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Experimental assessment of the impact of rapid evolution on population dynamics

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ABSTRACT

Background: It is generally assumed that short-term population dynamics are too slow to be influenced by evolution.

Question: Can evolution occurring within only weeks (four to five generations) impact concurrent population dynamics?

Organism: Green peach aphid (*Myzus persicae*) growing on an undomesticated host (*Hirschfeldia incana*). Aphid clones were collected from a single wild population.

Site of experiment: Greenhouse experiment lasting 4 weeks.

Methods: A preliminary experiment found that aphid clonal lineages differed by up to 17% in intrinsic growth rate (fitness). Using a subset of clones, we manipulated the amount of genetic variation in intrinsic growth rate within replicated aphid populations by manipulating aphid clonal composition. We compared the population dynamics of evolving populations (clonal frequencies free to vary) with that of non-evolving controls (populations without clonal variation).

Results: Two of the three evolution treatments rapidly evolved by changing in clonal frequencies. These evolving populations grew 28–34% faster, reaching higher densities, compared with non-evolving control populations.

Keywords: aphids, clonal selection, contemporary evolution, eco-evolutionary dynamics, ecological genetics, evolution enlightened management, experimental evolution, population growth rate.

INTRODUCTION

Few studies or models of short-term population dynamics consider the possibility that evolution could alter population parameters during the study period (as noted by Thompson, 1998; Fussmann *et al.*, 2003; Levins, 2003). For example, life tables are commonly used to estimate intrinsic growth rate, a key parameter in models forecasting population dynamics (Kocourek *et al.*, 1994; Guldemond *et al.*, 1998; Ro and Long, 1999). Although some studies take into account changes in population parameters due to changes in age structure or spatial distribution, they rarely

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consider the possibility that there could be genetic variation for life table parameters or that changes in these parameters over time might be attributable to evolution (e.g. Cappuccino and Price, 1995; Sibly and Hone, 2002), even though some ecologists have advocated such a consideration for half a century (Pimentel, 1961, 1968; Chitty, 1967; Anderson and King, 1970; Berry, 1979). Assertions as to the importance of evolutionary change in short-term population dynamics are, however, increasing in frequency and are now supported by some empirical data (Hairston *et al.*, 2005; Saccheri and Hanski, 2006; Kinnison and Hairston, 2007; Hughes *et al.*, 2008; Pelletier *et al.*, 2009; Schoener, 2011).

The justification for ignoring evolution in short-term ecological studies has been based on two assumptions. First, populations are often assumed to be genetically homogeneous, at least in traits that impact population dynamics (Roughgarden, 1979; Cappuccino and Price, 1995). Yet, within-population genetic variation in ecologically important traits has been demonstrated repeatedly since the 1960s (reviewed by Ayala, 1968; Berry, 1979). Moreover, such standing genetic variation can have a significant influence on population dynamics in the laboratory (Schlager, 1963; Leips *et al.*, 2000) and in nature (Hazell *et al.*, 2006; Saccheri and Hanski, 2006). Second, evolution is often assumed to occur on a much slower (and thus separate) time scale (i.e. over hundreds or thousands of generations) than short-term ecological processes (i.e. over a dozen or fewer generations) (Slobodkin, 1980). Thus, even if genetic variation in traits is considered, evolution of these traits is ignored because it is perceived to be too slow to have an ecological effect (Endler, 1991; Thompson, 1998; Hairston *et al.*, 2005). Studies falsifying this assumption by identifying 'rapid evolution' occurring on 'ecological time', often within a few generations, in both natural and human disturbed environments, have recently become common (see reviews in Dyer, 1968; Thompson, 1998; Hendry and Kinnison, 1999; Bone and Farres, 2001; Reznick and Ghalambor, 2001; Ashley *et al.*, 2003). Yet evolution might be considered to be 'rapid' by several criteria, often implicitly (as discussed in Hendry and Kinnison, 1999). Here we use Hairston and colleagues' (2005) definition whereby evolution is deemed rapid only if it has a large impact on concurrent ecological dynamics relative to other ecological factors (e.g. resource availability). Thus an important and yet unresolved issue is whether such evolution actually impacts short-term ecological dynamics (Saccheri and Hanski, 2006; Kinnison and Hairston, 2007; Pelletier *et al.*, 2009; Schoener, 2011).

Theoretical models have shown that rapid evolution in population parameters can significantly alter population growth trajectories (Pimentel, 1961; Anderson and King, 1970; Sinervo *et al.*, 2000; Fussmann *et al.*, 2003; Duffy and Sivars-Becker, 2007). These effects could be straightforward. For example, if a genotype with a higher growth rate becomes more common, it could accelerate the whole population's growth rate. On the other hand, more complex effects are also possible. For example, a common genotype's relative fitness advantage might decrease with its increasing density or frequency, thus altering the growth of an evolving population (Agrawal, 2004). Because of such potentially non-intuitive interactions, it is important to experimentally assess the impact of rapid evolution.

Some of these modelling studies suggest that evolution might only impact ecological dynamics under certain conditions (Ellner *et al.*, 2011). Both the ecological context (e.g. community composition, level of disturbance) and evolutionary context (e.g. amount of genetic variation present, rate of evolution, mechanism of evolution) could influence the strength of the impact of rapid evolution on population dynamics. For example, Yoshida *et al.* (2003) showed that rapid evolution within their rotifer–algal chemostat system causes predator–prey cycles to become almost perfectly out-of-phase and not one-quarter out-of-phase as predicted by non-evolutionary ecological theory. Yet this predator–prey cycling in chemostats is highly dependent on nutrient flow and will not occur if the dilution

rate is too low or too high (Shertzer *et al.*, 2002). This implies that empirical studies over a range of conditions are required to quantify the importance of rapid evolution in population dynamics.

A small but growing number of eco-evolutionary dynamics experimental studies have begun quantifying the impact of rapid evolution on population dynamics (reviewed by Fussmann *et al.*, 2007). Bohannan and Lenski (2000) observed changes in mean density, and a fluctuation in density in both bacteria and phage, as the bacteria evolved resistance to this phage. Fussmann *et al.* (2003) used a combination of modelling and an experimental verification to show that rapid evolution of asexual reproduction in a rotifer qualitatively changed population dynamics within 30 days. Without evolution the populations had a single peak in density, then crashed; however, with evolution a second peak in density occurred. Other studies have experimentally compared the population dynamics of evolving populations with those that cannot evolve due to (1) replacement of the population with unselected individuals (Pimentel *et al.*, 1963; Pimentel and Al-Hafidh, 1965; Pimentel, 1968), (2) a lack of genetic variation (Yoshida *et al.*, 2003; Agashe, 2009), or (3) because the non-evolving population is in an environment without the key selective pressure, such as predation (Agrawal, 2000; terHorst *et al.*, 2010). These studies demonstrate that evolutionary dynamics can influence the dynamics and outcome of short-term ecological phenomena and argue convincingly for causality.

Surprisingly, there have been no experimental studies in which evolution has been manipulated and its impact on population dynamics quantified in plant–insect interactions. Plant–insect interactions are thought to be one of the most common and important ecological interactions, generating much of the species and phenotypic diversity in nature as well as having immense economic importance (Ehrlich and Raven, 1964; Futuyma and Agrawal, 2009). Short-term eco-evolutionary dynamic interactions seem likely given a growing body of research showing that plant genetic factors influence insect communities on individual plants ['community genetics' (reviewed in Whitham *et al.*, 2006; Hughes *et al.*, 2008)]. This lack of research is striking given that Wallner (1987), in his highly cited review of the causes of insect pest outbreak, strongly advocated for a consideration of the role of evolution. The ecological effects of rapid evolution in plant–herbivore systems could differ greatly from those observed in predator–prey or host–parasitoid interactions for two reasons. First, the patterns of population dynamics often differ. In many plant–herbivore systems, especially in agricultural pests, the pest dynamics are often in a non-equilibrium state consisting of very low densities until large outbreaks occur (reviewed in Wallner, 1987; Karley *et al.*, 2004), as opposed to equilibrium or predator–prey cycles observed in the eco-evolutionary dynamics studies listed previously. Second, herbivores might have a weaker effect on host population dynamics than would a predator on predator–prey dynamics because herbivores do not necessarily kill their host. The magnitude of impact of insect herbivores on plant population dynamics has been debated for years (Crawley, 1989) and only in the last decade have a dozen or so studies found strong evidence for an impact of insect herbivores on plant population dynamics (Maron and Crone, 2006).

Our goal in this study was to develop a model plant–insect herbivore system to study how rapid evolution through natural selection acting on genetic variation present within natural populations impacts concurrent population dynamics. We selected the generalist green peach aphid (*Myzus persicae*) and a local wild invasive annual mustard host (*Hirschfeldia incana*). We first characterized genetically and ecologically an aphid population by collecting and maintaining multiple clonal lineages to quantify variation in fitness as measured by intrinsic growth rate. To study the impact of rapid evolution on population

dynamics, we then experimentally manipulated the aphid population's genetic composition and evolutionary potential by controlling which clones were present in replicated populations. By selecting different pairs of clones, we thus altered the level of genetic variation in an ecologically important trait and could thereby test how the evolutionary context might alter the impact of rapid evolution on concurrent population dynamics. Our main objectives were to test the following hypotheses and predictions: (1) If rapid evolution impacts population dynamics, then the observed population dynamics of evolving aphid populations will differ significantly from those of non-evolving aphid populations. (2) If evolutionary context is important, then the impact of rapid evolution on population dynamics will differ between the different evolution treatments (different pairs of clones). (3) If rapid evolution in aphids impacts their host plant, then plant fitness will differ significantly when exposed to evolving versus non-evolving aphids.

MATERIALS AND METHODS

Study system

Myzus persicae is considered the world's most important crop pest, due to its enormous host-range (over 40 families of plants) and its ability to transmit over 100 plant viruses (Mackauer and Way, 1976; Blackman and Eastop, 2000). As a consequence, its life history and ecology are well studied. It is highly amenable to experimental evolution because of its short generation time (5–10 days), ease of culture, and the large magnitude of genetic variation in multiple traits (Vorburger, 2005). This aphid is a cyclical parthenogen; in the fall it undergoes one generation of sexual reproduction so that the following spring populations are replete with multiple clonal lineages that reproduce asexually until the fall (Mackauer and Way, 1976). Aphid populations rapidly evolve through natural clonal selection within months, changing gene frequencies and mean trait values (Via and Shaw, 1996; Vorburger, 2006). This parthenogenetic lifestyle permits the experimental manipulation of the genetic variation within a population by controlling the initial frequency of clones therein, which permits the experimenter to manipulate the rate of evolution. We selected the short-pod mustard *Hirschfeldia incana* (Brassicaceae), formerly *Brassica geniculata*, as a host species. This mustard, probably of Mediterranean origin, has invaded Western Europe, Australia, New Zealand, and the South Western United States (Horovitz and Galil, 1972). Although these species have been interacting locally for a limited time, they have probably been interacting for long periods of time given this aphid's global distribution, including the Mediterranean, and its ability to grow on many species of Brassicaceae (Mackauer and Way, 1976).

Population sampling, clone identification, and ecological characterization

In March 2008, we collected 22 adult female apterous (non-winged) *M. persicae* feeding on *H. incana* from the University of California Motte-Rimrock Reserve in Perris, California. Isofemale colonies were established on individually caged *H. incana* seedlings under 16 h light/8 h dark conditions that maintain asexual reproduction (Blackman, 1974). Using six published microsatellite markers (Sloane *et al.*, 2001; Wilson *et al.*, 2004), we identified 10 unique aphid clonal lineages (see evolutionary-ecology.com/data/2658App.pdf for detailed mircosatellite methods in Appendix 1, and the aphid clonal lineages' unique genotypes in Appendix 2).

For adaptive evolution to occur within a population composed of different clones, the clones must differ in relative fitness. Fitness was measured with a preliminary experiment by measuring clonal intrinsic growth rate (r_m), which is a good index of absolute fitness in aphids because populations typically grow exponentially and then crash as their host plant senesces (Wallner, 1987; Karley *et al.*, 2003, 2004). Intrinsic growth rate varies greatly between aphid clones within and between species (Weber, 1985; Vorburger, 2005). It is also a key component in predictive models used in integrative pest management (Guldemond *et al.*, 1998; Ro and Long, 1999). Fitness during the asexual phase is a key component in yearly fitness because clones with lower rates of reproduction during the summer months are eliminated from the population or contribute less to the sexual reproduction phase in the fall. Intrinsic growth rate, although a good indicator of absolute fitness, does not account for interactions between aphid clones and might not predict relative clone fitness when mixed.

We extracted r_m for each aphid clone by fitting population growth models to exponentially growing populations (Vehrs *et al.*, 1992; Guldemond *et al.*, 1998) (see evolutionary-ecology.com/data/2658App.pdf for detailed methods in Appendix 3). This experiment revealed significant clonal variation in intrinsic growth rate (see Appendix 4 at evolutionary-ecology.com/data/2658App.pdf; $P = 0.002$). Clonal lineages differed by as much as 17% in r_m, which gives a range in doubling time of 41–48 h. Over a period of 10 days of exponential growth, the fastest clone should reach 85% higher population size than the slowest clone (see Appendix 4 at evolutionary-ecology.com/data/2658App.pdf). For the focal experiment, we selected three clones that differ in growth rate, which we coded as A, B, and C.

Design of focal experiment

Evolving and non-evolving *M. persicae* populations on *H. incana* plants were studied in a partly cooled greenhouse (mean daytime temperature = 31°C, range = 16–47°C; mean nighttime temperature = 19°C, range = 13–30°C). Asexual reproduction was maintained by using additional lighting providing 16 h light/8 h dark (Blackman, 1974). To minimize genotype × genotype interactions, plants used in the experiment were grown from the seeds of a single *H. incana* individual collected in 2008 at the Motte-Rimrock Reserve. Such genotype × genotype interactions might be important in nature but to properly quantify them would require a second much larger experiment. These seeds were planted in 4-L pots, using University of California Riverside soil mix III, a sand/peat moss mix supplemented with micronutrients, and watered every 3 days. Cages were constructed to individually house each plant. Cages consisted of an 8-L pot with a wire frame creating a 75-cm high dome that supported thin transparent mesh (Bridal Organza, #664-7242, Jo-Ann Fabrics and Crafts). On Day 0 of the experiment, the 6-week-old seedlings in the rosette stage, approximately 10–15 cm in diameter, were inoculated with one of the seven different aphid treatments by placing 20 third instar aphids onto each plant. Replicates were initiated on three consecutive days starting 1 October 2009. On Day 1, missing aphids were replaced with fourth instar aphids. We established seven aphid treatments and each was assigned in a randomized block design and replicated 10 times.

Three evolution treatments consisted of aphid populations (on a single plant) that have two different clones and we created all three two-way combinations of clones A, B, and C (A–B, B–C, and A–C). Based on the ranking of growth rate in preliminary experiments, we initiated these evolving populations with 5 individuals of the faster growing clones and 15 individuals of the slower growing clones. The A clone in the A–B and A–C evolution

treatments as well as the B clone in the B–C evolution treatment represented 25% of the initial clonal frequency. These mixed populations have genetic variation in fitness (e.g. the r_m of clone A is greater than that of clone C) and thus could evolve by changing in clonal frequency (away from the 25% : 75% initial ratio). Three different non-evolution (pure clone) treatments received 20 aphids of only one of the three clones. Because all individuals were of the same genotype within these pure populations, gene frequencies could not change barring novel mutations, thus preventing evolution. Finally, a 'no-aphid control' treatment did not receive any aphids.

Rates of evolution

On Day 28, we collected aphids from every population to track changes in clonal frequencies (evolution). Between 16 and 24 aphids from each population were genotyped (for a total of 497 aphids). All aphids were genotyped for three of the six microsatellite loci. This was done in multiplex PCR reactions identical to those used to genetically characterize all clones (see Appendix 1 at evolutionary-ecology.com/data/2658App.pdf), except for the use of a mixed primer solution that contained all primers. Signal strength was normalized with primer concentrations of 1, 3, and 5 μM for loci myz2, M40, and M86 respectively. For each treatment, we calculated the frequency of the faster clone for each replicate and then determined whether the mean frequency of the faster clone differed significantly from the initial clonal frequency of 25% using one-sample *t*-tests on raw data. We also explored whether the frequency of clones when mixed could be predicted from the population dynamics observed in the pure clone treatments. Thus we compared the observed frequency of clones on Day 28 with the expected frequency determined by the ratio of corresponding pure clone densities on that day using one-sample *t*-tests.

Aphid population dynamics

Aphid population dynamics were quantified by counting all aphids on Days 3, 7, 10, 14, 17, 21, 24, 28, and 33, and all counts were made by MMT. When populations rose above 2000 aphids per plant, they were sub-sampled by counting one half of every leaf. Plant senescence caused the aphid populations to crash after Day 28, and so Day 33 results were excluded. Residuals, for each treatment, from an exponential population growth model suggested that exponential growth lasted until Day 14. Afterwards populations grew linearly, as determined by fitting a separate linear model on this portion of the time-series, Days 14–28.

The exponential growth phase, Days 0–14, was analysed with a linear mixed-effect (LME) model where the dependent variable was $\ln(x)$ transformed number of aphids, the fixed effect was aphid treatments, and day was the main covariate. Because the repeated aphid counts on the same plant violated the assumption of independent observations, we set unique plant identity as a random effect on population growth rate and intercept and used an autoregressive correlation error structure (Pinheiro and Bates, 2000). We modelled increasing variance through time by using a variance function that increases with the power of the variance covariate (varPower). Block (day of initiation of the replicate and spatial position in the greenhouse) and initial plant rosette diameter, measured on Day 3, did not improve model fit and were not included in the final model. For the linear growth phase, Days 14–28, the same LME model was applied except that the number of aphids was not

ln(x) transformed. All analyses were implemented in R v.2.11.1 (R Development Core Team, 2009) using the 'nlme' package (Pinheiro *et al.*, 2011).

The impact of evolution on population dynamics

Our objective was to statistically test the impact of changes in clonal frequency on concurrent population dynamics. Ideally, one would compare the observed aphid population dynamics in the evolution treatment to that of a non-evolving mixed population containing the same two clones that remain at a frequency of 25% : 75%. This is impossible since clones will change in frequency because of fitness differences. We thus generated the expected population growth parameters of such a non-evolving population by using the pure aphid treatments. We tested three *a priori* null hypotheses that the population growth rate (slope) and density (intercept) do not differ between each evolution treatment and their corresponding pure treatments (e.g. A–C evolution treatment vs. pure A and pure C treatments). We did so with the use of planned contrasts that were orthogonal comparisons between a subset of the aphid treatment levels within the LME analysis. We tested different hypotheses by assigning weight coefficients to treatment levels. We set the planned contrast coefficients of the no-evolution expectation to match those of the initial clonal frequency (e.g. pure A = −0.25 and pure C = −0.75) and these were compared with the A–C evolution treatment (weight = +1) such that the sum of all weights was zero. Appendix 5 (see evolutionary-ecology.com/data/2658App.pdf) shows a table with the planned contrast weights for each hypothesis. Thus differences in growth rate or density between the evolution treatment and the no-evolution expectation represent the impact of changes in the frequency of clones (rapid evolution) on population dynamics.

Host plant fitness

Finally, to quantify the impact of aphid rapid evolution on its host's fitness, we measured the above-ground dry biomass of the plants at the end of the experiment as a proxy for host fitness (Mitchell-Olds and Bradley, 1996). We fit a general linear model on ln(x) transformed plant weight measurements. The factors were aphid treatment, block, and initial plant size (rosette diameter on Day 3). The interactions between these factors were non-significant and thus removed from the final model. We again used weighted planned contrasts, proportional to the pure clone treatments, to determine whether plants with evolving aphid populations were smaller than expected from no-evolution treatments.

RESULTS

Pure clone treatments

Pure clone treatments differed in their population dynamics as the rank order of growth rates changed throughout the experiment (Fig. 1). In the exponential phase (Days 0–14), the B clone grew fastest (5.6% faster than A) and the A clone was second fastest (4.8% faster than the C clone; Fig. 1a). In the linear growth phase (Days 14–28), the B clone grew significantly slower than the other two clones. The A clone grew 80% faster and the C clone grew 37% faster than the B clone, respectively, and the A clone grew 12% faster than the C clone (Fig. 1b).

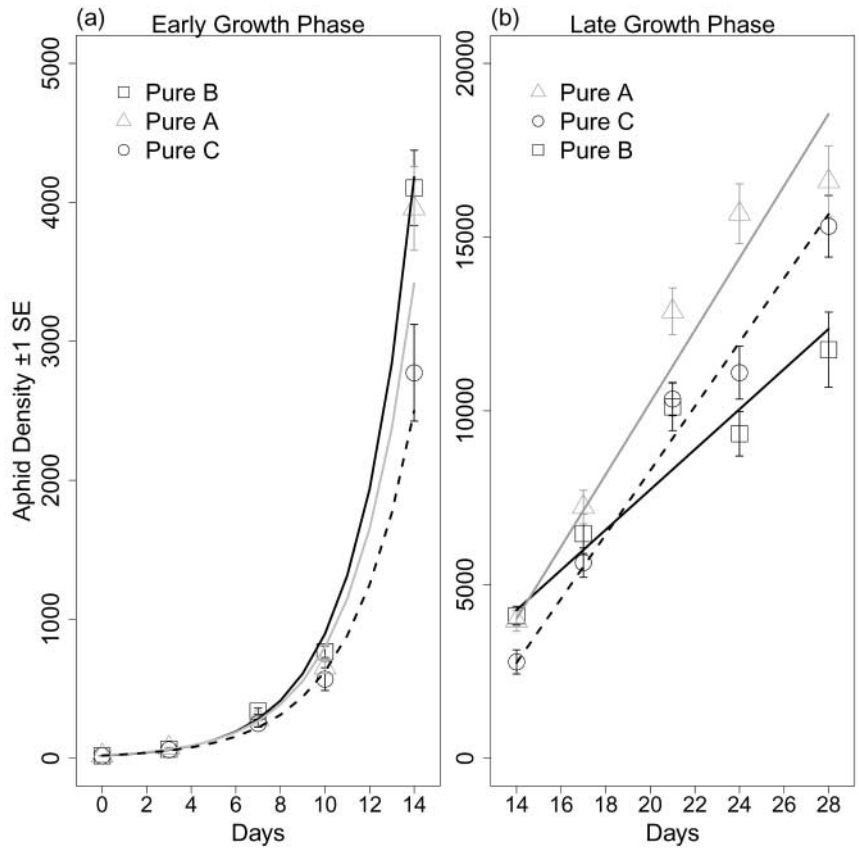


Fig. 1. Population dynamics of pure clonal treatments. Values represent mean number of aphids (± 1 standard error) through time separated into two periods for easier visualization: (a) the early growth phase (Days 0–14) and (b) the late growth phase (Days 14–28). The y-axes differ between panels.

Evolution treatments – genetic analyses

On Day 28, we tested for changes in the frequency of clones in the evolution treatments away from the initial frequency of 25% : 75%. Although the final frequency of clones in the A–B evolution treatment did not differ on Day 28 (frequency of A clone = 23%, one-sample *t*-test, $P = 0.63$; Fig. 2), it is possible that frequencies increased then decreased before Day 28. We did document significant evolution on Day 28 for the B–C and A–C evolution treatments as the frequency of the C clone decreased. The B clone reached 44% ($P = 0.031$) and the A clone reached 47% ($P = 0.001$), almost doubling their initial frequency of 25% (Fig. 2). These mixed populations evolved differently than expected based on the final density of the corresponding pure clones (Table 1). The A clone in the A–B evolution treatment did not increase in frequency as expected, whereas the A clone in the A–C treatment and the B clone in the B–C treatment evolved significantly faster than expected (Table 1).

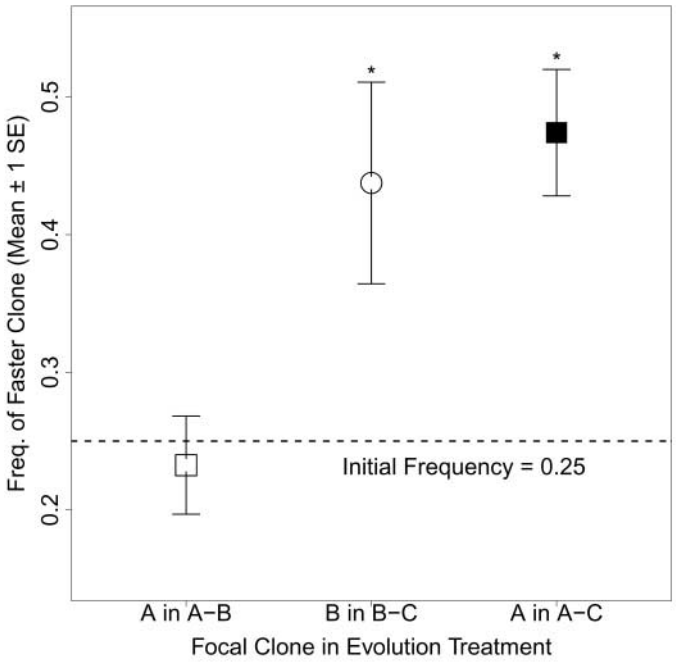


Fig. 2. Rapid clonal evolution as shown by the mean frequency of the faster growing aphid clone on Day 28 in each evolution treatment (± 1 standard error). The x-axis shows which clone's frequency is illustrated in each evolution treatment. Dashed horizontal bar indicates initial clonal frequency of 25% and asterisks indicate significant divergence from initial frequency.

Table 1. One-sample *t*-test analyses comparing observed frequencies of faster growing clone in each evolution treatment on Day 28 to expected clonal frequencies based on pure clone densities on Day 28

Evolution treatment (focal clone in population)	Observed frequency	Expected frequency	*t*-value	d.f.	*P*-value
A in A–B	0.233	0.32	–2.45	9	0.036
B in B–C	0.437	0.204	3.19	9	0.011
A in A–C	0.474	0.265	4.55	8	0.002

Note: All *P*-values are for two-tailed tests. Significant *P*-values imply that observed frequencies are not predictable from pure clone treatments.

Impact of aphid evolution on aphid population dynamics

To test the impact of rapid evolution on concurrent population dynamics, we compared the observed population dynamics in evolving populations to that observed in both corresponding pure treatments by using planned contrasts proportional to the initial frequency of clones (i.e. population dynamics without evolution). For example, in the exponential growth phase the A–C evolution treatment grew with an exponential rate of 0.321, which is 8.5% slower than the expected growth rate of a population at a constant

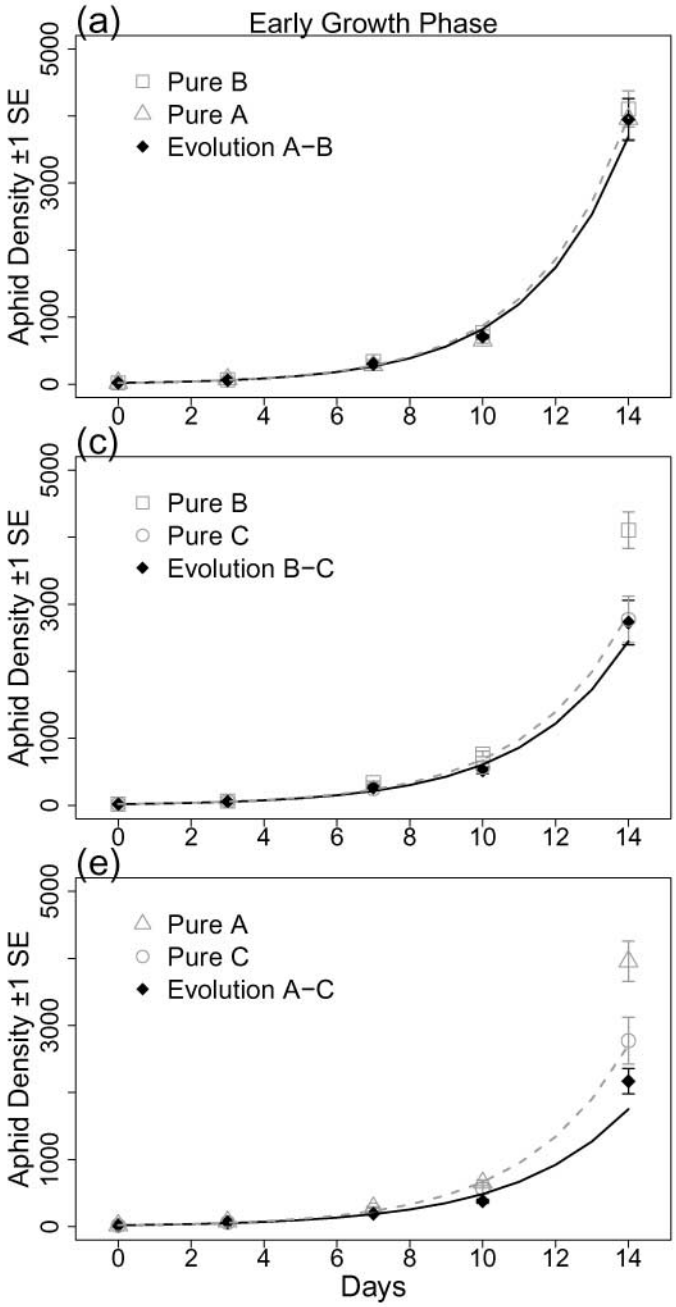
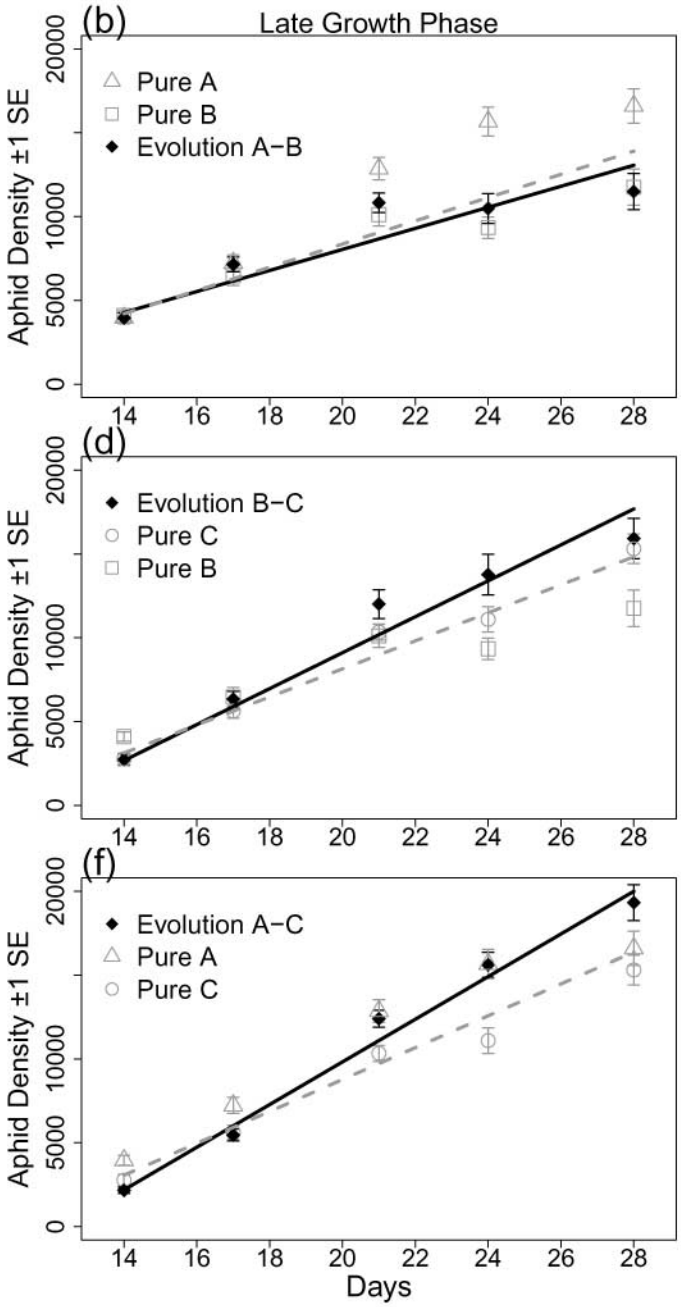


Fig. 3. Population dynamics of the three observed evolution treatments (black diamonds) with the best-fit model from LME analysis (black line). The dashed grey line represents the best-fit model that combines both pure treatments using the constant (non-evolving) frequency of clones



(25% : 75%). For each treatment we added the corresponding pure clone treatments (grey symbols) used to generate the no-evolution expectation. Values represent mean number of aphids (± 1 standard error) and the y-axes differ between left and right panels. Evolution treatments have two letters.

Table 2. Planned contrasts from a linear mixed-effect model comparing each type of evolving population with its corresponding no-evolution expectation, generated from the pure aphid treatments following the initial frequency of clones (see Methods for details)

Evolution treatment (clones)		Days 0–14				Days 14–28			
		d.f.	*t*	*P*	% change	d.f.	*t*	*P*	% change
A–B	Intercept	50	−0.51	0.613	−2.6	50	0.16	0.875	+1.7
	Slope	216	−0.38	0.703	−0.9	215	−0.77	0.440	−9.4
B–C	Intercept	50	−0.77	0.448	−3.7	50	−1.33	0.187	−14.4
	Slope	216	−0.89	0.375	−2.2	**215**	**2.67**	**0.008**	**+28.2**
A–C	Intercept	50	−0.35	0.723	−1.8	**50**	**−2.77**	**0.009**	**−28.5**
	Slope	**216**	**−3.22**	**0.002**	**−8.5**	**215**	**3.41**	**0.001**	**+33.8**

Note: The percent change represents the change in slope or intercept from the non-evolving expectation to that of the observed evolution treatment. Thus positive changes represent higher values in the evolution treatments. Slope represents the rate of growth of aphid populations and intercept represents density at the start of each time period. All *P*-values are for two-tailed tests. Significant results are shown in bold for ease of identification.

(non-evolving) frequency of 25% (for clone A with a growth rate of 0.363) and 75% (for clone C with a growth rate of 0.347), which has an expected growth rate of 0.351.

In the exponential phase (Days 0–14), the only evolution treatment that differed in population growth rate from its no-evolution expectation was the A–C treatment (Fig. 3a, c, e, Table 2). In the second growth phase (Days 14–28), the A–B evolution treatment did not differ from the no-evolution expectation in either intercept (density on Day 14) or growth rate (Fig. 3b, Table 2). Evolution in the B–C treatment did not alter density on Day 14 but significantly accelerated population growth rate compared with the no-evolution expectation afterwards (+28.2%, $P = 0.008$, LME; Fig. 3d, Table 2). Finally, although the A–C evolution treatment grew slower in the exponential stage leading to a significant decrease in density at Day 14 (−28.5%, $P = 0.009$), evolution significantly accelerated population growth rate in the second growth phase (+33.8%, $P < 0.001$; Fig. 3f, Table 2).

Impact of aphid evolution on host plant fitness

Aphid feeding severely reduced plant size. The no-aphid control plants were five times heavier than plants with aphids ($P < 0.001$). Final plant weight did not differ significantly between the three evolution treatments and their corresponding no-evolution expectations (ANOVA, all *P*-values > 0.1). Although in certain treatments evolution led to higher aphid densities, this did not magnify the impact of aphids on the host plant's above-ground biomass.

DISCUSSION

We present a new natural system to study eco-evolutionary dynamics, in which we experimentally assessed the impact of rapid evolution on concurrent ecological dynamics in

an insect herbivore. We found that rapid evolution, occurring within weeks, significantly accelerated population growth rates and density as clonal frequencies changed. The presence of aphids had a large and significant impact on plant growth, but faster-growing evolving aphids did not damage their host more than non-evolving aphids. These results have important implications for the study of population dynamics and pest management.

Over the course of only 28 days, approximately 4–5 aphid generations, natural selection significantly altered aphid clonal frequencies in two of the three evolution treatments. Similar changes have been observed in non-experimental aphid infections in greenhouses (Fuller *et al.*, 1999), as well as in wild populations (de Barro *et al.*, 1995; Vorburger, 2006).

The density of pure clone treatments on Day 28 did not accurately predict the observed frequency of clones in the evolution treatments (Table 1). This suggests that growth rate differences measured in the pure clone treatments was not the only mechanism influencing evolution when clones were mixed. Other potential mechanisms include clonal interactions and density- or frequency-dependent growth or competition. Some aphid clones are known to influence each other's growth (Rochat *et al.*, 1999) and some aphid species show intraspecific variation in the strength of density-dependent growth (Agrawal *et al.*, 2004). Another non-mutually exclusive hypothesis is that final aphid density was not a good predictor of evolutionary outcome because most evolutionary change occurred in the exponential growth phase (Days 0–14). This was a likely possibility because in the early growth phase the population expanded approximately 170-fold but only 4.5-fold in the later growth phase. This hypothesis helps to explain why the A–B treatment did not evolve, since clones A and B had more similar growth rates in the early growth phase, and why clone B increased in frequency so quickly in the B–C evolution treatment even though it had slower growth in the later growth phase when in pure culture (Figs. 1 and 2).

The novelty of our study is to assess the impact of these evolutionary changes on concurrent population dynamics and not to evaluate the exact mechanism that caused evolution. Our first hypothesis was supported; we observed strong impacts of rapid evolution on population dynamics. Rapid evolution accelerated population growth rate by 28% and 33% respectively in the two mixed treatments where evolution occurred (Fig. 3, Table 2). Evolution increased population density by as much as 17% for the B–C treatment and 19% for the A–C treatment compared with no-evolution expectations (best linear approximations on Day 24 where maximum differences are seen in the raw data). These effects are similar in magnitude to other ecological forces usually deemed to be important. For example, increasing temperature from 20°C to 25°C and from 25°C to 30°C causes *M. persicae*'s intrinsic growth rate to increase by 14% and 3.6% respectively (Davis *et al.*, 2007). Gurevitch and colleagues' (2000) factorial meta-analysis of dozens of experiments manipulating predation and competition revealed impact sizes similar to those reported here. The strong effects we observed suggest that population density and growth rate might not be predictable simply by averaging the demographic parameters of a mixed genotype population (Wallner, 1987; Endler, 1991). As Hairston *et al.* (2005) suggested, we consider the rate of evolution we observed to be 'rapid' because it had a relatively large impact on concurrent ecological dynamics compared with other ecological factors (e.g. temperature). Moreover, significant genetic changes occurred within only a few generations and less than a single growing season of its hosts.

Even stronger effects of rapid evolution might have been observed if clones with larger fitness differences were selected. Such variation would be likely if we had (1) sampled more clones from the natural population, (2) collected aphids from different populations or

(3) from different host species. Other studies in *M. persicae* have reported greater genetic variation between clones. Weber (1985) found up to eight-fold variation in population size after 12 days in 1137 unreplicated isofemale lines, whereas Vorburger (2005) found 60% variation in his measure of fitness using 19 clones.

Our results suggest that evolutionary context (identity of genotypes and rate of evolution) influences the impact of rapid evolution on population dynamics (hypothesis 2). In general, as the rate of evolution increased so did the impact of evolution (Table 2, Fig. 2); however, this result is only based on three rates of evolution. In the Introduction we discussed that rapid evolution might impact population growth rate in a simple or complex manner. Our results suggest that both types are occurring. In the A–C evolution treatment, the population growth rate accelerates as the frequency of the faster growing A clone becomes more common. A more complex effect occurs in the B–C treatment, where the population growth rate accelerates as the B clone increases in frequency, but this clone has lower growth rate in the second growth phase. Why exactly this occurs remains unresolved but suggests that changes in clonal frequency during evolution altered clonal interactions that then impacted population growth rate.

An obstacle we faced was how to statistically compare evolving and non-evolving populations. If one compares an evolving mixed population (e.g. clones A and B) to either pure treatment (pure A or pure B), then genetic variation, clonal identity, and evolution are confounded. Our aim was to assess the impact of evolution itself and not the former factors. Hence we created planned contrasts that compared the observed population parameters in the evolution treatment to those of both corresponding pure treatments in the ratio of the initial frequency of clones (25% : 75%). This is akin to having a population composed of a constant (non-evolving) ratio of clones. The limitation of this approach is that it assumes that inter-clonal interactions are equivalent to intra-clonal interactions.

The limitations of our methods make it possible that aphid clonal interactions influenced the change in population growth rate observed, since the evolution treatment had interacting clonal lineages but the no-evolution controls did not. Although some clonal interactions were noted, our results generally suggest that rapid evolution is the main driving force that caused the acceleration of growth rate we observed. First, only the evolution treatments that evolved significantly (B–C and A–C) showed a significant difference in population dynamics compared with their no-evolution controls. If aphid interactions were more important, then we would expect an effect in all evolution treatments, including A–B, since they all had interacting clones. Second, if rapid evolution was the main driver, we would expect a delay before evolution altered growth rate because it takes time for clonal frequencies to change. This pattern was observed in the B–C evolution treatment where evolution only had an impact in the second growth phase (Fig. 3c vs. 3d, Table 2). This suggests that clonal interactions are weak, since they had no effect between Days 0 and 14. The A–C evolution treatment, however, presents mixed support. Clonal interactions seem to have reduced growth rate in the first growth phase but then evolution, through an increase in the frequency of the faster growing clone, compensated for this reduction and accelerated population growth rate significantly (Fig. 3e, f). Complex interactions are possible between evolutionary changes and frequency-dependent clonal interactions and might be specific to each clonal pairing but we here focus on the general effects of evolution.

It is difficult to compare the effect of rapid evolution on population dynamics between different study systems. Rapid evolution in certain predator–prey or host–parasite systems

has been shown to alter the mean density and the pattern and magnitude of density cycles (Pimentel, 1968; Bohannan and Lenski, 2000; Fussmann *et al.*, 2003; Yoshida *et al.*, 2003; terHorst *et al.*, 2010). These systems might inherently have more opportunity for qualitative changes in population dynamics, since the two interacting species usually undergo multiple generations within the experiment. Thus the population dynamics and potentially the evolutionary dynamics of both species might be altered. This is not what occurs in many plant–herbivore systems. Many insect populations grow rapidly and then crash because of plant senescence, predation, parasitism or climate (Wallner, 1987; Ro and Long, 1999; Karley *et al.*, 2004). Such dynamics often occur within one generation of the plant. Given differences in these types of interspecific interactions in nature, it is important to investigate the impact of rapid evolution in different model systems.

Our results directly challenge the assumption that evolutionary change happens on a much longer time scale than the change wrought by ecological interactions (Thompson, 1998; Hendry and Kinnison, 1999). The large acceleration of growth rate within only a few weeks suggests that rapid evolution on naturally occurring genetic variation can be a strong driver of population dynamics on 'ecological time scales'. In effect, this study quantified one-half of the potential eco-evolutionary feedback cycle and showed that rapid evolution can significantly alter population density. Whether this occurs under different ecological conditions remains to be tested. Another unanswered question is whether changes in density reciprocally influence future bouts of evolution. If this is the case, it would complete the eco-evolutionary feedback cycle (Fussmann *et al.*, 2007; Kokko and López-Sepulcre, 2007). Although selection within aphids is known to be density-dependent (Agrawal *et al.*, 2004), direct tests of this hypothesis in this system should be undertaken.

Accurate predictions of population dynamics are crucial in many applied fields, such as fisheries, pest management, conservation biology, invasion biology, and epidemiology. Rapid evolution in many ecologically relevant traits has been documented repeatedly in these systems (Ashley *et al.*, 2003), yet evolution is usually not considered in population dynamics studies. Our experimental results strongly challenge this approach and suggest that rapid evolution can have a large effect on growth rate and density. It follows that investigating the impact of rapid evolution can improve predictions and population management (Stockwell *et al.*, 2003; Hufbauer and Roderick, 2005; Duffy and Sivars-Becker, 2007; Kinnison and Hairston, 2007).

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REFERENCES

Agashe, D. 2009. The stabilizing effect of intraspecific genetic variation on population dynamics in novel and ancestral habitats. *Am. Nat.*, **174**: 255–267.

Agrawal, A.A. 2000. Host-range evolution: Adaptation and trade-offs in fitness of mites on alternative hosts. *Ecology*, **81**: 500–508.

Agrawal, A.A. 2004. Plant defense and density dependence in the population growth of herbivores. *Am. Nat.*, **164**: 113–120.

Agrawal, A.A., Underwood, N. and Stinchcombe, J.R. 2004. Intraspecific variation in the strength of density dependence in aphid populations. *Ecol. Entomol.*, **29**: 521–526.

Anderson, W.W. and King, C.E. 1970. Age-specific selection. *Proc. Natl. Acad. Sci.*, **66**: 780–786.

Ashley, M.V., Willson, M.F., Pergams, O.R.W., O'Dowd, D.J., Gende, S.M. and Brown, J.S. 2003. Evolutionarily enlightened management. *Biol. Conserv.*, **111**: 115–123.

Ayala, F.J. 1968. Genotype, environment, and population numbers. *Science*, **162**: 1453–1459.

Berry, R.J. 1979. Genetical factors in animal population dynamics. In *Population Dynamics: The 20th Symposium of the British Ecological Society* (R.M. Anderson, B.D. Turner and L.R. Taylor, eds.), pp. 53–80. London: Scientific Publications.

Blackman, R.L. 1974. Life-cycle variation of *Myzus persicae* (Sulz.) (Hom., Aphididae) in different parts of the world, in relation to genotype and environment. *Bull. Entomol. Res.*, **63**: 595–607.

Blackman, R.L. and Eastop, V.F. 2000. *Aphids on the World's Crops: An Identification and Information Guide*. New York: Wiley.

Bohannan, B.J.M. and Lenski, R.E. 2000. Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. *Ecol. Lett.*, **3**: 362–377.

Bone, E. and Farres, A. 2001. Trends and rates of microevolution in plants. *Genetica*, **112/113**: 165–182.

Cappuccino, N. and Price, P.W. 1995. *Population Dynamics: New Approaches and Synthesis*. San Diego, CA: Academic Press.

Chitty, D. 1967. The natural selection of self-regulatory behaviour in animal populations. *Proc. Ecol. Soc. Aust.*, **2**: 51–78.

Crawley, M.J. 1989. Insect herbivores and plant population dynamics. *Annu. Rev. Entomol.*, **34**: 531–562.

Davis, J.A., Radcliffe, E.B. and Ragsdale, D.W. 2007. Effects of high and fluctuating temperatures on *Myzus persicae* (Hemiptera: Aphididae). *Physiol. Ecol.*, **35**: 1461–1466.

de Barro, P.J., Sherratt, T.N., Brookes, C.P., David, O. and Maclean, N. 1995. Spatial and temporal genetic variation in British field populations of the grain aphid *Sitobion avenae* (F.) (Hemiptera: Aphididae) studied using RAPD-PCR. *Proc. R. Soc. Lond. B*, **262**: 321–327.

Duffy, M.A. and Sivars-Becker, L. 2007. Rapid evolution and ecological host–parasite dynamics. *Ecol. Lett.*, **10**: 44–53.

Dyer, K.F. 1968. Evolution observed – some examples of evolution occurring in historical times. *J. Biol. Educ.*, **2**: 317–338.

Ehrlich, P.R. and Raven, P.H. 1964. Butterflies and plants: A study in coevolution. *Evolution*, **18**: 586–608.

Ellner, S.P., Geber, M.A. and Hairston, N.G. 2011. Does rapid evolution matter? Measuring the rate of contemporary evolution and its impacts on ecological dynamics. *Ecol. Lett.*, **14**: 603–614.

Endler, J.A. 1991. Genetic heterogeneity and ecology. In *Genes in Ecology: The 33rd Symposium of the British Ecological Society* (R.J. Berry, T.J. Crawford and G.M. Hewitt, eds.), pp. 315–334. Oxford: Blackwell Scientific Publications.

Fuller, S.J., Chavigny, P., Lapchin, L. and Vanlerberghe-Masutti, F. 1999. Variation in clonal diversity in glasshouse infestations of the aphid, *Aphis gossypii* Glover in southern France. *Mol. Ecol.*, **8**: 1867–1877.

Fussmann, G.F., Ellner, S.P. & Hairston, N.G.J. 2003. Evolution as a critical component of plankton dynamics. *Proc. R. Soc. Lond. B*, **270**: 1015–1022.

Fussmann, G.F., Loreau, M. and Abrams, P.A. 2007. Eco-evolutionary dynamics of communities and ecosystems. *Funct. Ecol.*, **21**: 465–477.

Futuyma, D.J. and Agrawal, A.A. 2009. Macroevolution and the biological diversity of plants and herbivores. *Proc. Natl. Acad. Sci. USA*, **106**: 18054–18061.

Guldemond, J.A., Brink, W.J. and Belder, E. 1998. Methods of assessing population increase in aphids and the effect of growth stage of the host plant on population growth rates. *Entomol. Exp. Appl.*, **86**: 163–173.

Gurevitch, J., Morrison, J.A. and Hedges, L.V. 2000. The interaction between competition and predation: a meta-analysis of field experiments. *Am. Nat.*, **155**: 435–453.

Hairston, N.G.J., Ellner, S.P., Geber, M.A., Yoshida, T. and Fox, J.A. 2005. Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.*, **8**: 1114–1127.

Hazell, S.P., McClintock, I.A.D. and Fellowes, M.D.E. 2006. Intraspecific heritable variation in life-history traits can alter the outcome of interspecific competition among insect herbivores. *Basic Appl. Ecol.*, **7**: 215–223.

Hendry, A.P. and Kinnison, M.T. 1999. The pace of modern life: measuring rates of contemporary microevolution. *Evolution*, **53**: 1637–1653.

Horovitz, A. and Galil, J. 1972. Gynodioecism in East Mediterranean *Hirschfeldia incana* Cruciferae. *Bot. Gaz.*, **133**: 127–131.

Hufbauer, R.A. and Roderick, G.K. 2005. Microevolution in biological control: mechanisms, patterns, and processes. *Biol. Control*, **35**: 227–239.

Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, N. and Vellend, M. 2008. Ecological consequences of genetic diversity. *Ecol. Lett.*, **11**: 609–623.

Karley, A.J., Pitchford, J.W., Douglas, A.E., Parker, W.E. and Howard, J.J. 2003. The causes and processes of the mid-summer population crash of the potato aphids *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera: Aphididae). *Bull. Entomol. Res.*, **93**: 425–437.

Karley, A.J., Parker, W.E., Pitchford, J.W. and Douglas, A.E. 2004. The mid-season crash in aphid populations: why and how does it occur? *Ecol. Entomol.*, **29**: 383–388.

Kinnison, M.T. and Hairston, N.G. 2007. Eco-evolutionary conservation biology: contemporary evolution and the dynamics of persistence. *Funct. Ecol.*, **21**: 444–454.

Kocourek, F., Havelka, J., Berankova, J. and Jarosik, V. 1994. Effect of temperature on development rate and intrinsic rate of increase of *Aphis gossypii* reared on greenhouse cucumbers. *Entomol. Exp. Appl.*, **71**: 59–64.

Kokko, H. and López-Sepulcre, A. 2007. The ecogenetic link between demography and evolution: can we bridge the gap between theory and data? *Ecol. Lett.*, **10**: 773–782.

Leips, J., Travis, J. and Rodd, F.H. 2000. Genetic influences on experimental population dynamics of the least killifish. *Ecol. Monogr.*, **70**: 289–309.

Levins, R. 2003. Towards a population biology, still. In *The Evolution of Population Biology* (R.S. Singh and M.K. Uyenoyama, eds.), pp. 21–47. Cambridge: Cambridge University Press.

Mackauer, M. and Way, M.J. 1976. *Myzus persicae* Sulz. an aphid of world importance. In *Studies in Biological Control* (V.L. Delucchi, ed.), pp. 51–119. Cambridge: Cambridge University Press.

Maron, J.L. and Crone, E. 2006. Herbivory: effects on plant abundance, distribution and population growth. *Proc. R. Soc. Lond. B*, **273**: 2575–2584.

Mitchell-Olds, T. and Bradley, D. 1996. Genetics of *Brassica rapa*. 3. Costs of disease resistance to three fungal pathogens. *Evolution*, **50**: 1859–1865.

Pelletier, F., Garant, D. and Hendry, A.P. 2009. Eco-evolutionary dynamics. *Phil. Trans. R. Soc. Lond. B*, **364**: 1483–1489.

Pimentel, D. 1961. Animal population regulation by the genetic feed-back mechanism. *Am. Nat.*, **95**: 65–79.

Pimentel, D. 1968. Population regulation and genetic feedback. *Science*, **159**: 1432–1437.

Pimentel, D. and Al-Hafidh, R. 1965. Ecological control of a parasite population by genetic evolution in the parasite–host system. *Ann. Entomol. Soc. Am.*, **58**: 1–6.

Pimentel, D., Nagel, W.P. and Madden, J.L. 1963. Space–time structure of the environment and the survival of parasite–host systems. *Am. Nat.*, **97**: 141–167.

Pinheiro, J.C. and Bates, D.M. 2000. *Mixed-Effects Models in S and S-PLUS*. New York: Springer-Verlag.

Pinheiro, J.C., Bates, D.M., DebRoy, S., Sarkar, D. and R Development Core Team. 2011. *nlme: Linear and Nonlinear Mixed Effects Models*, R package version 3.1-98. Vienna: R Development Core Team.

R Development Core Team. 2009. *R: A Language and Environment for Statistical Computing*, version 2.10.1. Vienna: R Development Core Team.

Reznick, D.N. and Ghalambor, C.K. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, **112/113**: 183–198.

Ro, T.H. and Long, G.E. 1999. GPA-Phenodynamics, a simulation model for the population dynamics and phenology of green peach aphid in potato: formulation, validation, and analysis. *Ecol. Model.*, **119**: 197–209.

Rochat, J., Vanlerberghe-Masutti, F., Chavigny, P., Boll, R. and Lapchin, L. 1999. Inter-strain competition and dispersal in aphids: evidence from a greenhouse study. *Ecol. Entomol.*, **24**: 450–464.

Roughgarden, J. 1979. *Theory of Population Genetics and Evolutionary Ecology: An Introduction.* Upper Saddle River, NJ: Prentice-Hall.

Saccheri, I. and Hanski, I. 2006. Natural selection and population dynamics. *Trends Ecol. Evol.*, **21**: 341–347.

Schlager, G. 1963. The ecological genetics of the mutant Sooty in populations of *Tribolium castaneum. Evolution*, **17**: 254–273.

Schoener, T.W. 2011. The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science*, **331**: 426–429.

Shertzer, K.W., Ellner, S.P., Fussmann, G.F. and Hairston, N.G. 2002. Predator–prey cycles in an aquatic microcosm: testing hypotheses of mechanism. *J. Anim. Ecol.*, **71**: 802–815.

Sibly, R.M. and Hone, J. 2002. Population growth rate and its determinants: an overview. *Phil. Trans. R. Soc. Lond. B*, **357**: 1153–1170.

Sinervo, B., Svensson, E. and Comendant, T. 2000. Density cycles and an offspring quantity and quality game driven by natural selection. *Nature*, **406**: 985–988.

Sloane, M.A., Sunnucks, P., Wilson, A.C.C. and Hales, D.F. 2001. Microsatellite isolation, linkage group identification and determination of recombination frequency in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Genet. Res.*, **77**: 251–260.

Slobodkin, L.B. 1980. *Growth and Regulation of Animal Populations*. New York: Dover.

Stockwell, C.A., Hendry, A.P. and Kinnison, M.T. 2003. Contemporary evolution meets conservation biology. *Trends Ecol. Evol.*, **18**: 94–101.

terHorst, C.P., Miller, T.E. and Levitan, D.R. 2010. Evolution of prey in ecological time reduces the effect size of predators in experimental microcosms. *Ecology*, **91**: 629–636.

Thompson, J.N. 1998. Rapid evolution as an ecological process. *Trends Ecol. Evol.*, **13**: 329–332.

Vehrs, S.L., Walker, G.P. and Parrela, M.P. 1992. Comparison of population growth rate and within-plant distribution between *Aphis gossypii* and *Myzus persicae* (Homoptera: Aphididae) reared on potted chrysanthemums. *J. Econ. Entomol.*, **85**: 799–807.

Via, S. and Shaw, A.J. 1996. Short-term evolution in the size and shape of pea aphids. *Evolution*, **50**: 163–173.

Vorburger, C. 2005. Positive genetic correlations among major life-history traits related to ecological success in the aphid *Myzus persicae. Evolution*, **59**: 1006–1015.

Vorburger, C. 2006. Temporal dynamics of genotypic diversity reveal strong clonal selection in the aphid *Myzus persicae. J. Evol. Biol.*, **19**: 97–107.

Wallner, W.E. 1987. Factors affecting insect population dynamics: differences between outbreak and non-outbreak species. *Annu. Rev. Entomol.*, **32**: 317–340.

Weber, G. 1985. Genetic variability in host plant adaptation of the green peach aphid, *Myzus persicae. Entomol. Exp. Appl.*, **38**: 49–56.

Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster, S.M., Bangert, R.K., LeRoy, C.J. *et al.* 2006. A framework for community and ecosystem genetics: from genes to ecosystems. *Nat. Rev. Genet.*, **7**: 510–523.

Wilson, A.C.C., Massonnet, B., Simon, J.C., Prunier-Leterme, N., Dolatti, L., Llewellyn, K.S. *et al.* 2004. Cross-species amplification of microsatellite loci in aphids: assessment and application. *Mol. Ecol. Notes*, **4**: 104–109.

Yoshida, T., Jones, L.E., Ellner, S.P., Fussmann, G.F. and Hairston, N.G. 2003. Rapid evolution drives ecological dynamics in a predator–prey system. *Nature*, **424**: 303–306.