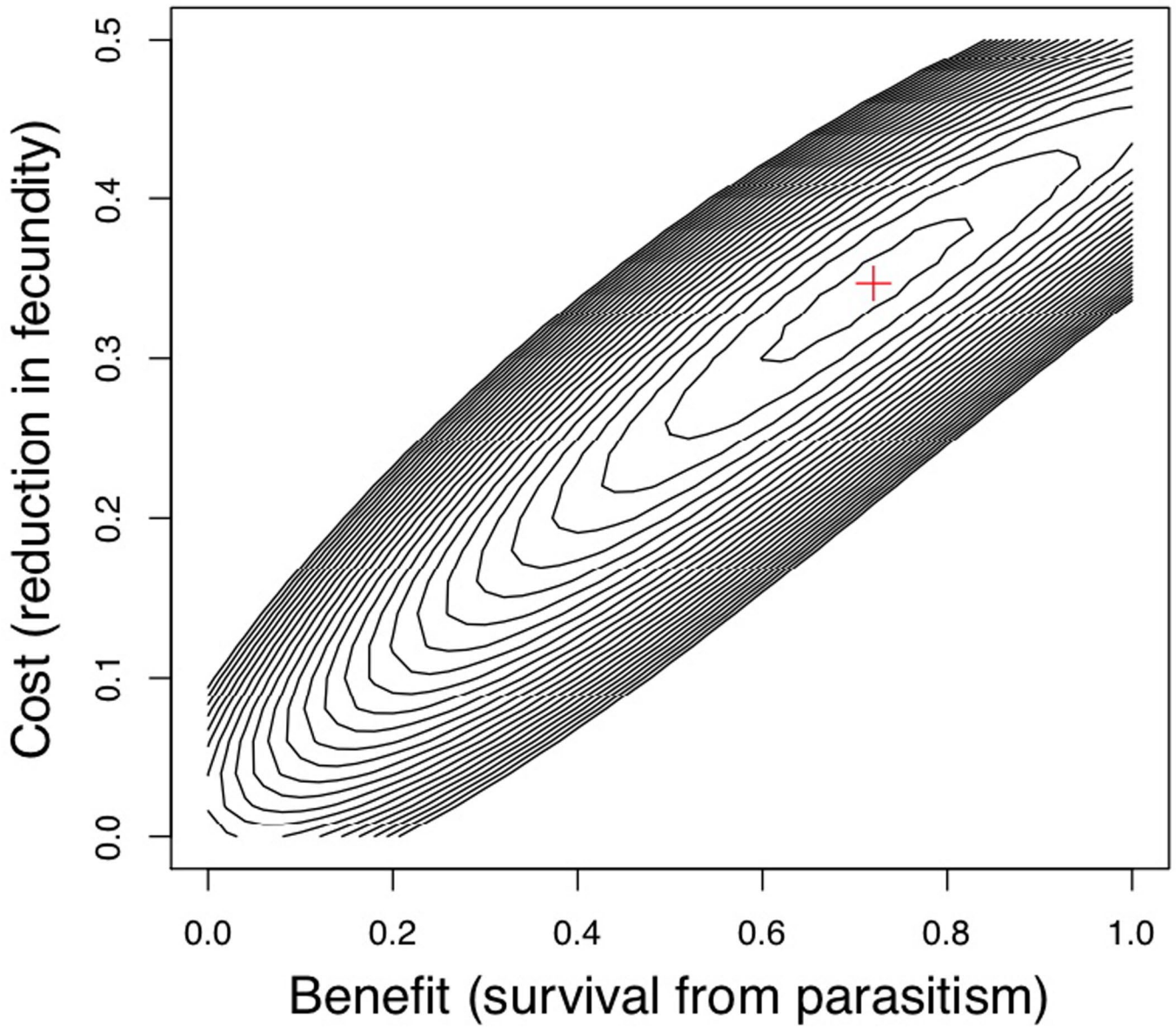
Extended Data Fig. 2 | Assay for phenotypic resistance of aphid clones. Normal-Poisson GLMM for the number of aphids recovered in the assays for resistance depending on whether the line contains or does not contain *Hamiltonella*-APSE3 (Ham+ or Ham-) and whether parasitoids were added (parasitoid+) or not (parasitoid-), with random effects for the focal line, the competing line and the observation. See Supplementary Information: 'Assays for resistance in experimental aphid clones'.



Extended Data Fig. 3 | Experiment to measure phenotypic resistance among *Hamiltonella*-containing aphid clones. In a field experiment investigating resistance to parasitism, final aphid abundances for clones with and without *Hamiltonella*-APSE3 (Ham+ and Ham-) when parasitic wasps were not supplemented (F) or were supplemented (T). In the top panel all trials are included, whereas in the bottom panel only trials containing the clones used to inoculate the hoop houses are included. See Supplementary Information: 'Assays for resistance in experimental aphid clones'.

sample	hoop house	treatment	APSE1	APSE2	APSE3	uninfected	total
2015	1	sync	13	26	6	54	99
2015	2	async	4	14	51	28	97
2015	3	sync	8	9	7	66	90
2015	4	async	0	4	63	35	102
2015 cage	1	sync	3	0	9	2	14
2015 cage	1	sync	1	8	8	8	25
2015 cage	2	async	0	0	17	16	33
2015 cage	2	async	0	0	5	5	10
2015 cage	3	sync	0	0	5	1	6
2015 cage	3	sync	0	1	4	10	15
2015 cage	4	async	0	0	5	2	7
2015 cage	4	async	0	1	8	2	11
2016 early	1	sync	0	10	1	39	50
2016 early	2	async	0	5	21	24	50
2016 early	3	sync	0	2	0	48	50
2016 early	4	async	0	4	24	22	50
2016 late	1	sync	0	17	2	77	96
2016 late	2	async	0	3	39	54	96
2016 late	3	sync	0	7	1	88	96
2016 late	4	async	0	4	52	40	96

Extended Data Fig. 4 | Results from symbiont assays. Numbers of assayed pea aphids containing *Hamiltonella* and the numbers containing no symbionts in hoop houses and cages within hoop houses at three sampling dates. See 'Hoop house experiment'.



Extended Data Fig. 5 | Likelihood function for the model fit to hoop house data from summer-autumn 2015 graphed for the benefits and costs of resistance. The benefit of resistance is the probability that an aphid attacked by a parasitoid kills the parasitoid egg, given by (1—*b*) in the fitted model. The cost of resistance is the proportional reduction in fecundity, given by (1—*c*). The maximum likelihood estimates of both parameters are marked by the red cross and the contour lines are at intervals of ΔlogLik = 5.99/2; 5.99 is the value of a chi-square distribution with df = 2, so the first contour corresponds to the joint approximate 95% confidence interval given by a Likelihood Ratio Test.

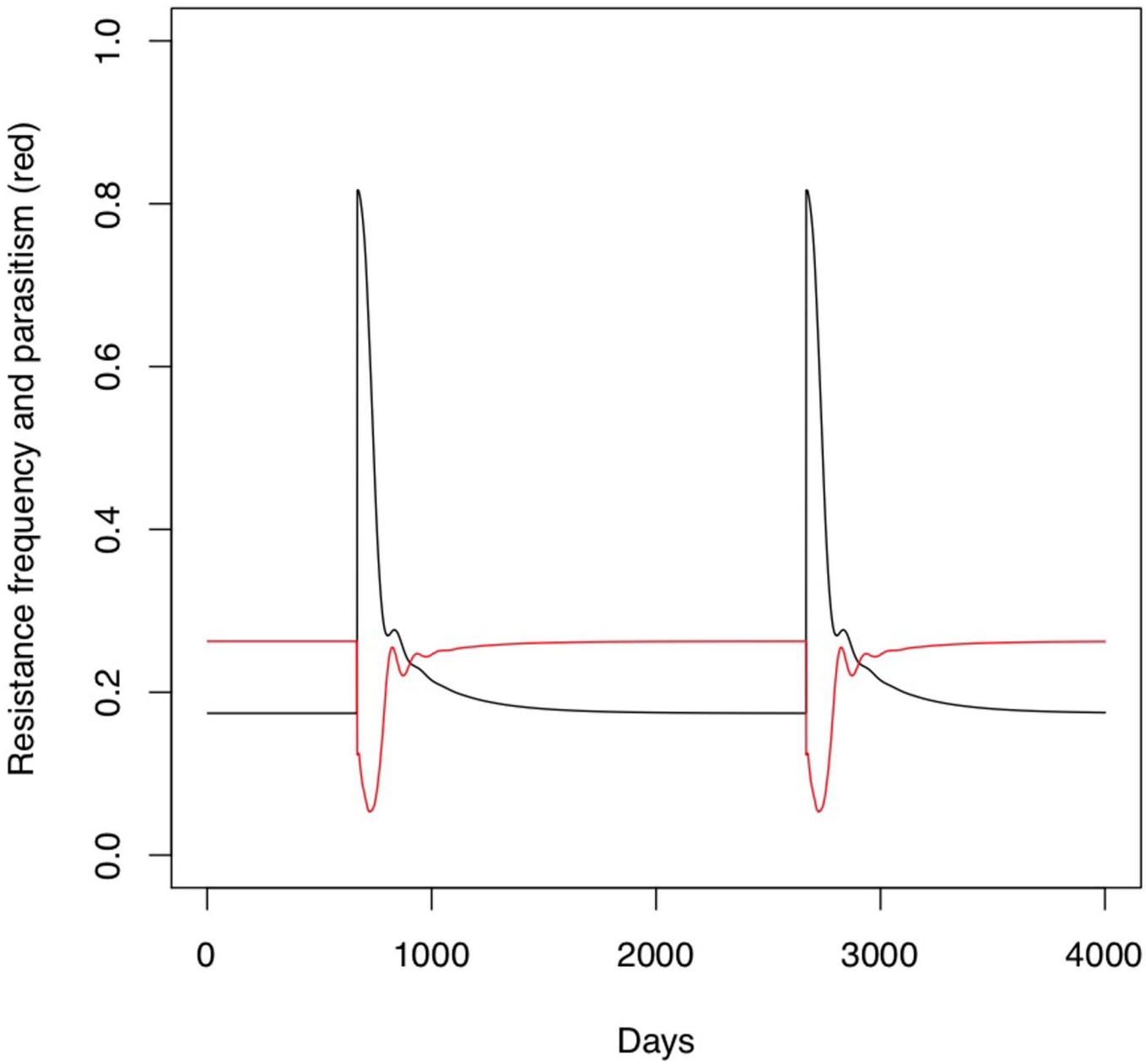
(A)

Factors	χ^2	df	Pr(>χ^2)
Date	10.40	1	0.00126
Hoop house	167.79	3	<<0.001

(B)

Factors	χ^2	df	Pr(>χ^2)
Proportion in fall	0.23	1	0.63
Treatment	1.79	1	0.18

Extended Data Fig. 6 | Analyses of changes in the proportion of pea aphid clones containing *Hamiltonella*-APSE3 relative to uninfected clones between 23 September, 2015, and 22 April, 2016. a, Binomial ANOVA analysis of the proportion of aphid clones with Hamiltonella-APSE3, showing a decrease in infections over winter. **b**, Binomial ANOVA analysis of the proportion of aphid clones with Hamiltonella-APSE3 in spring including the proportion infected in autumn as a predictor, showing that harvesting treatment (synchronous versus asynchronous) does not affect this change. See Supplementary Information: 'Hoop house experiment—Additional statistical analyses (ii) Levels of resistance'.



Extended Data Fig. 7 | Regional stability of the simulation model showing the frequency of resistant aphid clones (black line) and the proportion parasitism (red line) calculated from all 40 simulated fields. To illustrate regional dynamics of the simulation model (Fig. 3), we removed the log-normal variation in aphid survival within fields and iterated the model for 4000 days. On days 667 and 2667, a perturbation was applied in which the proportion of the resistant clone was sharply increased. This caused parasitism to decrease, and with decreased parasitism selection favoured the non-resistant clone. After roughly 1000 days the proportion of resistant clones and proportion parasitism returned to their regional equilibrium values.

parameter	2015 estimate	2016 estimate
c	0.65	-
b	0.28	-
a_0	–2.04	–2.77
z_0	0.87	1.01
σ^2_a	0	0
σ^2_z	0.00014	0.00064
σ^2_e	0	0.11
Δa	1.33	–2.67
$\rho\sigma^2_m$	4.86	-
x_0	-	6.65
y_0	-	27.5
ρy_0	-	0.003

Extended Data Fig. 8 | Model parameter estimates. Model parameter estimates. Parameter estimates from the state-space model fit to the hoop house experiment for summer-autumn 2015 and spring 2016. See 'Model of resistance fitted to hoop house experiment data' and Supplementary Information: 'Model of resistance fitted to hoop house experiment data—Model fitting'.

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Study description	The study involves field experiments, field observation data, and mathematical simulations. The statistical analyses include nonlinear state-space models, hierarchical models, and simpler statistics. Full details are given in the Methods and Supplementary Information.
Research sample	Populations of Acyrthosiphon pisum and Aphidius ervi were sampled in Dane Co, Wisconsin. The extent of the population is unknown but probably represents the upper Midwest.
Sampling strategy	Sampled sizes for field experiments and observations were as large as feasible.
Data collection	Data were collected by Anthony Ives, Brandon Barton, Rachel Penczykowski, Jason Harmon, and Kyungsun Kim. In addition, field experiment data were collected by Ian Chen, Sebastian van Bastelaer, Sal Divita , and Pat Uphues. The long-term field data were collected by about 15 undergraduate students.
Timing and spatial scale	Full details are given in the Methods and Supplementary Information.
Data exclusions	No data were excluded.
Reproducibility	All observational and experimental data used in this work are publicly available on figshare.com. All of the computer code used for analyses and plotting are available with the data. The identifier for data and code is https://doi.org/10.6084/m9.figshare.11828865.v1.
Randomization	N/A
Blinding	No formal data blinding was used.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Experiments and observations were performed over five years from April to October.
Location	Experiments and field observations were performed at the Arlington Agricultural Station, WI. 43°18’9.47"N 89°20’43.32' W
Access and import/export	No permits were needed or obtained.
Disturbance	No disturbance was made.

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