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Source: *The American Naturalist*, Vol. 181, No. S1, A Critical Look at Reciprocity in Ecology and Evolution (May 2013), pp. S46-S57
Published by: The University of Chicago Press for The American Society of Naturalists
Stable URL: http://www.jstor.org/stable/10.1086/668078
Accessed: 24/04/2013 09:37

SYMPOSIUM

Experimental Test of an Eco-Evolutionary Dynamic Feedback Loop between Evolution and Population Density in the Green Peach Aphid

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ABSTRACT: An eco-evolutionary feedback loop is defined as the reciprocal impacts of ecology on evolutionary dynamics and evolution on ecological dynamics on contemporary timescales. We experimentally tested for an eco-evolutionary feedback loop in the green peach aphid, *Myzus persicae*, by manipulating initial densities and evolution. We found strong evidence that initial aphid density alters the rate and direction of evolution, as measured by changes in genotype frequencies through time. We also found that evolution of aphids within only 16 days, or approximately three generations, alters the rate of population growth and predicts density compared to nonevolving controls. The impact of evolution on population dynamics also depended on density. In one evolution treatment, evolution accelerated population growth by up to 10.3% at high initial density or reduced it by up to 6.4% at low initial density. The impact of evolution on population growth was as strong as or stronger than that caused by a threefold change in intraspecific density. We found that, taken together, ecological condition, here intraspecific density, alters evolutionary dynamics, which in turn alter concurrent population growth rate (ecological dynamics) in an eco-evolutionary feedback loop. Our results suggest that ignoring evolution in studies predicting population dynamics might lead us to over- or underestimate population density and that we cannot predict the evolutionary outcome within aphid populations without considering population size.

Keywords: density-dependent selection, community genetics, rapid evolution, experimental evolution, eco-evolutionary dynamic cycle, clonal selection.

Introduction

The reciprocal causal influences between evolution and ecological dynamics, termed "eco-evolutionary dynamics," are currently receiving much attention because such dynamics can alter the predicted outcome of ecological interactions (reviewed in Day 2005; Fussmann et al. 2007; Johnson and Stinchcombe 2007; Pelletier et al. 2009; Schoener 2011). This interest emanates from recent empirical studies quantifying how contemporary evolution can impact ecological dynamics over short timescales, or within a few dozen generations (Fussmann et al. 2003; Yoshida et al. 2003; Hairston et al. 2005; Van Doorslaer et al. 2009; Turcotte et al. 2011*b*). Eco-evolutionary dynamics occurring on these timescales have traditionally been ignored because of a widely held assumption that evolution is too slow to influence ecological dynamics (Hairston et al. 2005; Carroll et al. 2007; Fussmann et al. 2007; Kinnison and Hairston 2007). Thus, much of the work in evolutionary ecology and ecological genetics focused on how ecological conditions (abiotic, competitive environment) and ecological dynamics (density fluctuations) impose selection and cause evolution (Levins 2003; Carroll et al. 2007; Johnson and Stinchcombe 2007). The reciprocal arrow of causality—how evolution impacts ecological dynamics—was rarely studied, but important exceptions do exist (Pimentel 1961, 1968; Chitty 1967).

The assumption that evolution is too slow to have an impact on contemporary ecological interactions has been challenged by many reviews listing examples of contemporary evolution in both natural and human-disturbed habitats (Thompson 1998; Hendry and Kinnison 1999; Bone and Farres 2001; Reznick and Ghalambor 2001). These examples inspired a growing number of empirical studies that quantify how contemporary evolution alters ecological dynamics (Bohannan and Lenski 2000; Yoshida et al. 2003; Post et al. 2008; Van Doorslaer et al. 2009; Terhorst et al. 2010; Turcotte et al. 2011*a*, 2011*b*). For example, in this special issue, Agrawal et al.'s (2012) field experiment found that evolution of flowering time in evening primrose, *Oenothera biennis*, over 3 years significantly alters the density of a common lepidopteran seed predator. When both ecological and evolutionary dynamics reciprocally influence each other on contemporary and commensurate timescales, the system is said to demonstrate

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Am. Nat. 2013. Vol. 181, pp. S46–S57.
0003-0147/2013/181S1-53712$15.00.
DOI: 10.1086/668078

an eco-evolutionary feedback loop, or, as it is sometimes called, an eco-evolutionary dynamic cycle (Le Galliard et al. 2005; Kinnison and Hairston 2007; Kokko and López-Sepulcre 2007; Schoener 2011). Theoretical studies suggest that cyclical causality between evolution and ecology (e.g., density) on short timescales can greatly impact the ecological and evolutionary outcomes when compared to systems with linear causality (Pimentel 1961; Anderson 1971; Abrams and Matsuda 1997; Witting 2000; Shertzer et al. 2002; Day 2005; Hanski 2011). Eco-evolutionary feedback loops permit even more complex dynamics because neither process is independent of the other.

Few eco-evolutionary dynamics study systems have confirmed an eco-evolutionary feedback loop on short timescales. One confirmatory example involves the cyclical dynamics observed in the density of side-blotched lizards and the frequency of life-history strategies (Sinervo et al. 2000; Svensson and Sinervo 2000). Females that produce many small progeny are favored when population density is low. As they increase in frequency, so does density, which favors females that produce few larger, more competitive offspring, which eventually lowers population density, which leads the observed ecological and evolutionary cycles. Another example is that of changes in density of algae and predatory rotifers in chemostats. These density changes were caused by, and also lead to, changes in the frequency of algae clones that are either slow growing but less palatable or fast growing and more palatable (Yoshida et al. 2003). A similar cycle was also demonstrated in another rotifer algae system, but in that case the defensive trait was cell clumping (Becks et al. 2012). These studies show how evolution alters the ecological properties of the system and that these ecological changes alter future bouts of selection and evolution.

This study aims to experimentally test for an eco-evolutionary feedback loop in green peach aphids, *Myzus persicae*. Our previous experimental evolution studies manipulated the evolutionary potential of aphid populations and tracked their ecological dynamics. We found that evolution occurring over only four to five generations (approximately 1 month) significantly accelerated the population growth rate, compared to nonevolving control populations in the greenhouse, by up to 34% (Turcotte et al. 2011*a*). These results showed that evolution can be a strong driver of concurrent population dynamics. We then conducted this experiment in the wild on caged populations as well as on uncaged populations that were open to migration, interspecific competitors, and enemies. Uncaged plants were harmed by leaf herbivores, and the aphids showed higher intraspecific competition on these plants than on the caged plants. Intriguingly, only in the uncaged treatments did evolution alter population growth rate, increasing it by up to 42% compared to nonevolving control populations (Turcotte et al. 2011*b*). The uncaged populations also evolved faster than the caged ones. We suspected that these results were driven by differences in the strength of intraspecific competition as opposed to the occurrence of predation or migration, because a correlational analysis suggested that aphid clonal selection changed with density (Turcotte et al. 2011*b*). To investigate this hypothesis in this study, we manipulated both evolution and population density simultaneously to test for the presence and ramifications of an eco-evolutionary feedback loop that might have driven the results observed in the wild.

This study extends previous ones by experimentally testing (1) whether the rate and direction of evolution depend on density. We know evolution alters density and population growth, but does this modify the selective environment? To test for such a feedback loop, we investigated (2) whether the impact of evolution on concurrent population dynamics depends on initial density. By conducting these experiments with different aphid clones, we tested (3) whether the evolutionary context (genotypic composition of the population) influences eco-evolutionary dynamics. Finally, because simply showing that eco-evolutionary dynamics occur fails in telling us how important they are, it is imperative to compare their effect size with other commonly studied ecological forces (Hairston et al. 2005; Johnson and Stinchcombe 2007; Pelletier et al. 2009; Schoener 2011). Thus, we also tested (4) whether evolution had a relatively large impact on population growth rate compared to the effect of initial density.

Material and Methods

Experimental Evolution with the Green Peach Aphid

The focal study species is the green peach aphid, *Myzus persicae*, growing on one of its many hosts, the annual mustard, *Hirschfeldia incana*. *Myzus persicae* is a globally distributed species best known for its tremendous impact as an agricultural pest (Blackman 1974; Mackauer and Way 1976). We developed this experimental evolution system specifically to study eco-evolutionary dynamics because the aphid naturally evolves quickly through changes in clonal frequency during its asexual growth phase in the spring–summer months (Foster et al. 2002; Vorburger 2006; Turcotte et al. 2011*b*). The cyclical parthenogenesis of aphids makes for an excellent experimental evolution system because one can create nonevolving control populations (see below) without issues of inbreeding and measure evolution by tracking changes in genotypic frequency. Our study utilizes the economically important period of asexual reproduction in their life cycle that lasts from spring to fall and represents a large fraction of their yearly

generations (Blackman 1974). Changes in frequency and extermination of clonal lineages in this period alter the gene pool available during the sexual phase and thus have lasting impacts on population genetic structure (Vorburger 2006).

In 2008, multiple clonal lineages were collected from a single wild population at the Motte Rimrock Reserve in Perris, California. *Myzus persicae* populations in the spring contain many clonal lineages, and we usually found more than one clone per plant (M. M. Turcotte, unpublished data). Although a cyclical parthenogen in most of its range, this population of aphids in Southern California could have lost the sexual phase altogether since winters are so mild (Vorburger et al. 2003). We identified clones using six microsatellite markers and selected three of these clones that differ in intrinsic growth rate for this and previous experiments (for details and methods, see Turcotte et al. 2011*a*). A life table experiment also found that these clones differ in lifetime fecundity and survival rates (M. M. Turcotte, unpublished data).

Experimental Design

We manipulated initial aphid density and evolutionary potential by creating populations with a single aphid clone (pure) or two clones (mixed). As populations grew until their host senesced, we took a genetic sample and measured the rate of evolution as the change in genotype, or clonal, frequencies over time in the mixed treatments. We repeatedly counted aphids in all treatments to compare their population growth rates.

Myzus persicae populations growing on *H. incana* plants were studied in a partly cooled greenhouse (mean daytime temperature = 33°C, range = 21°–47°C; mean nighttime = 21.5°C, range = 16°–28.5°C). Asexual reproduction was maintained by artificial lighting providing 16L : 8D (Blackman 1974). Variation in host plants was minimized by using the seeds of a single *H. incana* collected in 2008 at the reserve. These seeds were planted in 4-L pots using soil mix 3, a sand/peat moss mix, from the University of California, Riverside, supplemented with micronutrients, and watered every 3 days. Plants in 8-L pots were caged using a wire frame that created a 75-cm-high dome holding up thin, transparent mesh (Bridal Organza, no. 664-7242, Jo-Ann Fabrics and Crafts).

Replicates were initiated on three consecutive days starting July 25, 2010, when the plants were 8 weeks old. Each plant received third-instar aphids from stock greenhouse clonal populations (regularly tested for contamination) that were maintained on *H. incana*. On day 1, aphids that failed to settle were replaced with fourth-instar aphids.

We used three aphid clonal lineages, identified as A, B, and C, that differ in microsatellite markers and exponential growth rate (Turcotte et al. 2011*a*, 2011*b*). We created 12 aphid treatments in a factorial design that manipulated initial density and evolution (i.e., the possibility of evolution or not). Three different mixed treatments consisted of aphid populations (on a single plant) that had two different clones at an initial frequency of 50 : 50. We established all possible two-way combinations (A-B, B-C, and A-C). These populations have genetic variation in fitness (e.g., clone A's intrinsic growth rate is greater than that of clone C) and thus could evolve by changing in clonal frequency (away from the initial ratio). Three different nonevolution (pure clone) treatments received aphids from only one of the three clones. Because these populations contained only one genotype, evolution could not occur. This method assumes that mutations during the experiment are too rare or too weak to spread in the population within a few generations and influence population dynamics.

We also manipulated population density by initiating these six treatments with either 20 or 60 aphids on each plant. We tripled the initial density because in our field collections we often observed such a range of density on single plants early in the growing season (M. M. Turcotte, personal observation). We did not create larger initial populations because the plant might senesce too soon. Each treatment combination was assigned in a randomized block design and replicated seven times for a total of 84 aphid populations (plants).

Rates of Evolution and the Impact of Density

On day 22 we collected 50 aphids from every population in order to track changes in clonal frequencies (evolution). Between 16 and 40 aphids from each sample were genotyped (for a total of 892 aphids) at three microsatellite loci using a multiplex approach (for detailed methods, see Turcotte et al. 2011*a*). We tested whether final frequency of the faster clone in the seven replicates of each evolution treatment differed significantly from the initial clonal frequency of 50% using one-sample *t*-tests. We also tested whether initial density altered the rate of evolution in each aphid pairing using two-sample *t*-tests.

Aphid Population Dynamics

Aphid population dynamics were quantified using methods similar to previous studies (Turcotte et al. 2011*a*, 2011*b*). Aphids were counted on days 3, 6, 10, 13, 16, and 22. All counts were made by M. M. Turcotte. When aphid populations rose above 2,000, they were subsampled by counting one-half of every leaf. On day 22, plants had begun senescing, having multiple yellow and/or drying leaves. Aphid population density had also begun to crash

by day 22, and all replicates died soon afterward. Aphid populations in nature are well known for growing exponentially and then suddenly crashing within a few days (Karley et al. 2003). In our experience these populations never approach equilibrium density. By inspecting the residuals of each treatment, we found that exponential growth models from days 0 to 16 best fit the observed population dynamics. Including day 22 led to large overestimates of population size. Logistic models were also tested with and without day 22 but generated even weaker fits to multiple data points.

Differences in population growth rate between treatments were tested using the exponential growth model

$$N_t = N_0 \cdot e^{(r_m \cdot \text{day})},$$

where N_t is the number of aphids on day t, N_0 is the number of aphids on day 0, and r_m is the daily intrinsic growth rate. This model was analyzed with a nonlinear mixed-effect model (nlme), implemented in R (ver. 2.11.1, R Development Core Team 2009) using the nlme package (Pinheiro et al. 2011). The fixed effect for N_0 was initial density (low or high), and the fixed effects for r_m were initial density, aphid composition, and their interaction. Because 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). Block (day of initiation of the replicate and spatial position in the greenhouse) did not improve model fit and was not included in the final model. Covariates were included to control for differences in initial plant size and stage of development. Initial plant size was quantified as the first principal component, explaining 70% of the variation in the number of true leaves and rosette width. Stage of development was quantified into an ordinal scale (1 = low bolt, 2 = bolt, 3 = flowers).

The Impact of Evolution on Population Dynamics

One of our main objectives is to quantify the impact of the evolution of aphids on population dynamics. We cannot directly compare identical mixed clone populations that do and do not evolve without manipulating the selective environment (which could impact the population dynamics). Instead, we developed a method to compare the observed population growth rate in the mixed (evolving) treatments to the expected growth rate of a population that has the same two clones but does not evolve (remains at a frequency of 50 : 50) by using the pure clone aphid treatment data (Turcotte et al. 2011*a*, 2011*b*). We tested a priori hypotheses that the population growth rate does not differ between evolution treatments and their corresponding pure treatments, for example, A-C mixed treatment versus pure A and pure C treatments. We did so with the use of planned orthogonal contrasts between a subset of the treatments within the nlme analysis. We set weighed, planned contrast coefficients of the no-evolution expectation to match those of the initial clonal frequency (e.g., pure A low density = −0.5 and pure C low density = −0.5, compared to A-C mixed low density = +1). Thus, the differences in growth rate between the mixed treatment and the no-evolution expectation represent the impact of changes in the frequency of clones (evolution) on population dynamics. To enhance interpretation of the magnitude of differences in population growth rate, we also calculated expected densities on day 16 from the model parameters and set common covariate values (mean values). This approach implicitly assumes that interactions within and between clones are similar since different clones are not actually interacting in the no-evolution prediction.

Results

Pure Clone Treatments

Pure clone treatments differed in their intrinsic growth rate, and these differences changed in magnitude and rank with initial density (fig. 1). At low initial density, differences between pure treatments were smaller. Clone A grew fastest ($r_m \pm 1$ SE, 0.297 ± 0.013); its r_m value was 2.4% higher than clone B's (0.290 ± 0.012) and 5.3% higher than clone C's (0.282 ± 0.015; fig. 1*a*). Clone B grew faster than clone C by 2.9%. At high density, however, there was a change in rank order (fig. 1*b*). Clone A (0.281 ± 0.010) grew 14% faster than clone C (0.246 ± 0.011) and 21% faster than clone B (0.232 ± 0.012), and clone B grew 6% slower than clone C. Expected density on day 16 based on the best-fit growth estimates predict that the pure population could differ by as much as 27% in low density and 117% at high density.

Genetic Analyses of Density-Dependent Selection

Clear evidence of density-dependent clonal selection was observed as the evolutionary rate and outcome was altered by initial aphid density. By day 22, within only three to four aphid generations, five of the six mixed aphid treatments evolved away from the initial frequency of clones (fig. 2). The A-B mixed treatments significantly evolved but only at high density (final frequency of clone A = 47%, one-sample t-test, $P = .630$; 57%, $P = .020$, for low and high density, respectively). In the B-C mixed treatments, density altered the direction of evolution; at low density clone B became more frequent, reaching 69% ($P = .017$), whereas at high density clone C reached 70%

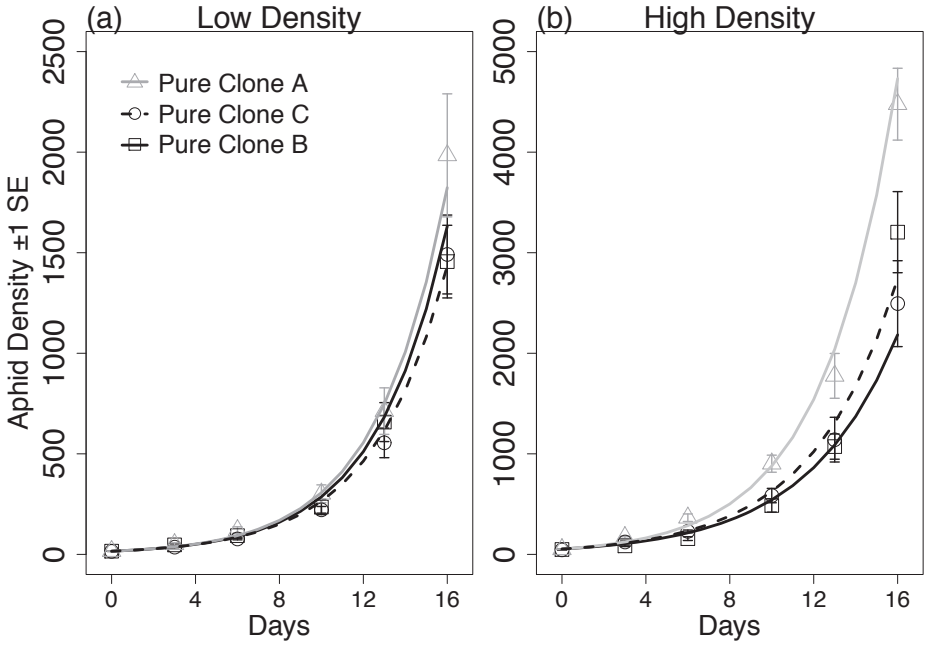

Figure 1: Partial residual plots of population dynamics of pure clonal treatments at low and high initial density. Values represent the mean number of aphids (±1 SE) through time, once all explained variation in the model has been removed. Panels show low initial density treatments (*a*) and high initial density treatments (*b*); notice that the *Y*-axes differ. Functions represent the best-fit exponential model for each treatment (clone A is the gray line, clone B is the full black line, and clone C is the dashed black line).

($P = .004$). Finally, in the A-C mixed treatments, clone A had an advantage over clone C and there was no effect of density on the outcome. Clone A reached a frequency of 71% ($P = .002$) at low density and 65% ($P = .044$) at high density (fig. 2). We also explored whether the frequency of clones on day 22 could be predicted from the population dynamics observed in the pure clone treatments. Using the growth parameters for each clone from the model, we created an expected frequency of clones in mixed treatments on day 22. Table 1 presents *t*-tests comparing each evolution treatment to its expected state. In general, pure clone growth rates predicted the direction but not the rate of evolution.

Impact of Density and Evolution on Population Dynamics

Initial aphid density and evolution causally impacted population growth. Although exponential growth models best represented the population dynamics of all treatments, high initial aphid density did reduce population growth rate by 9.5% on average (nlme, $F = 25$, $P < .0001$). To test for the impact of evolution on concurrent population dynamics, we compared the observed population dynamics in mixed populations to those observed in both corresponding pure treatments by using planned contrasts proportional to the initial frequency of clones (i.e., population dynamics without evolution). Given that the interaction between initial density and aphid treatment was significant (nlme, $F = 4.8$, $P = .0003$), we conducted planned contrasts for each density separately (table 2). Evolution in the A-B treatments did not significantly alter population dynamics at either density (fig. 3*a*, 3*b*; table 2; both $P > .2$). The impact of evolution in the B-C treatments was highly influenced by initial density. At low density, evolution (i.e., an increase in the frequency of clone B) slowed population growth by 6.4% ($P = .0004$; fig. 3*c*), whereas, at high density, evolution (i.e., an increase in clone C) accelerated population growth by 10.3% ($P = .003$; fig. 3*d*; table 2). Expected density on day 16, based on the best-fit growth estimates, predicts that evolution causes differences in population size of −25% and +48%, respectively (table 2). Finally, in the A-C mixed treatments, initial density magnified the effect of evolution. At low density, evolution had a small but statistically significant positive impact (1.1%, $P = .022$) on population growth rate (fig. 3*e*), yet at high density evolution strongly accelerated population growth by 9.4% ($P = .0001$; fig. 3*f*; table 2). These differences in population growth rate due to evolution are predicted to cause differences on day 16 of +4.3% and +43% in population size, respectively (table 2).

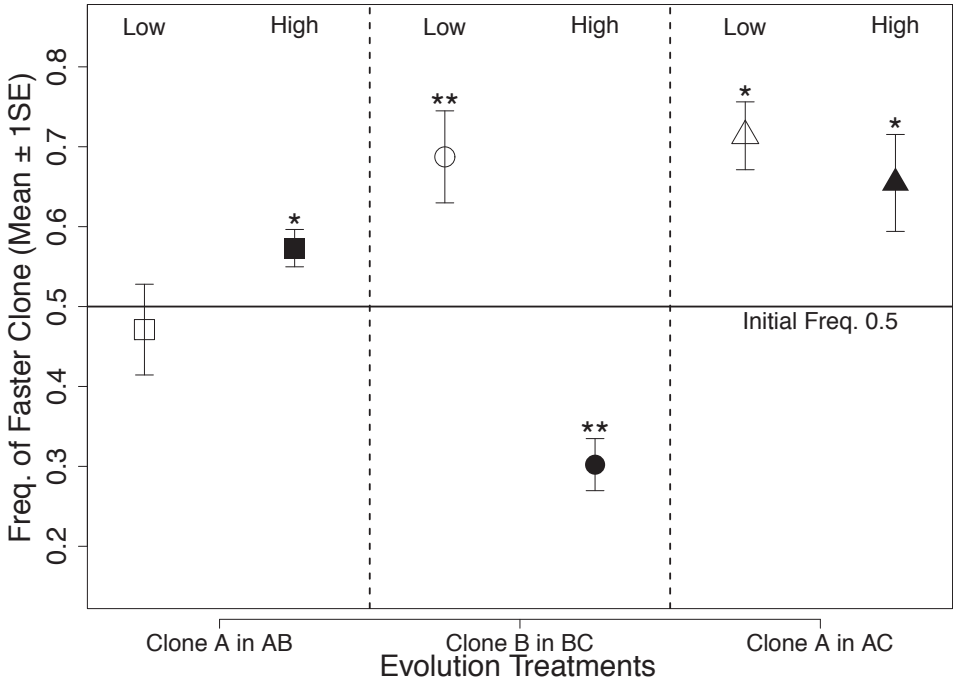

Figure 2: Clonal evolution as shown by the mean frequency (±1 SE) of the faster-growing aphid clones on day 22 of the experiment in all six mixed treatments. The horizontal bar indicates the initial clonal frequency of 0.5 on day 0. The *X*-axis shows which clone's frequency is being tested in each treatment. Dashed vertical lines separate the evolution treatments for which both initial density treatments are shown. The asterisk indicates significant divergence from initial frequency, and two asterisks indicate that clone frequency significantly differs between low and high initial density within an evolution treatment.

Discussion

This experiment tested for causal interactions between evolutionary and ecological dynamics on short timescales. We found that the density of aphids significantly altered the rate and direction of evolution and that these evolutionary changes significantly altered population growth rate and final predicted density. Given that both ecological dynamics and evolutionary dynamics causally influenced each other on commensurate timescales, this system demonstrates an eco-evolutionary feedback loop (Kokko and López-Sepulcre 2007; Schoener 2011). The implications of such loops, as our results demonstrate, are that accurate ecological predictions must incorporate evolution and, conversely, that accurate evolutionary predictions require knowledge of the way evolution modifies ecological context.

The Mechanisms of Eco-Evolutionary Dynamics

Three mechanisms drove the eco-evolutionary feedback loop in this system. First, evolution altered the populations' intrinsic growth rates through changes in clonal composition. This evolutionary change occurred so rapidly that significant changes in population growth rate were observed as the population evolved. Most experimental studies of eco-evolutionary dynamics compare the ecological impacts between already diverged populations (Kinnison et al. 2008; Post et al. 2008; Van Doorslaer et al. 2009; Bassar et al. 2010) and, in doing so, might miss interesting interactions between ecological and evolutionary dynamics. The use of experimental evolution is growing in popularity because it permits researchers to quantify the immediate ecological effects of evolution as it is occurring (Pimentel et al. 1963; Pimentel 1968; Yoshida et al. 2003; Terhorst et al. 2010; Turcotte et al. 2011*a*, 2011*b*; Becks et al. 2012). The second mechanism driving the eco-evolutionary feedback loop is that the evolution of the aphids depends on the same ecological context (i.e., density) that is modified by evolution. Initial density determined whether evolution occurred (A-B treatments) and changed the outcome (B-C treatments; fig. 2). This evolutionary sensitivity to density is due to variation in how strongly density impacts different aphid clones, as observed in the milkweed aphid (Agrawal et al. 2004). Thus, as the aphid populations grew, the relative fitness of clones changed, as we hypothesized previously (Turcotte et al. 2011*b*). Although our predictions of the evolutionary out-

Table 1: One-sample *t*-test analyses comparing the observed frequencies of the faster-growing clone in each evolution treatment on day 22 to predicted frequencies based on model fits of pure clones

Focal clone in evolution treatment	Mean observed frequency	Predicted frequency	*t* value	*P* value
Low density:				
A in A-B	.471	.538	−1.17	.286
B in B-C	**.687**	**.541**	**2.54**	**.044**
A in A-C	**.714**	**.578**	**3.20**	**.019**
High density:				
A in A-B	**.573**	**.749**	**−7.51**	**.000**
B in B-C	**.302**	**.416**	**−3.49**	**.025**
A in A-C	.655	.679	−.40	.703

Note: All *P* values are for two-tailed tests. Significant *P* values imply that observed frequencies differ from predictions. All *P* values are for two-tailed tests. Significant results were bolded for easier identification.

come of mixed populations using pure clonal population dynamics were usually accurate in direction, when accounting for initial density, evolution was usually faster than predicted (table 1). This suggests a third mechanism: that aphid clones seem to interact when mixed, leading to asymmetrical competitive impacts (e.g., Hazell et al. 2005). For example, we found that, at low density, clone A, when grown with clone C, should have reached 58% frequency but actually reached 71% on average. This suggests that clone A is somehow reducing clone C's growth. The mechanisms driving such interactions remain untested in this system but could include differential efficiency in resource acquisition, which could occur through finding better feeding sites, through aggressive behavior, or by variation in sensitivity to alarm pheromones (Muller 1983). These clonal interactions influenced both the outcome of evolution and how evolution impacts population growth rates by changing the strength of intraspecific competition, making it more difficult to quantify the impact of evolution on its own given our experimental design. Truly disentangling the complex web of interactions would require additional experiments that manipulate the clonal composition (i.e., their initial density and frequency). Such complexity further highlights the importance of applying eco-evolutionary dynamics approaches when predicting population dynamics.

This factorial experiment was motivated by our previous study in the wild where evolution accelerated population growth in uncaged treatments but not in caged treatments (Turcotte et al. 2011*b*). We hypothesized that the most important factor driving these results was that uncaged populations were denser and had stronger intraspecific competition, as opposed to other differences between these treatments, such as the occurrence of predation, interspecific competition, and migration (Turcotte et al. 2011*b*). In support of this hypothesis, we found that, in general, higher initial density led to stronger effects of evolution on population dynamics (fig. 3; table 2) because the clones differed more in growth rate at high density (fig. 1), which caused a greater change in population dynamics as the frequency of the faster clones increased. Yet, because we conducted this experiment in controlled greenhouse conditions to carefully test how evolution and density interact, we excluded other ecological factors (e.g., predation). Thus, we can conclude only that intraspecific competition represents a plausible explanation for our results in the wild.

Relation of This Study to Prior Studies of Eco-Evolutionary Dynamics

Most experimental studies of eco-evolutionary dynamics consist of small communities where prey evolve resistance that alters the ecological dynamics of the enemy, which then changes future evolution of the prey (Pimentel et al. 1963; Yoshida et al. 2003; Bull et al. 2006; Terhorst et al. 2010; Becks et al. 2012). Of the systems with eco-evolutionary dynamic feedback loops that occur within a single species, all seem to operate through intraspecific density. As discussed previously, density cycles of the side-blotched lizard lead to and are caused by cycles in the frequency of different life-history strategies (Sinervo et al. 2000; Svensson and Sinervo 2000). The Glanville fritillary butterfly system undergoes eco-evolutionary feedback loops within a metapopulation context (Hanski 2011). Butterflies with a known genetic polymorphism for higher dispersal ability and higher fecundity at low density are favored in small populations and in areas with many unoccupied patches, versus low dispersal and low fecundity types. Their frequency in the metapopulation increases as they disperse and colonize unoccupied patches (Zheng et al. 2009). The dispersive types also accelerate population

Table 2: Analysis of population dynamics comparing evolving and nonevolving populations in low- and high-density treatments

Evolution treatment (clones)	Low density				High density			
	df	*F*	*P*	% change	df	*F*	*P*	% change
A-B	397	.3	.577	+1.0	397	1.6	.205	+3.6
Density day 16				+4.8				+7.6
B-C	**397**	**12.8**	**.0004**	**-6.4**	**397**	**9.1**	**.0028**	**+10.3**
Density day 16				−25.6				+47.5
A-C	**397**	**5.3**	**.022**	**+1.1**	**397**	**15.2**	**.0001**	**+9.4**
Density day 16				+4.3				+43.1

Note: The effect of evolution on population growth rate compared to nonevolving controls at low and high initial density. Results of planned contrasts from a nonlinear mixed-effect model comparing growth rates of each type of evolving population to its corresponding no-evolution expectation, generated from the pure aphid treatments following the initial frequency of clones (see "Material and Methods" for details). The percent change represents the change in population growth rate from the nonevolving expectation to that of the observed evolution treatment. Thus, positive changes represent increases due to evolution. We also added the percent difference in predicted density on day 16 based on the best-fit model parameters. All *P* values are for two-tailed tests. Significant results were bolded for easier identification.

growth (Hanski and Saccheri 2006), increase the size of the metapopulation, and cause a decline in available patches. This ecological change causes selection to favor nondispersing genotypes because these types do better when resources are scarce (Zheng et al. 2009; Hanski 2011). As the frequency of dispersers declines, so does population growth, and fewer patches are recolonized after extinction, leading to a smaller metapopulation size until conditions favor the dispersers once again (Hanski 2011). In both of these systems, as well as with the green peach aphids, intraspecific density seems to provide the link between ecological and evolutionary dynamics. Yet, with such a small number of empirical systems, it is too soon to suggest that intraspecific eco-evolutionary feedback loops act only in this fashion.

The Role of Density in Shaping Eco-Evolutionary Dynamics

Interactions between density and evolution are often key drivers of eco-evolutionary dynamics, as suggested in the earliest studies on the topic; however, density can also impede such dynamics. Chitty (1960, 1967) suggested that genetically based changes in the quality of voles, *Microtus agretis*, regulate population cycles. His hypothesis states that high density selects for aggressive but slow-reproducing voles, which slows population growth, leading to selection for nonaggressive, fast-reproducing voles, thus regulating population density. Later empirical evidence failed to support this hypothesis (Tamarin and Krebs 1969). Pimentel (1961) created one of the first eco-evolutionary feedback loop models to show how evolution of defenses in a plant to herbivores would feed back and reduce the density of those herbivores. This would in turn reduce selection for resistance and eventually enable larger herbivory populations. Continued feedback between herbivory pressure and plant evolution led to dampened population fluctuations. Although density is a key player in the eco-evolutionary feedback loop in our system and in others, it might, under some circumstances, prevent such dynamics. In systems with strong density regulation, evolutionary changes in growth rate could have little impact on the population dynamics of a population near carrying capacity, unless carrying capacity itself evolves (Saccheri and Hanski 2006; Kinnison and Hairston 2007). In aphids, populations grow quickly and crash (Karley et al. 2003) and rarely reach stable dynamics. Although we observed that higher intraspecific density lowered population growth rate, our system is not strongly density regulated. Thus, it is not surprising that we observed stronger impacts of evolution on population growth at higher initial density, given that the differences in growth rate between clones are larger at high densities.

Most systems showing eco-evolutionary dynamic loops have cyclical ecological and evolutionary dynamics where intra- and or interspecific density fluctuations alternately favor different genotypes (Sinervo et al. 2000; Yoshida et al. 2003; Hanski 2011; Becks et al. 2012; but see Terhorst et al. 2010). In our system, this is not the case. We observed how evolution and density influenced each other during a single increase in population size until a crash. Two factors might explain this difference. First, the eco-evolutionary dynamics can be dampening. In the case of the A-C treatments, we see that clone A always increased in frequency at both low and high density (fig. 2). As population growth accelerated due to evolution, clone A remained favored and eventually should exclude clone C completely. Thus, the potential for evolution to influence

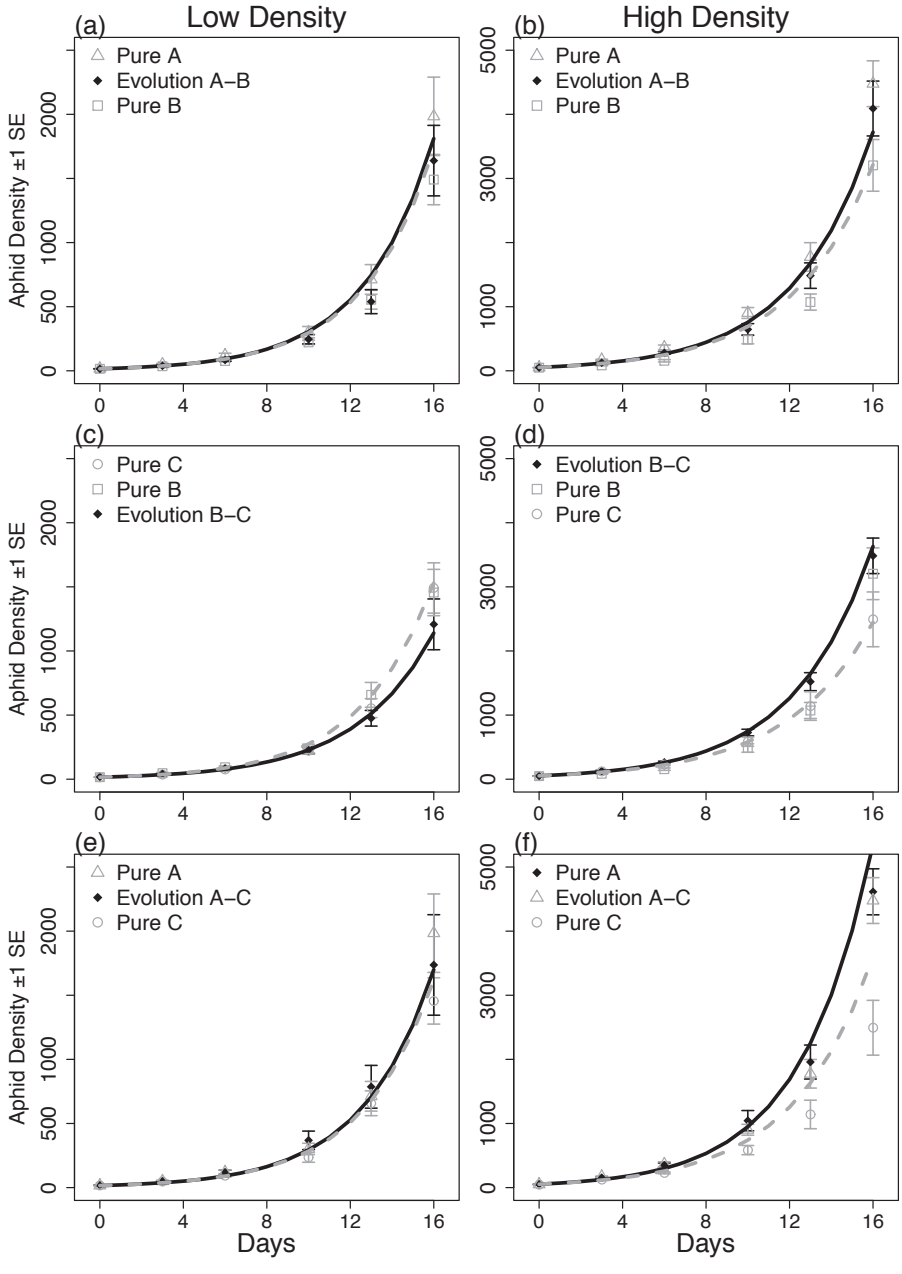

Figure 3: Population dynamics of evolving and nonevolving aphids at different initial densities. Partial residual plots of population dynamics of the observed evolution treatments (black diamonds) with the best-fit model from nlme analysis (black line). Left panels are for the low initial density treatments, whereas the right panels are for the high initial density treatments. The *Y*-axes differ between panels. The dashed gray line represents the best-fit model that combines both pure treatments using the constant (nonevolving) initial frequency of clones (50 : 50). We added the corresponding pure clone treatments (gray symbols) used to generate the no-evolution expectation. Values represent mean number of aphids (± 1 SE) once explained variation in the model was removed.

ecological dynamics diminished through time as the favored genotype approached a frequency of 100%. In the cycling systems reviewed earlier, genotypes have strong trade-offs, where one is favored at low density (e.g., side-blotched lizards, butterflies) or high predation pressure (clumping algal cells), whereas the other genotypes are favored at high density or low predation pressure. In our system, we observed a trade-off in competitive ability between clone B and clone C at high and low density (fig. 2), but the eco-evolutionary dynamics were always damp-

ening. Clone B did better at low density, and in those conditions evolution slowed the population growth rate (fig. 3), thus reducing the likelihood that the ecological context will ever favor clone C. A similar scenario occurs at high density, where C was favored and evolution altered growth rate in a manner that further favors clone C. In addition, our experimental design has no ability to detect cycles. Unlike in the other experimental systems, we did not renew resources for the aphids, and eventually the plants died and the aphid populations crashed. This more closely mimics the population dynamics of aphids in the wild (Karley et al. 2003). Aphids will eventually colonize different nearby plants or become winged and fly to different crops or their winter hosts (Blackman 1974). In these cases, the populations usually start at a low density. It is thus possible that cycles of density and genetic composition would occur over multiple growing seasons.

How Important Are Eco-Evolutionary Dynamics?

A question of growing interest in evolutionary ecology is to quantify the importance of eco-evolutionary dynamics (Johnson and Stinchcombe 2007; Pelletier et al. 2009). Hairston et al. (2005) developed methods to calculate the relative impact of evolutionary and ecological changes on an ecological responses variable repeatedly measured through time. They suggested that we should qualify evolution as "rapid" only when it has a large relative impact. Our factorial experimental design lends itself to addressing this question. We found that a threefold increase in initial density reduced exponential population growth rate by 9.5% but did not alter the pattern of population growth, that is, it did not become logistic. We found that evolution in two different treatments had equal or greater effect sizes on exponential growth rate (table 2). This suggests that eco-evolutionary dynamics can be relatively important forces in population dynamics on short timescales and that the evolution we observed was "rapid." Our previous experiment argues that evolution is a strong driver of short-term population dynamics in the wild, as it altered growth rate by up to 42% in the face of other ecological processes and abiotic variation (Turcotte et al. 2011*b*). Yet, this greenhouse experiment excluded many ecological processes (e.g., predation). Thus, to truly resolve how important eco-evolutionary dynamics are requires factorial manipulations of key ecological processes and of evolution in the wild, which presents an interesting avenue of future research.

Our study has important implications for the burgeoning field of eco-evolutionary dynamics because it presents a rare experimental test of a feedback loop (reviewed in Fussmann et al. 2007; Schoener 2011). Since population density influences the outcome and rate of evolution and evolution alters population growth, our results suggest that ecological and evolutionary predictions would be improved if eco-evolutionary dynamics were explicitly considered. We found that evolution can accelerate or slow population growth on short timescales, which has important, tangible implications for the nascent subdiscipline of evolutionary enlightened management (Ashley et al. 2003). Kinnison and Hairston (2007) discuss how contemporary evolution could help rescue endangered populations by increasing population growth rate but also that selection could favor more competitive genotypes, leading to reduced population growth rate. Our study provides valuable experimental data that support some of the mechanisms required for evolution to influence population dynamics and, potentially, persistence.

Acknowledgments

We thank V. La and K. Russell for assistance with genetic analyses and greenhouse work. We also thank two anonymous reviewers for providing constructive comments that greatly improved the manuscript. Funding for M.M.T. from the Natural Sciences and Engineering Research Council–Fonds de Recherche Nature et Technologies and a Mildred E. Mathias Grant supported this work, as did National Science Foundation DEB-0623632EF for D.N.R.

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Editor: Troy Day