

LETTER

The impact of rapid evolution on population dynamics in the wild: experimental test of eco-evolutionary dynamics

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Abstract

Rapid evolution challenges the assumption that evolution is too slow to impact short-term ecological dynamics. This insight motivates the study of 'Eco-Evolutionary Dynamics' or how evolution and ecological processes reciprocally interact on short time scales. We tested how rapid evolution impacts concurrent population dynamics using an aphid (*Myzus persicae*) and an undomesticated host (*Hirschfeldia incana*) in replicated wild populations. We manipulated evolvability by creating non-evolving (single clone) and potentially evolving (two-clone) aphid populations that contained genetic variation in intrinsic growth rate. We observed significant evolution in two-clone populations whether or not they were exposed to predators and competitors. Evolving populations grew up to 42% faster and attained up to 67% higher density, compared with non-evolving control populations but only in treatments exposed to competitors and predators. Increased density also correlates with relative fitness of competing clones suggesting a full eco-evolutionary dynamic cycle defined as reciprocal interactions between evolution and density.

Keywords

Aphid dynamics, brassica pest, clonal selection, contemporary evolution, eco-evolutionary feedback, ecological genetics, evolution enlightened management, experimental evolution, population growth rate.

Ecology Letters (2011)

INTRODUCTION

Ecological and evolutionary forces are usually thought to influence each other asymmetrically – ecology shapes evolution but not vice versa (Levins & Lewontin 1980; Hairston *et al.* 2005; Kokko & López-Sepulcre 2007). Ecological changes are often assumed to occur independently of evolution, such as when an organism's population size is reduced due to a drought. Evolutionary changes, however, are usually a consequence of the environment, e.g. desiccation resistance evolves in response to droughts. Most ecological models and studies make the simplifying assumption that evolution does not impact short-term ecological processes (as discussed in Endler 1991; Thompson 1998; Hairston *et al.* 2005; Pelletier *et al.* 2009) because evolution is thought to be slow relative to ecological interactions (Slobodkin 1980). This assumption has now been challenged by dozens of studies documenting rapid evolution in nature occurring on 'ecological time scales', sometimes within a few generations (reviewed by Thompson 1998; Hendry & Kinnison 1999; Reznick & Ghalambor 2001; Ashley *et al.* 2003).

Given the convergence of time scales, we now ask whether rapid evolution and concurrent ecological dynamics influence each other reciprocally (Pelletier *et al.* 2009; Schoener 2011). This reciprocal interaction defines 'Eco-Evolutionary Dynamics' (Hairston *et al.* 2005; Bull *et al.* 2006; Kokko & López-Sepulcre 2007). We focus on quantifying the impact of rapid evolution on concurrent ecological dynamics. This aspect of eco-evolutionary dynamics has received less attention than the impact of ecology on evolution (Bull *et al.* 2006; Pelletier *et al.* 2009). A growing body of theory, based on very different biological assumptions, concludes that when rapid evolution occurs during the course of an ecological interaction, it can significantly alter quantitative and qualitative ecological predictions (reviewed in Day 2005; Fussmann *et al.* 2007). Theoretical models

suggest that such eco-evolutionary dynamics can influence the trajectory of growth of single populations (Anderson & King 1970), the density and stability of victim-exploiter systems (Pimentel 1961; Fussmann *et al.* 2003; Duffy & Sivers-Becker 2007), the structure of multi-species communities (Loeuille & Leibold 2008) and even ecosystem processes (Loeuille *et al.* 2002).

Empirical studies are beginning to quantify the impact of rapid evolution on ecological dynamics. One approach consists of modelling the relative impacts of ecological and evolutionary processes on ecological dynamics. Evolution is usually strongly correlated with ecological dynamics and ecological predictions are usually improved by including evolution (Hairston *et al.* 2005; Duffy & Sivers-Becker 2007). Another approach compares the ecological properties of ancestral populations vs. populations that have undergone evolution. Researchers have identified impacts of evolution on life history properties (Reznick & Bryga 1996), population dynamics (Hanski & Saccheri 2006), community structure and ecosystem processes (Harmon *et al.* 2009; Bassar *et al.* 2010) demonstrating the strength and generality of rapid evolution's ecological effects.

The method we employ is to experimentally manipulated the occurrence of evolution and assessed ecological impacts of ongoing evolution by comparing evolving and non-evolving populations. Pimentel first used this approach and found that the population dynamics of a parasitoid wasp changed as its housefly host evolved resistance compared with a non-evolving control (Pimentel *et al.* 1963; Pimentel 1968). Rapid evolution in the host reduced the parasitoid's mean and variance in population size even though host population size was held constant. This experimental approach has only been attempted a handful of times and only in the laboratory (Tuda 1998; Bohannan & Lenski 1999; Yoshida *et al.* 2003; Agashe 2009; Terhorst *et al.* 2010; Turcotte *et al.* 2011).

Fussmann *et al.* (2007), and more recently (Schoener 2011), emphasised the need to test the ecological effects of evolution in real time. They set criteria that would ideally test for such effects. The study should: (1) track changes in ecological dynamics over many generations, (2) track changes in genetic frequencies, (3) provide a plausible mechanistic link between ecology and evolution and (4) include non-evolving control populations. They concluded that no single study has met all these criteria though some have come close. The only study to our knowledge that fulfils all conditions is our greenhouse study using green peach aphids, *Myzus persicae* [Sulzer] (Turcotte *et al.* 2011). We manipulated the genetic composition of populations and hence evolutionary potential, then tracked population dynamics as the populations evolved. Rapid aphid evolution significantly accelerated aphid population growth rate by as much as 34% compared with non-evolving control populations (Turcotte *et al.* 2011).

While recognising the value of laboratory studies, field experiments are crucial because ecological context can influence both ecological and evolutionary processes. Here, we test for eco-evolutionary dynamics using methods that follow the criteria set out by Fussmann *et al.* (2007), but in the wild. We define rapid evolution as significant changes in genotypic frequencies occurring within a growing season, a time period usually deemed too short for evolution to have an impact.

Experiments conducted in the wild within natural communities incorporate biotic and abiotic variation as well as gene flow. These confounding factors could impose different selective pressures, altering the rate or direction of evolution. They could also interfere with or mask the manner in which rapid evolution impacts population dynamics, e.g. by altering the strength of density regulation. These problems imply that eco-evolutionary dynamics should be studied in the wild because results could differ significantly from predictions based only upon laboratory experiments.

By creating treatments that allow or prevent interactions with a natural spectrum of herbivores and predators, we also test the importance of ecological context in eco-evolutionary dynamics. Finally we quantify interspecific effects of aphid evolution. Our previous greenhouse experiment found that aphids significantly harm their host plant, an undomesticated annual mustard, *Hirschfeldia incana* [Lagrèze-Fossat], reducing above ground biomass by a factor of five. Yet aphid evolution, although accelerating population growth rate, did not cause additional reduction of host growth (Turcotte *et al.* 2011). Field populations permit better quantification of host plant fitness differences because natural seed production can occur, thus providing a stronger test of aphid impacts.

MATERIALS AND METHODS

We first present the study system and describe the field experiment where we created replicated aphid populations, half of which remained caged and the other half did not. Aphid populations were composed of a single clone (pure) or two clones (mixed). As the populations grew, we tested whether the mixed populations evolved. We then counted aphids repeatedly and tested whether evolution alters the aphid's population dynamics and host fitness compared with non-evolving populations.

Study system

Myzus persicae is considered the world's most important crop pest thus its life history and ecology are well studied (Mackauer & Way 1976).

It is amenable to experimental evolution because of its short generation time, ease of culture and genetic variation identified in multiple traits (Vorburger 2005). This cyclically parthenogenetic aphid undergoes one generation of sexual reproduction in the fall. The following spring, populations are replete with multiple clonal lineages that reproduce asexually until the fall (Mackauer & Way 1976). Aphid populations rapidly evolve through natural clonal selection within months, changing gene frequencies and mean trait values (Via & Shaw 1996; Vorburger 2006; Turcotte *et al.* 2011).

In 2008, we collected multiple clonal lineages from a single wild population feeding on an invasive mustard host *H. incana* from the Motte-Rimrock Reserve in Perris California. We identified clones using six microsatellite markers and characterised their intrinsic per capita growth rates in the greenhouse (for details see Turcotte *et al.* 2011). We conducted the current study with three clones from the former study with known differences in growth rate.

Field experiment design

The experiment was conducted in a wash (20 m by 12 m), cleared of vegetation, at the Reserve. We deployed a wire fence try to exclude vertebrate herbivores. To minimise variation in host plants, they were grown from the seeds of a single *H. incana* collected in 2008 at the Reserve. Two-week old seedlings were planted 1.4 m apart in the field site. Plants were watered three times a week because there was no measurable precipitation during the experiment. Plants were caged in a thin transparent mesh (Bridal Organza, #664-7242, Jo-Ann Fabrics and Crafts, Riverside, CA, USA) held up by 75 cm tall wire frame to prevent insect damage and permit aphid populations to become established.

We initiated the experiment on three consecutive days starting June 30th 2009 by placing 20 third instar aphids onto each plant (day 0). Aphids came from stock greenhouse clonal populations, regularly tested for contamination and maintained on *H. incana*. On day 1, aphids that failed to settle and died (*c.* 9% of the total) were replaced with fourth instar aphids. On day 13, the mesh was lifted from half of the plants, then tied to the top of the wire frame. This maintained consistent shade between treatments but gave the arthropod community access to the plant. Thus aphid treatments were fully crossed with caging treatments. In uncaged plants, competitors, predators, pollinators and other herbivores were seen interacting with the aphids and their host.

We used three aphid clonal lineages, identified as 'A', 'B' and 'C' that differ in microsatellite markers and in exponential growth rate under greenhouse conditions (Turcotte *et al.* 2011). Three evolution treatments consisted of aphid populations (on a single plant) that were initially composed of two different clones (10 individuals of each clone for a total of 20 aphids). We created all three-two-way combinations of clones (A–B, B–C and A–C). These populations have genetic variation in fitness (e.g. clone A's r_m is greater than that of clone C) and the population could evolve by changing in clonal frequency (away from the initial ratio of 50% : 50%). Three different non-evolution (pure clone) treatments received aphids from only one of the three clones. Because all individuals were of the same genotype within these pure populations, gene frequencies could not change, thus preventing evolution. The evolution non-evolution distinction assumes that aphid clones do not mutate and evolve so quickly as to impact population dynamics within the 31 days of the experiment. Finally, 'no-aphid control' treatments

did not receive any aphids. Treatments were assigned in a randomised block design and replicated eight times for a total of 112 plants.

Rates of evolution

On days 13, 20 and 31, we collected 20, 50 and 70 aphids respectively from every population to track changes in clonal frequencies (evolution). Between 16 and 32 aphids from each sample were genotyped (for a total of 2213 aphids) at three microsatellite loci (for detailed genetic methods see Turcotte *et al.* 2011). We tested whether the mean frequency of the faster clone differed significantly from the initial clonal frequency of 50% using one sample *t*-tests. We also tested how caging, evolution treatment and their interaction impacts the final frequency of clones using a general linear model.

Aphid population dynamics

Aphid population dynamics were quantified by counting all aphids on days 3, 6, 10, 14, 17, 20, 24, 27, 31 and 36. All counts were made by MMT. When populations rose above 2000 aphids, we sub-sampled by counting one half of every leaf. The removal of cages on day 13 for half the treatments qualitatively altered population dynamics. We thus analysed caged and uncaged treatments separately. Treatments that remained caged for the entire experiment grew exponentially until day 27 (Fig. 1a). On day 31, population growth slowed and by day 36 populations crashed due to plant senescence. The uncaged treatments grew exponentially for days 1–13, when caged (Fig. 1b). Once cages were removed, many lower leaves were damaged or consumed by vertebrate herbivores, which temporarily reduced aphid population growth rate. However, their exponential growth resumed and was

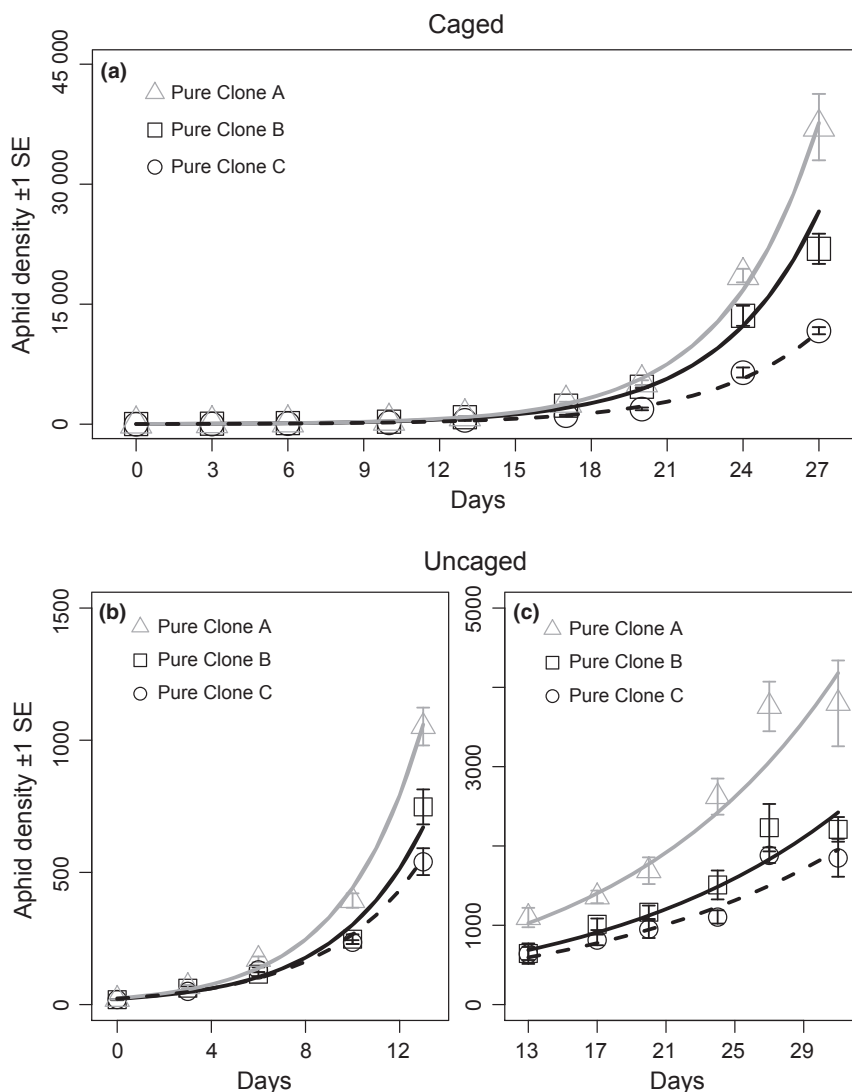


Figure 1 Partial residual plots of population dynamics of pure clonal treatments, over the time periods used in the analyses. Values represent mean number of aphids (± 1 SE) through time once all explained variation in the model has been removed. Panel (a) represents the caged treatments during days 0–27, (b) early growth phase of the uncaged plants before cages were removed on day 13, and (c) late growth phase of uncaged plants once cages were removed. Panels differ in y-axis scales. Functions represent the best-fit exponential model for each treatment (clone A is grey, clone B is the full black line and clone C is the dashed black line).

sustained until day 31 (Fig. 1c). Thus we analysed these time periods separately (days 0–13 and then starting day 13–31) for the uncaged treatments. This greatly improved the normality of residuals in all treatment groups.

We thus had three separate analyses; caged days 0–27, uncaged days 0–13 and uncaged 13–31. We compared treatments by fitting linear mixed-effect models (LME) where the dependent variable was $\text{LN}(x)$ transformed number of aphids and the fixed effects were aphid treatment, day (as the main covariate) and their interaction. Because repeated aphid counts on the same plant violated the assumption of independent observations, we set plant identity, nested within block, as a random effect on population growth rate and intercept and used an autoregressive correlation error structure (Pinheiro & Bates 2000). To account for differences in initial plant size and stage of development on day 3, we added these covariates to the model. Initial plant size was quantified as the first principal component, explaining 73% of the variation, composed of the number of true leaves and rosette width. Stage of development was quantified into an ordinal scale (1 = rosette, 2 = low bolt, 3 = bolt). Only in the caged treatment analysis did the covariates improve fit and were kept in the final model. All analyses were implemented in R using the *nlme* package (v. 2.11.1 R Development Core Team 2009).

The impact of evolution on population dynamics

Our main objective is to statistically test the impact of changes in clonal frequency on population dynamics. Ideally one would compare the observed aphid population dynamics in the evolution treatment to those of a mixed population, containing the same clones, but that remain at a frequency of 50% : 50%. This is not possible because of differences in fitness. We thus generated the *expected* population growth parameters of a non-evolving population by using the pure aphid treatments. We tested *a priori* 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 orthogonal contrasts between a subset of the aphid treatment levels within each LME analysis. We set weighed planned contrast coefficients of the no-evolution expectation to match those of the initial clonal frequency (e.g. pure A = –0.5 and pure C = –0.5 and these are compared with the A–C evolution treatment = +1; see Appendix S1 for contrasts used to test 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.

The effect of density and clonal frequency on natural selection

We also tested for density- and frequency-dependent selection that would suggest more complex eco-evolutionary dynamics. For each population, we calculated the quantity of each clone on days 0, 13, 20 and 31 by multiplying their genotypic frequencies by total population size. Next we calculated per capita growth rate in each time period (days 0–13, 13–20 and 20–31) using $\ln(N_2) - \ln(N_1)/(\text{day}_2 - \text{day}_1)$, where N = number of aphids of this clone (Agrawal *et al.* 2004). This was done for each clone in each evolution treatment. We then fitted a LME model on these growth rates. We fitted separate models for caged and uncaged populations. Fixed effects were the treatment

(combination of identity of the focal clone, identity of the competitor; e.g. clone A competing with clone B), initial total density and clonal frequency at the start of that time period and their interactions. We included plant identity and block as a random effect as well as autocorrelation error. With the use of planned contrasts, we determined if density and frequency differentially reduced growth rate between competing clones.

Host plant growth and fitness

We first determined whether aphid treatments differ in their impact on host plant growth by measuring final above ground dry biomass. We fit a general linear model with plant weight as the response variable and aphid treatment as a fixed effect, including the 7th treatment, which did not receive any aphids. We included initial plant stage of development and the first principal component of plant size as covariates. We used the same planned contrasts described above to test specific hypotheses.

We also quantified plant fitness traits. We calculated flower-days by summing counts of the number of flowers present on every sampling day multiplied by the length of that counting period. We estimated the number of seeds and mean seed dry mass by sub-sampling. We performed a similar analysis as for plant biomass but within a MANCOVA framework.

RESULTS

Pure clone treatments

Pure clone treatments differed greatly in their population dynamics yet the rank order of clonal growth rates was consistent across treatments (Fig. 1). Growth rates differed by 4.7–19.3% (Appendix S2). Caged treatments reached ≈ 10 times higher density than uncaged treatments (Fig. 1). This difference occurred because once cages were removed, there was predation on aphids and vertebrate herbivory, which reduced plant biomass. Counts of predatory and competing arthropod taxa are reported in Appendix S3.

Evolution treatments – genetic analyses

Faster growing clones rapidly increased in frequency and by day 31 (within 4–5 generations) all evolution treatments showed significant evolution (one sample *t*-tests, all *P*-values < 0.01, except for caged A–B *P* = 0.1). The faster growing clones in the caged populations reached on average 71% frequency, significantly less than in the uncaged populations, which reached 79% (*P* = 0.04, Fig. 2). This implies that uncaged treatments evolved 38% faster than caged treatments (29% average increase in frequency vs. 21%). The A–C and B–C treatments evolved significantly faster than A–B (*P*-values < 0.001) but did not differ from each other (Fig. 2). Caging did not interact with evolution treatment (LME, *P* = 0.46). Thus clone A in the A–C evolution treatments reached on average 85%, clone B in the B–C treatments reached 80%, and clone A in the A–B treatments reached 62% (Fig. 2).

Impact of aphid evolution on aphid population dynamics

In the caged treatments, although rapid evolution occurred, increasing the frequency of the faster growing clone, evolution did not impact

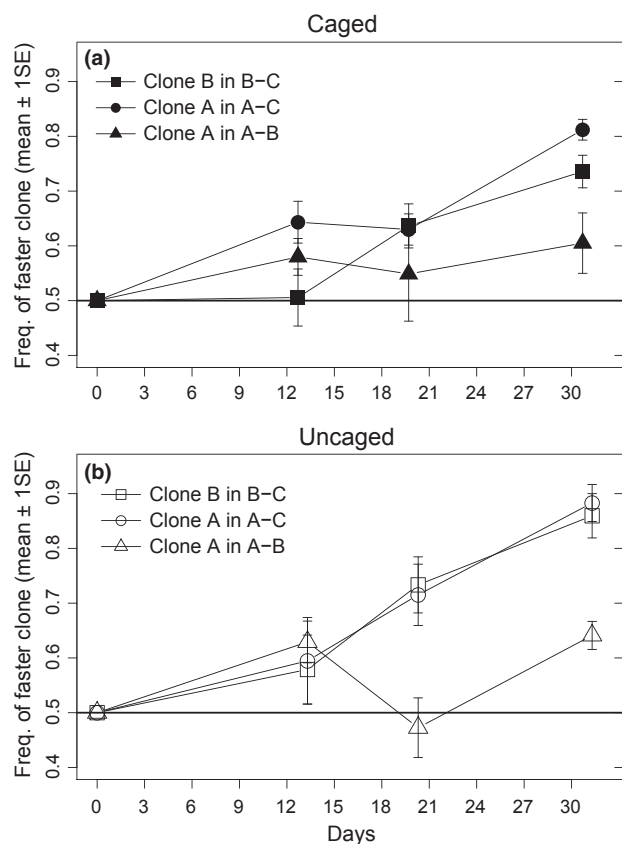


Figure 2 Rapid clonal evolution as shown by the mean frequency of the faster growing aphid clones in each evolution treatment (± 1 SE). Panels separate caged (a) and uncaged (b) treatments. Horizontal bar indicates initial clonal frequencies of 0.5.

population growth rate. Evolving populations grew as quickly as the expected growth rate of the corresponding pure treatments at a constant 50% : 50% frequency (Table 1, Fig. 3). In early growth

phase of the uncaged treatments, evolution did not impact population growth rate (Table 1, Fig. 4a,b,c). After day 13, all three evolution treatments grew significantly faster than their corresponding no-evolution expectations (between 33 and 42% faster, Table 1, Fig. 4d,e,f). Evolution had a stronger impact on population dynamics in populations that evolved more rapidly (Fig. 2; Table 1).

We also calculated predicted population densities, based on the model fitted parameters, which is more reliable than simply comparing densities on day 31 because the fitted values incorporate all the time series data. Evolution treatments reached higher densities than expected without evolution (A–B treatment +13.7%, B–C +67%, and A–C +17.5%, Table 1).

The effect of density and clonal frequency on natural selection

The relative growth rates of competing clones were negatively influenced by total density and clonal frequency. Three way interactions (with treatment) were not significant and these were removed. Increases in density more severely reduced the growth of clone C compared with clone A and also compared with clone B in mixed populations (P -values < 0.05, Table 2). Thus the relative fitness of clone C decreases in response to increased population density when in competition. Increases in clonal frequency significantly reduced the relative fitness of clone A when competing with clone B but only when uncaged (Table 2).

Impact of aphid evolution on host plant growth and fitness

Because rapid evolution did not impact aphid dynamics in the caged treatments, we only present results for the uncaged treatments. First, our analysis revealed that aphid herbivory significantly reduced final host biomass (mean $\pm$ SE weight with aphids = 15.3 g $\pm$ 2.6, weight without aphids = 20.8 g $\pm$ 2.2; GLM, P = 0.014). However, although rapid aphid evolution significantly increased aphid growth rate and density, this did not cause more damage to the host plant than the corresponding non-evolving aphid populations (GLM, all

Table 1 Analysis of population dynamics comparing evolving to non-evolving populations. Planned contrasts from linear mixed-effect models comparing each type of evolving population to its corresponding predicted non-evolving expectation, for the caged treatments (days 0–27), for the early growth phase (days 0–13) and the late phase (days 13–31) of the uncaged treatments. Evolution treatments are identified by their clonal composition. ‘Intercept’ represents density at the start of each time period, ‘slope’ represents aphid population growth rate and ‘predicted density’ represents expected density on the last day of the time period based on the best-fit model parameter estimates. The ‘percent change’ represents the change in intercept, slope, or final density, from the non-evolving expectation to that of the observed evolution treatment. Positive values represent increase due to rapid evolution. All P -values are for 2-tailed tests.

Evolution treatment (clones)	Caged (days 0–27)				Uncaged (days 0–13)				Uncaged (days 13–31)			
	d.f.	F	P	Change (%)	d.f.	F	P	Change (%)	d.f.	F	P	Change (%)
A–B												
Intercept	29	1.3	0.212	14.7	30	–1.3	0.208	–13.2	30	–1.9	0.071	–25.7
Slope	345	–0.8	0.443	–1.7	166	–0.9	0.361	–4.9	200	2.5	0.012	33.3
Predicted final density				0.19				–29.0				13.7
B–C												
Intercept	29	–0.7	0.506	–7.9	30	1.0	0.307	12.4	30	0.6	0.568	9.1
Slope	345	1.7	0.088	4.2	166	–0.7	0.487	–4.2	200	2.4	0.016	35.2
Predicted final density				12.6				–2.8				67.0
A–C												
Intercept	29	2.3	0.026	27.8	30	–1.3	0.188	–15.1	30	–1.8	0.079	–29.0
Slope	345	–0.6	0.581	–1.3	166	–0.1	0.905	–0.7	200	2.8	0.006	41.7
Predicted final density				0.1				–21.5				17.5

Significant results were bolded for easier identification.

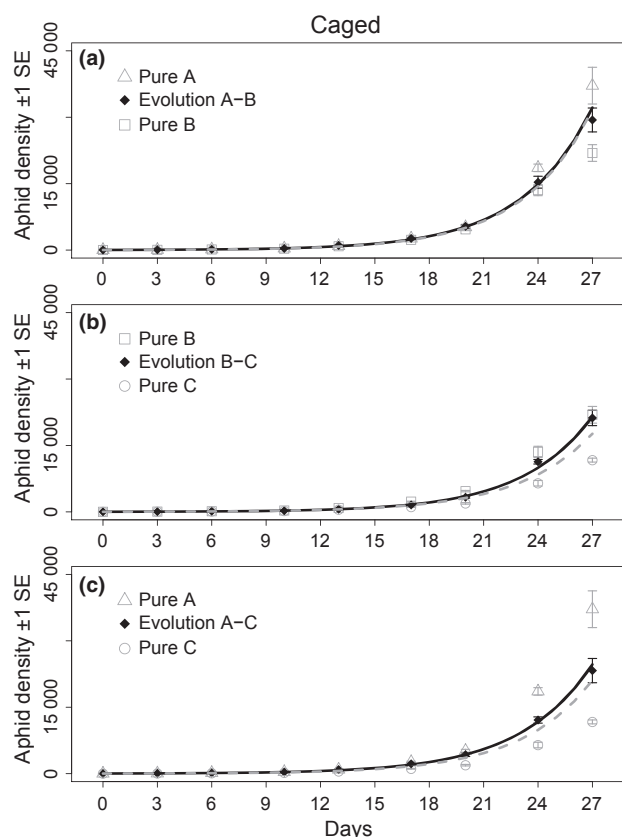


Figure 3 Partial residual plot of population dynamics of the three observed evolution treatments (black diamonds) with the best-fit model from LME analysis (black line) for caged treatments. The dashed grey line represents the best-fit model that combines both pure treatments using the constant (non-evolving) initial frequency of clones. For each treatment, we added the corresponding pure clone treatments (grey symbols) used to generate the no-evolution expectation. Evolution treatments have two letters. Values represent mean number of aphids (± 1 SE) once all explained variation in the model has been removed.

three P -values > 0.11). Our multivariate analysis of fitness for flower-days, seed number and seed weight found no difference between any aphid treatments including the no-aphid treatment (MANCOVA, Wilks $\lambda = 0.57$, $F = 1.1$, overall treatment P -value = 0.37, planned contrasts P -values all > 0.12). Given that even the no-aphid treatments had equal fitness, our results might be explained by the large variation between replicates overwhelming any trends (e.g. mean $\pm$ SE for flower-days for no-aphid treatment = 1683 ± 373 , mean for aphid treatments = 1542 ± 135). We also tested whether fitness traits were correlated with above ground dry biomass. Pearson correlations revealed a significant correlation between biomass and seed number and with total flower-days but mean seed weight was marginally non-significant ($P = 0.084$, Appendix S4).

DISCUSSION

Rapid evolution significantly accelerated population growth rate and density in the wild as the frequency of faster growing clones increased, yet this change in aphid density and growth rate did not impact host plant fitness. Rapid evolution only accelerated population growth rate in uncaged treatments. Our study has important implications for the burgeoning field of eco-evolutionary dynamics because it is experi-

mental and is conducted in the wild following the guidelines developed by Fussmann *et al.* (2007) and Schoener (2011).

In our field experiment, the three non-evolving, pure clone treatments grew at significantly different rates (Fig. 1, Appendix S2). Differences in clonal growth rates caused mixed populations to evolve, meaning that the frequency of genotypes changed rapidly (e.g. frequency of clone C was reduced by more than fourfold, Fig. 2b). We characterise this evolution as 'rapid' because it occurred within a growing season, of ≈ 4 –5 aphid generations. Similar large changes in clonal frequencies have been observed in other wild aphid populations (Vorburger 2006). This change in clonal frequency is not surprising nor is it novel. The novelty of our study lies in quantifying the effect of this evolutionary change on the population's growth rate. We first focus on the natural uncaged treatments where evolution had no impact on population growth rate between days 0 and 13. This is not unexpected given that time is required for evolution to occur and also for changes in the growth properties of the population to actually affect population growth rate. However, in the second growth phase evolving populations grew at significantly faster rates (up to 42% faster) than the expected rate if evolution is not taken into account (Fig. 4, Table 1). Evolution altered growth rate because selection favoured faster reproducing clones. The favoured clones are predicted from the pure clone treatments. These faster clones increased in frequency and increased the population's mean growth rate. Endler (1991) foreshadowed these results while discussing early ecological-evolutionary models: "*The time course of total population size is not predictable from the average of the demographic parameters of all genotypes. ... genotypes contribute unequally and differently to population size as they change in frequency during the course of natural selection.*"

Our results add to the growing body of evidence that evolution is sufficiently rapid to impact short-term ecological dynamics (Pimentel 1968; Fussmann *et al.* 2003; Yoshida *et al.* 2003; Turcotte *et al.* 2011). Here we observed a quantitative change, acceleration of population growth rate by up to 42%, which is similar in effect size to what we observed in greenhouse experiments (Turcotte *et al.* 2011). Rapid evolution can be a strong driver of population dynamics in the laboratory as well as in the field. However, some laboratory studies identify more complex qualitative changes in population dynamics. In Yoshida *et al.*'s (2003) study of rotifer and algae in chemostats, rapid evolution in algae caused the predator–prey population dynamics to change from being 1/4 out-of-phase to being perfectly out-of-phase. In another rotifer study, Fussmann *et al.* (2003) found that rapid evolution caused two peaks in population size but only a single peak when populations could not evolve. The absence of such dynamics in the current study may be attributable to lower number of generations and to the large difference in the generation time of aphids vs. *Brassica* relative to rotifers and algae. In the current study, and in most plant-herbivore systems in general, there are multiple herbivore generations during a single plant generation, which might potentially limit the opportunity for qualitative changes. Many insect populations grow rapidly then crash because of plant senescence, predation, parasitism or climate (Wallner 1987). More experiments in different types of model systems will be needed to determine whether the number of generations or asymmetry is more important in permitting complex eco-evolutionary dynamics.

The impact of rapid evolution in the uncaged treatments was evident even in the face of potentially confounding biotic factors including: severe herbivory when cages were first removed, the invasion of a competing aphid species (*Brevicoryne brassicae*), predators

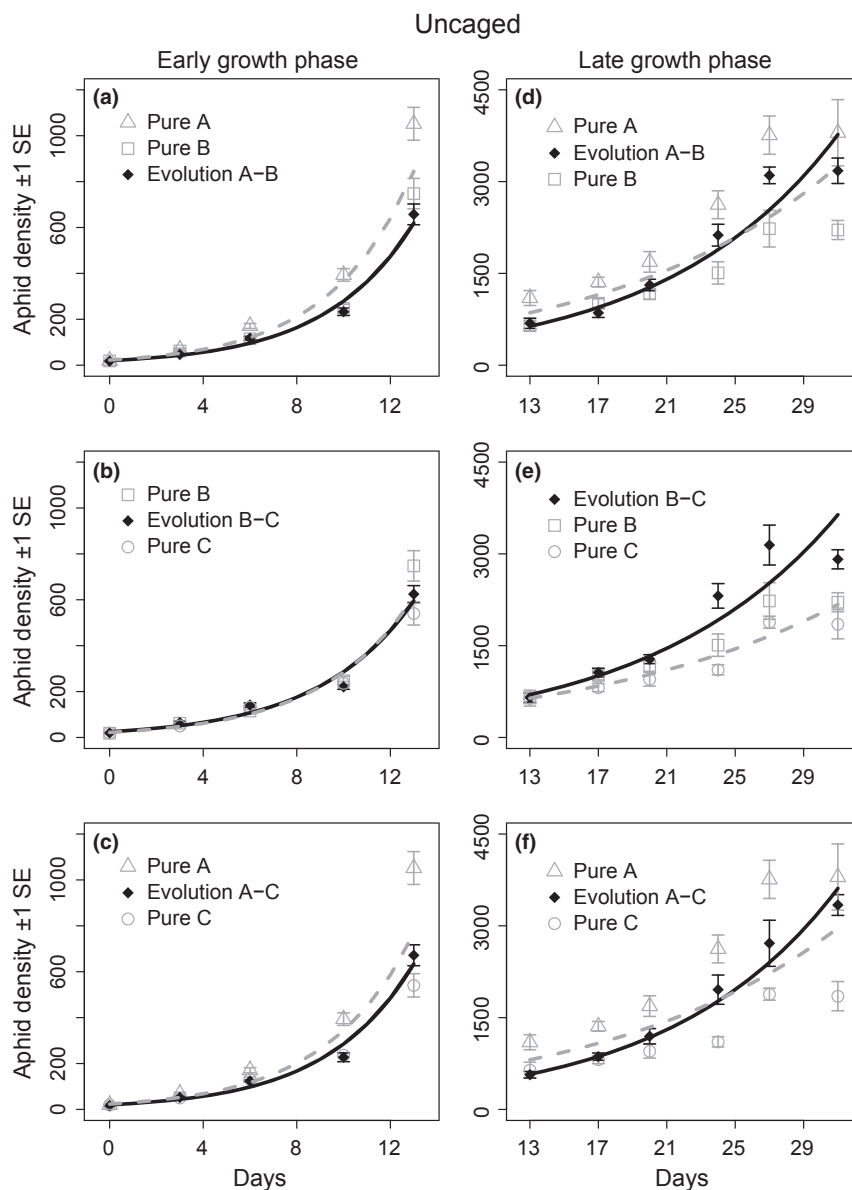


Figure 4 Partial residual plot of population dynamics of the three observed evolution treatments (black diamonds) with the best-fit model from LME analysis (black line) for uncaged treatments. The dashed grey line represents the best-fit model that combines both pure treatments using the constant (non-evolving) initial frequency of clones. For each treatment, we added the corresponding pure clone treatments (grey symbols) used to generate the no-evolution expectation. Evolution treatments have two letters. Values represent mean number of aphids (± 1 SE) once all explained variation in the model has been removed. The y-axes differ between panels. Left panels are for the early growth phase (days 0–13) and right panels are for the late growth analysis (days 13–31).

(e.g. spiders, coccinellids, Appendix S3) and aphid immigration by conspecifics (10% of the aphids genotyped were immigrants). These confounding factors and environmental variation strengthen the importance of our uncaged results because the impact of rapid evolution on population dynamics was still measurable and large even under these natural conditions.

Our experiment, however, demonstrated that ecological context could have an important and unexpected impact on rapid evolution's ecological effects. Our *a priori* hypothesis was that environmental biotic variation might overwhelm the effect of evolution and thus caged plants would be more likely to identify eco-evolutionary dynamics. This was not the case. It seems that these biotic factors might have magnified eco-evolutionary dynamics. Surprisingly, even

though populations rapidly evolved in the caged treatments, evolution did not significantly impact population dynamics (Fig. 3, Table 1). Our ability to detect an effect of evolution was reduced by the large population sizes reached in the caged treatments (up to 50 000 aphids). The coefficients of variation support this point; triplicate counts of the same plant at a density of 6000 aphids equaled 0.02 whereas for a plant with 26000 aphids it was 0.13.

A more likely explanation is that ecological context altered evolution. Caged treatments evolved 38% slower than uncaged treatments (Fig. 2), which could reduce the magnitude of evolution's impact on ecological dynamics and our ability to detect it. We posit two hypotheses for why evolution was slower in the caged treatment. Further experiments are required to identify the cause. Some aphid

Table 2 Summary of density- and frequency-dependent clonal selection analysis. Results of planned contrasts comparing whether increasing total density and increasing clonal frequency differentially reduce the growth rate of the clones in the evolution treatments. Percent differences represent the difference in slope (of relative growth rate versus density or frequency) between competing clones. Negative values imply that the faster growing clone, the first letter in each pairing, is less strongly negatively influenced by density or frequency compared with the slower clone. All *P*-values are for 2-tailed tests.

		Density		Frequency	
Treatments	d.f.	Difference in slope (%)	<i>P</i> -values	Difference in slope (%)	<i>P</i> -values
Caged					
<i>A</i> – <i>B</i>	77	–10.3	0.721	+212	0.267
<i>B</i> – <i>C</i>	77	–73.1	0.020	–62.3	0.182
<i>A</i> – <i>C</i>	77	–48.9	0.003	+328	0.126
Uncaged					
<i>A</i> – <i>B</i>	72	+23.9	0.268	+259	0.030
<i>B</i> – <i>C</i>	72	–38.5	0.048	–44.7	0.564
<i>A</i> – <i>C</i>	72	–51.6	0.003	–40.1	0.486

Significant results were bolded for easier identification.

clones are more susceptible to predation (Muller 1983; Pilson 1992), a selective force present in uncaged but not caged treatments. Another possibility is that caged populations were less strongly density-regulated than uncaged populations. Aphid clones are known to differ in how their growth rates respond to increased population density (Agrawal *et al.* 2004). We argue that caged aphid populations were under less severe population regulation because per capita growth rates were much higher throughout the experiment (mean daily $r_m = 0.25$) compared with uncaged populations in the second growth phase ($r_m = 0.08$). This difference can be attributed to the uncaged host plants being damaged by herbivores, which reduced plant size. Final mean dry weight for caged plants was 52% higher than uncaged (*t*-test, $P < 0.001$). Uncaged plants also had smaller leaves, most of which were lost by the end of the experiment in contrast to caged plants. Stronger competition among aphids might have caused more rapid evolution in uncaged treatments and hence a stronger impact of evolution on population growth rate.

Saccheri & Hanski (2006) proposed that population density is less likely to be influenced by natural selection or evolution if that population is under strong density regulation, which could overwhelm eco-evolutionary effects. Our results suggest that when regulation is weaker, density can still promote eco-evolutionary dynamics. Overall it seems the biotic environment played a significant role in this aphid species' eco-evolutionary dynamics, which highlights the importance of conducting such research in the wild.

The importance of genetic variation and rapid evolution on other members of the community is currently receiving much attention (Hughes *et al.* 2008; Johnson *et al.* 2009). Although aphid evolution increased aphid population growth rate and density this did not impact plant fitness or plant final biomass. One possibility is that non-aphid herbivore damage was a dominant determinant of plant fitness, overwhelming the aphid's impact. This is supported by the observation that plants attacked by aphids, although having lower biomass, did not differ in fitness from plants without aphids. Finally, micro-environmental variation might have increased variation between replicates in plant fitness reducing our ability to detect an effect (coefficients of variation among replicates were high: range of 0.1–1.9 with a mean of 0.6).

Our experiment focused on the less-studied half of the eco-evolutionary dynamics cycle. Rapid evolution impacts concurrent population dynamics but is the reciprocal causal process also occurring? Using a correlation approach, we found that the competitive advantage of clone A and clone B over clone C significantly increases at higher density, in both caged and uncaged treatments (Table 2). This suggests the possibility of a full eco-evolutionary feedback cycle where both rapid evolution and ecological dynamics influence each other on similar timescales (Kokko & López-Sepulcre 2007). Rapid evolution leads to higher densities and this alters natural selection thus modifying future bouts of evolution. Moreover, we found some evidence that rapid evolution also alters relative clonal fitness in one treatment suggesting more complex interactions (Table 2). We limit our interpretation of these results as our data cannot disentangle the effect of density and plant age. Explicit experimental tests, where density and frequency are manipulated, will be required to validate the occurrence and importance of the full eco-evolutionary dynamics cycle in this system.

Rapid evolution occurs in many applied fields such as fisheries, pest management, conservation biology, invasion biology and epidemiology (Ashley *et al.* 2003). Yet few of these fields incorporate evolution as a causal factor in population dynamic predictions (Hufbauer & Roderick 2005; Duffy & Sivars-Becker 2007; Kinnison & Hairston 2007). If evolution within 31 days can accelerate population growth rate by as much 42% in our system, it suggests that accounting for evolution might significantly alter optimal management strategies.

ACKNOWLEDGEMENTS

We thank V. La, T. Luong and K. Russell for assistance with genetic analyses and greenhouse work. A. López-Sepulcre and R. Bassar helped with statistical analyses and S. Zarate with molecular genetic techniques. We thank the University of California Reserve System for access to the field site. Funding for M. M. Turcotte from NSERC, FQRNT, Sigma Xi and a Mildred E. Mathias Grant supported this work, as did NSF DEB-0623632EF for D. N. Reznick.

AUTHOR CONTRIBUTIONS

The study was designed by all authors but performed by MT. MT wrote the manuscript and all authors contributed to revisions.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Planned contrast weight matrix used to test the *a priori* hypotheses comparing each evolution treatment to its corresponding no-evolution expectation that matched the initial clonal frequency.

Appendix S2 Differences in growth rate between pure clone populations.

Appendix S3 Arthropod taxa observed on the uncaged treatments during the experiment.

Appendix S4 Summary of Pearson correlation analyses between final plant dry above ground biomass and three fitness traits.

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Editor, Mark Vellend

Manuscript received 6 June 2011

First decision made 30 June 2011

Manuscript accepted 18 July 2011