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Experimental Tests of Rapid Eco-Evolutionary Dynamics in a Plant-Herbivore System

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Martin Mathieu Turcotte

March 2011

Dissertation Committee:
Dr. David N. Reznick, Co-Chairperson
Dr. J. Daniel Hare, Co-Chairperson
Dr. Derek A. Roff

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The Dissertation of Martin Mathieu Turcotte is approved:

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University of California, Riverside

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ABSTRACT OF THE DISSERTATION

Experimental Tests of Rapid Eco-Evolutionary Dynamics in a
Plant-Herbivore System

by

Martin Mathieu Turcotte

Doctor of Philosophy,
Graduate Program in Evolution, Ecology, and Organismal Biology
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Examples of rapid evolution, occurring within a few dozen generations or less, have recently increased substantially. Evolution on these timescales suggests the possibility that rapid evolution could reciprocally interact with short-term ecological dynamics, a process termed '*Eco-Evolutionary Dynamics'*. My dissertation experimentally tests these interactions in an aphid (*Myzus persicae*) and an undomesticated mustard host (*Hirschfeldia incana*).

Aphid clonal lineages were collected from a local population, and found to differ by up to 17% in intrinsic growth rate. This variation was used to conduct experimental evolution. Experiment 1 quantified how aphid rapid evolution impacts concurrent aphid population dynamics in the greenhouse. I manipulated the amount of genetic variation in intrinsic growth rate by manipulating the genetic composition of aphid populations, which altered rates of evolution.

Significant changes in clonal frequencies (or gene frequencies) occurred within a few weeks. Evolving populations grew significantly faster, up to 34%, and reached higher densities compared to non-evolving control populations.

I then tested whether rapid evolution significantly impacts population dynamics in the wild. Evolving populations grew significantly faster, up to 42%, and reached up to 67% higher densities compared to non-evolving controls even in the face of environmental variation. Yet evolution only had this impact in the natural uncaged treatments highlighting that ecological context alters the strength of eco-evolutionary dynamics.

Finally, the last experiment tested the full eco-evolutionary dynamic cycle of dual causality between ecological dynamics (density) and evolutionary dynamics in the greenhouse. Initial aphid density altered the rate and outcome of evolution. Density also quantitatively and qualitatively altered how rapid evolution impacts population growth rate sometimes accelerating or decelerating growth. This experiment also revealed that rapid evolution and intraspecific density have similar relative impact sizes on population growth rate.

My dissertation experimentally quantified strong reciprocal causal interactions between rapid evolution and population dynamics on short-timescales in both greenhouse and in the field in a plant-herbivore system for the first time. Such interactions strongly countermand the assumption that evolution is too slow to influence population dynamics and suggests that ecological and

evolutionary predictions would be improved if these interactions were integrated into predictive models.

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Chapter One: A General Introduction to the Study of Rapid Eco-Evolutionary Dynamics

Abstract

In this introductory chapter I first briefly review the historical development of the ‘new’ field of eco-evolutionary dynamics and summarize current theoretical and empirical findings. I then present the objectives of my dissertation and how these aim to address outstanding questions in this emerging field of study.

Background

Interactions between Ecology and Evolution

Ecology and evolutionary biology are fields that share a long and complex history with periods of either strong integration or independent development (Collins 1986). It has long been recognized that ecology and evolution influence one another. For example, Darwin (1859) described how ecological interactions, especially competition, shapes the selective environments in which species evolve, and how evolution will impact extinction and species distributions. Few biologists would argue that evolution and ecology do not interact at least on long timescales. As the fields of population ecology and population genetics developed in the 1920s, they also grew apart (Collins 1986). Population ecologists often ignored evolution, since evolution was perceived to have little

impact, and population geneticists focused on how the ecological environment causes evolution (Ford 1964). The classic evolutionary paradigm was established where ecology caused evolution (arrow 1 in Fig. 1.1). The alternate causal pathway, where evolution impacts ecology (arrow 2 in Fig. 1.1), was recognized but usually relegated to long-term effects. For example speciation will impact species diversity (Sax and Gaines 2003, Pelletier et al. 2009). This separation is still enduring today as population genetics and population ecology are still largely studied separately (Levins et al. 2003, Lewontin et al. 2003, Hairston et al. 2005, Saccheri and Hanski 2006, Pelletier et al. 2009).

Two common assumptions in the ecological literature often exclude any possible impact that evolution might have on short-term population dynamics, dynamics occurring within a few dozen generations or within 100 years (Thompson 1998, Hairston et al. 2005). First, populations are assumed to be genetically homogeneous (Cappuccino and Price 1995, Sibly and Hone 2002, Hairston et al. 2005). Yet, within population genetic variation in ecologically important traits has been demonstrated repeatedly since the 1960s (Ayala 1968, Berry et al. 1978). Moreover, such variation can significantly influence population dynamics in the laboratory (Schlager 1963, Leips et al. 2000) and in nature (Hanski and Saccheri 2006, Hazell et al. 2006). Another, common assumption is that evolution occurs on a much slower (and thus separate) timescale than short-term ecological processes. Even if genetic variation is considered, evolution is ignored because it is perceived to be too slow to have an effect (Slobodkin 1980,

Endler 1991, Cappuccino and Price 1995, Hairston et al. 2005, Pelletier et al. 2009). Thus most ecological studies assume that evolution is not occurring and utilize non-evolving trait values in their models.

This assumption has now been challenged by dozens of studies documenting rapid evolutionary changes in nature occurring on 'ecological timescales', sometimes within a few generations (Dyer 1968, Thompson 1998, Hendry and Kinnison 1999, Bone and Farres 2001, Reznick and Ghalambor 2001, Ashley et al. 2003). Rapid evolution has been documented in many ecologically important traits such as life-history traits, foraging traits, morphological traits, phenology, and enemy resistance traits and has been documented in many major taxonomic groups including fish, arthropods, microbes, mammals, vascular and non-vascular plants, lizards, amphibians, and mollusks. These findings have led some researchers to reexamine how evolution and ecology interact given that the arbitrary distinction of ecological and evolutionary time is no longer valid.

Rapid Eco-Evolutionary Dynamics

Although biologists have been studying the interactions between ecology and evolution the reason this topic is currently receiving renewed interest is that it focuses primarily on interactions occurring on short timescales that have traditionally been overlooked (reviewed in Hairston et al. 2005, Fussmann et al. 2007, Johnson and Stinchcombe 2007, Pelletier et al. 2009, Schoener 2011).

This current field of study is called '*Eco-Evolutionary Dynamics'* and emphasizes the reciprocal and concurrent interactions between ecology and evolution and is the focus of my dissertation. Eco-evolutionary dynamics differs from other sub-categories of evolutionary-ecology because it focuses not on how the ecological environment causes evolution, i.e. ecological genetics (Ford 1964), but on how genetic variation and rapid evolution impact ecology (population dynamics, community structure, ecosystem functioning…). Yet the ultimate goal of eco-evolutionary dynamics is to study how evolutionary and ecological dynamics causally influence each other at the same time and how this might alter both ecological and evolutionary outcomes (full cyclical causality, Fig. 1.1; Bull et al. 2006, Kokko and Lopez-Sepulcre 2007, Ezard et al. 2009, Pelletier et al. 2009).

Previous Studies of Eco-Evolutionary Dynamics

Theoretical studies, investigating eco-evolution dynamics, date back at least 50 years (Pimentel 1961) and since then have diversified into different approaches, based on very different biological assumptions (reviewed in Bergelson et al. 2001, Abrams 2005, Day 2005, Fussmann et al. 2007). Eco-evolutionary dynamic models allow ecological and evolutionary processes to interact and assess how such interactions influence ecological and evolutionary dynamics. Such eco-evolutionary dynamics have been shown to influence the trajectory of growth of single populations (Anderson and King 1970), the density and stability of victim-exploiter systems (Pimentel 1961, Fussmann et al. 2003,

Bull et al. 2006, Duffy and Sivars-Becker 2007), the structure of multi-species communities (Loeuille and Leibold 2008), and even ecosystem processes (Loeuille et al. 2002). Overall these theoretical studies, by comparing models with and without evolution, overwhelmingly demonstrate that eco-evolutionary dynamics can qualitatively and quantitatively alter ecological and evolutionary outcomes (Day 2005, Fussmann et al. 2007). Yet, empirical studies of such interactions are still very rare and most of this body of theory remains untested (Fussmann et al. 2007).

One sub-discipline of eco-evolutionary dynamics, called '*Community Genetics,*' (Antonovics 1992) explores how genetic variation, mostly in plants, influences the structure and composition of arthropod communities they support, how it influences competition with other plant species, and ecosystem fluxes (Agrawal 2003, Johnson and Agrawal 2005, Whitham et al. 2006, Hughes et al. 2008, Bailey et al. 2009). These studies show the strong influence of intraspecific genetically based variation and suggest that if evolution changed the frequency of plant genotypes then evolutionary dynamics would influence ecological dynamics (Johnson et al. 2009). A similar approach consists of *post-hoc* comparisons of the ecological properties of populations thought to have undergone recent evolutionary diversification. Such studies assess how evolution has altered life history traits (Reznick and Bryga 1996), population dynamics (Hanski and Saccheri 2006), community structure (Post et al. 2008) and ecosystem processes (Bassar et al. 2010). These two empirical approaches

however have yet to quantify the impact of evolution as it occurs and thus could miss dynamic aspects of the eco-evolutionary interactions.

Empirical studies have also studied try to explain ecological changes using models that include evolutionary change (Anderson and May 1982, Tuda 1998, Sinervo et al. 2000, Hairston et al. 2005, Duffy and Sivars-Becker 2007, Ezard et al. 2009). In such studies, rapid evolution is usually strongly correlated and ecological predictions are usually significantly improved by including evolutionary dynamics. For example, an ecological model correctly predicted the start date of epidemic parasitic outbreaks in natural *Daphnia* populations but failed to predict their termination (Duffy et al. 2005). An evolutionary-ecological model where susceptibility evolved, correctly predicted the date of termination (Duffy and Sivars-Becker 2007). Although these observational studies establish the generality of evolutionary-feedback, they remain correlational and only suggest causality.

The Experimental Approach in Eco-Evolutionary Dynamics

The experimental approach quantifies the causal impact of rapid evolution as populations evolve compared to populations that cannot. This approach addresses the limitations of the methods above since they tract evolution as it occurs and can establish causality. Pimentel first used this approach to show how the population dynamics of a parasitoid wasp were changed as its housefly host evolved resistance compared to a non-evolving control (Pimentel et al.

1963, Pimentel and Al-Hafidh 1965, Pimentel 1968). By replacing the control housefly population every generation, he prevented the evolution of resistance. Rapid evolution within a few years in the host reduced the parasitoid's population size and variance even though host population size was held constant. This experimental approach has only been attempted a handful of times (Bohannan and Lenski 1999, Fussmann et al. 2003, Agashe 2009, Terhorst et al. 2010). Some experimental systems have quantified the full feedback cycle, where both ecological and evolutionary dynamics influence each other concurrently. In Yoshida et al.'s (2003) study of rotifers 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. This was caused by a change in the frequency of resistant algal clones that increased the density of algae while reducing that of the rotifer predator (evolution impacting ecology). Then because of frequency and density-dependent clonal selection the faster growing, but less defended algal clone, increased in frequency leading to increased predation and lower algal density resetting the cycle (ecology impacting evolution; Shertzer et al. 2002, Yoshida et al. 2003, Yoshida et al. 2004). These empirical studies demonstrate how evolutionary dynamics and genetic variation influence the dynamics and outcome of short-term ecological phenomena and argue convincingly for causality, but only under carefully controlled laboratory conditions.

DISSERTATION OBJECTIVES

My overall objective was to develop a study system wherein I could manipulate rapid evolution experimentally, altering its occurrence and rate. I could then use this system to experimentally assess the conditions under which eco-evolutionary dynamics are occurring, dissect how they operate, quantify their impact, and eventually determine their importance compared to other ecological processes. Although much progress is being made in the study of eco-evolutionary dynamics many outstanding questions remain that my dissertation aims to address.

Plant-herbivore 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). Yet none of the experimental model systems used in eco-evolutionary dynamics, where evolution is manipulated, to my knowledge utilize a plant-herbivore system (except one study of herbivorous spider mites but it was not framed in this context Agrawal 2000). All other studies use predator-prey (Fussmann et al. 2003, Yoshida et al. 2003, Terhorst et al. 2010) or host-parasitoid systems (Pimentel 1968, Tuda 1998, Bohannan and Lenski 2000) exclusively.

The eco-evolutionary dynamics in plant-herbivore systems could differ greatly from those observed in predator-prey or host-parasitoid interactions. Herbivore dynamics, especially those of pest species can often be in non-

equilibrium states (Wallner 1987, Karley et al. 2004). Also insect herbivores usually have a weaker impact on plant population dynamics than do predators 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 support for this (Maron and Crone 2006). Finally, it is important to study eco-evolutionary dynamics in plant-herbivore systems because of the immense economic importance these processes might have if they alter our ability to accurately predict pest population dynamics and pest evolution. **Thus my first objective was to develop a plant-herbivore system to study eco-evolutionary dynamics.** My first chapter describes the selected study system, consisting of a local aphid-mustard population. I then genetically characterized the aphid population by identifying neutral genetic variation that could be used to identify and track aphid clones. My next objective was to ecologically characterize the clones and identify ecologically relevant trait variation that could be manipulated in order to prevent or induce rapid evolution in future experiments.

Chapter 2 addressed my second objective, which was to quantify the impact of rapid evolution on concurrent ecological dynamics (arrow 2 in Fig. 1.1). This aspect of eco-evolutionary dynamics has received less attention than the impact of ecology on evolution (Bull et al. 2006, Ezard et al. 2009, Pelletier et al. 2009). In order to establish causality I utilized the experimental

approach where evolution itself is manipulated and its impact on ecological dynamics quantified directly. Since this has never been done using plant-herbivores I did so under partially controlled laboratory conditions. This experiment will help determine whether rapid evolution can impact short-term ecological dynamics. Previous investigations of eco-evolutionary dynamics study periods representing dozens of generations since they mostly use very fast reproducing microorganisms (Bohannan and Lenski 2000, Fussmann et al. 2003, Yoshida et al. 2003, Terhorst et al. 2010). I will explore the impact of even faster bouts of rapid evolution. My experiments focus on a single growing season of the host (5-6 aphid generations or less), which could have important implications for pest management in agricultural systems

To my knowledge all experimental test of eco-evolutionary dynamics, where evolution is manipulated, have been conducted under highly controlled and simplified laboratory environments (Fussmann et al. 2007). Although laboratory experiments establish the potential impact of rapid evolution, field experiments are crucial because ecological context can influence both ecological and evolutionary processes (Holt 2005). Experiments conducted in the wild within realistic communities encompass more realistic levels of biotic and abiotic variation as well as gene flow. These confounding factors could impose different selective pressures, altering the rate or direction of evolution itself, or they could interfere with the manner in which rapid evolution impacts population dynamics, e.g. by altering the strength of density regulation (Saccheri and Hanski 2006). All

of these problems imply that eco-evolutionary dynamics should ideally be studied in the wild since non-intuitive results could occur that differ significantly from predictions based only upon laboratory experiments. Even strong laboratory results could be overwhelmed by environmental variation in nature.

An important issue to consider is the source of the study population. To properly study the importance of eco-evolutionary dynamics it is important to use genotypes that actually interact in nature. Some studies (for an example see Agashe 2009) magnify the genetic variation in their experimental populations by using genotypes from multiple independent populations. If more genetic variation is used than is commonly found in wild populations, then the experimental populations might evolve more quickly which could overestimate the importance of eco-evolutionary dynamics. To avoid such a bias I only collected genotypes from a single population where the experiments were conducted. **Thus my third goal, the focus of Chapter 3, is to address whether eco-evolutionary dynamics have significant impacts in the wild in the face of environmental variation using a local population**. If eco-evolutionary dynamics have strong impacts in nature this has important implications for the study of population dynamics since evolution is traditionally not considered in these studies. For example I know of no pest population dynamic models that incorporate pest evolution within the growing season.

Experimental studies quantifying interspecific evolutionary-feedback have only been attempted in the laboratory (Fussmann et al. 2007) and those in nature

have been observational or focus on genetic variation and not evolution *per se*. It thus remains an open issue whether rapid evolution can have interspecific impacts in plant-herbivore systems. **Chapter 3 will also address my fourth objective, which is to experimentally determine whether aphid rapid evolution significantly impacts their host plant's fitness.** This objective will be addressed in all experiments but especially in Chapter 3 since a field experiment permits more accurate quantification of host fitness.

Many ecological forces have strong influences on ecological dynamics (e.g. interspecific competition, population density…). Ecologists should focus their limited resources on understanding and quantifying important drivers of ecological dynamics. Recent studies and many reviews claim that rapid evolution should be included in this list (Thompson 1998) yet very few studies have addressed this question explicitly (Johnson and Stinchcombe 2007). In community genetics only a few experiments quantify the relative importance of genotypic variation versus other ecological forces such as habitat variation and induced plant resistance (Johnson and Agrawal 2005, McGuire and Johnson 2006). The only eco-evolutionary dynamic study that compares the relative impact of evolution itself compared to ecological processes, is a correlation approach proposed by Hairston (2005). **Thus my fifth goal is to experimentally test the relative impact of rapid on population growth rate compared to that of intraspecific density (Chapter 4).**

Eco-evolutionary dynamics are defined as the reciprocal interactions between short-term ecological and evolution dynamics timescales (Kokko and Lopez-Sepulcre 2007). My objectives thus far have focused on quantifying the less studied half of that interaction, how rapid evolution impacts ecology. Yet much more complicate dynamics are possible if both arrows of causality are occurring concurrently (Fig. 1.1). Ecological changes induced by rapid evolution could alter future bouts of evolution by changing the selective environment experienced by the target organism. **Thus my sixth and final objective is to assess both arrows of causality in the same experiment and determine whether both ecological and evolutionary dynamics are influencing each other (Chapter 4).** If such an interaction is occurring this implies that much more complex dynamics are possible in this system and would put into question models that do not couple ecological and evolutionary changes together.

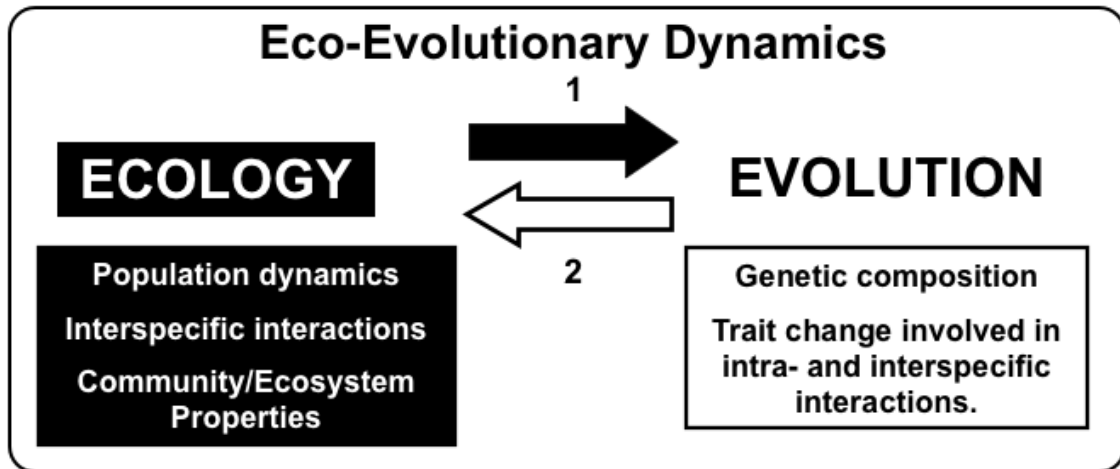
Importance of Eco-Evolutionary Dynamics

The growing interest in eco-evolutionary dynamics stems from its potential implications for many aspects of biology. Theoretical studies suggest that such a process could greatly alter not only evolutionary but also ecological interactions, dynamics, and outcomes. Acquiring a better understanding of this process should improve our understanding and our predictive ability (Duffy and Sivars-Becker 2007, Pelletier et al. 2009). Given the ever increasing examples of rapid evolution, considering evolutionary-ecological interactions as a working

hypothesis (Thompson 1998) might also provide important insight into many applied issues, e.g. disease epidemics (Real et al. 2005), fisheries management (Law 2000), bio-control (Hufbauer and Roderick 2005), and conservation biology (Ashley et al. 2003).

Figure 1.1: Diagram of Eco-Evolutionary Dynamics

Diagram representing eco-evolutionary dynamics illustrating the cyclical causality between evolution and ecology dynamics.



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CHAPTER TWO: MOLECULAR AND ECOLOGICAL CHARACTERIZATION OF STUDY SYSTEM

ABSTRACT

I developed a system to experimentally study eco-evolutionary dynamics in both the laboratory and in the field. I selected the green peach aphid, *Myzus persicae* (Sulzer), and a wild mustard host *Hirschfeldia incana* (Lagrèze-Fossat), system because the aphid's reproductive biology makes it an excellent candidate for experimental evolution. The preliminary studies presented in this chapter introduce and characterize the study system for the experiments presented in future chapters. I first justify why I selected an asexually reproducing model system. I then present the study system and describe how I sampled a local population and genetically identified multiple clonal lineages that were maintained in the greenhouse. I then ecologically characterized these clones by assessing differences in fitness using two experiments. I found that aphids differed by as much as 17% in intrinsic growth rate and selected a subset of three clones to use in subsequent experiments that directly quantify eco-evolutionary dynamics.

INTRODUCTION

Experimentally Testing Eco-Evolutionary Dynamics

The overall objective of my dissertation is to test experimentally the conditions under which rapid evolution interacts with concurrent ecological dynamics. The key to accomplish this end is to manipulate the rate of evolution. One commonly used approach is to compare non-evolved and evolved populations under common garden conditions in a *post-hoc* manner (Hanski and Saccheri 2006, Post et al. 2008, Harmon et al. 2009, Bassar et al. 2010). A related approach consists of repeatedly testing changes in ecological parameters as a population is evolving (e.g. fitness on a new host Agrawal 2000, the strength of predation Terhorst et al. 2010) compared to unselected control populations.

A different approach consists of manipulating only the response to selection without changing the selective environment. One can thus compare the ecological properties (e.g., population dynamics, interspecific interactions, ecosystem effects…) of populations as they are evolving to non-evolving controls. One method used to prevent evolution consists of continually replacing the control population under selection with unselected individuals (Pimentel 1968). This is unfeasible in most systems because it would alter population dynamics. An alternate approach, used here, is to manipulate available genetic variation (see also Yoshida et al. 2003, Agashe 2009). Populations with less or no genetic variation will evolve more slowly or not at all (Fisher 1930). The

population dynamics in these treatments can be directly compared to those of populations that have natural levels of genetic variation. Yet, this approach also has its limitations, including the confounding effect of inbreeding if the study organism is sexual. Inbreeding as well as genetic variation is known to impact ecological dynamics (Haag et al. 2002, Hanski and Saccheri 2006, Hughes et al. 2008). However, naturally asexual organisms do not suffer from these issues. Evolution in these asexual systems occurs through changes of clonal frequencies, which also can change mean population trait values (Via and Shaw 1996). This form of evolution is common in many taxa including aphids (Via and Shaw 1996, Vorburger 2006), algae (Fussmann et al. 2003, Yoshida et al. 2003), crustaceans (Lynch 1984), protozoans (Terhorst et al. 2010), 'genotypic selection' in clonally reproducing plants (Pan and Price 2001), bacteria (Bohannan and Lenski 2000), and has been observed in snails (Jokela et al. 2003) and fish (Vrijenhoek and Pfeiler 1997).

Study System

I developed a local aphid-mustard study system specifically for this dissertation research. To my surprise no other eco-evolutionary experimental system uses an insect-plant model system where the focal species is the insect (although Agrawal (2000) used herbivorous spider mites). Other studies use predator-prey (Fussmann et al. 2003, Yoshida et al. 2003, Terhorst et al. 2010) or host-parasitoid systems (Tuda and Iwasa 1998, Bohannan and Lenski 2000).

This is surprising given that a growing body of research in eco-evolutionary dynamics is focused on how plant genetics impacts insect communities ('community genetics' reviewed in Whitham et al. 2006, Hughes et al. 2008).

I selected the green peach aphid *Myzus persicae* (Sulzer) (Insecta: Hemiptera: Aphididae) for my studies. *M. persicae* has a global distribution but probably originated in China (Blackman 1974). It 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). Given its importance, its population dynamics, basic biology and ecology are well studied (Van Emden et al. 1969, Mackauer and Way 1976, Ro and Long 1999, Blackman and Eastop 2000, Karley et al. 2003). Moreover many genetic resources are available (Sloane et al. 2001, Wilson et al. 2003, Wilson et al. 2004). This host-alternating aphid reproduces through cyclical parthenogenesis which is the most common reproductive mode in aphids (Dixon et al. 1989). It reproduces sexually on its primary host (*Prunus* species) and the offspring migrate to secondary hosts (crops or wild plants) where they reproduce asexually for multiple generations or remain asexual switching between a series of secondary hosts (Van Emden et al. 1969). The asexual reproductive phase in aphids is apomictic, i.e. that offspring are exact genetic copies of their mothers except for rare point mutations (Wilson et al. 2003). This implies that microsatellite markers can reliably identify clonal lineages. Clonal lines differ in their propensity to reproduce sexually (Blackman

1974) and in certain warmer climates these clones are favored since the sexual over-wintering egg stage is not required for survival (Vorburger et al. 2003).

Myzus persicae is highly amenable to experimental evolution because of its short generation time (5-8 days depending on temperature), high fecundity, ease of culture, and facultative asexual reproduction. Aphids in their asexual phase have telescoping generations, i.e. grand-daughters are developing within adult females thus shortening development time from birth to maturity (Dixon et al. 1989). Also this aphid has a large magnitude of genetic variation between clones identified in multiple traits, including intrinsic growth rate (Weber 1985b, a, Vorburger 2005). Given their cyclical parthenogenetic reproduction and migration of clones over larger distances, spring populations are replete with clonal variation (Dickson and Laird 1967, Vorburger 2006). These populations naturally undergo rapid evolution, through clonal selection, within a matter of weeks leading to significant changes in clonal frequencies (Vorburger 2006). This occurs in many aphid species (de Barro et al. 1995, Sunnucks et al. 1997, Fuller et al. 1999). Such changes in clonal frequencies alter mean phenotypic trait values within weeks (Via and Shaw 1996). Multiyear temporal studies have identified highly successful and widespread genotypes (Wilson et al. 2003, Vorburger 2005, Vorburger 2006). In agricultural settings certain clones have evolved pesticide resistance and rapidly increase in frequency (Foster et al. 2002). Although evolution and ecological dynamics are often studied in aphids, to my knowledge no studies have looked at how rapid evolution within a season

might impact the aphid's population dynamics (e.g., growth rate, peak densities, peak density date) even in the laboratory (Roush and McKenzie 1987).

I selected the short-pod mustard *Hirschfeldia incana* (Lagreze-Fossat) (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). It was selected for its local abundance throughout the field site and because it is easily reared. This primarily self-incompatible (Horovitz and Galil 1972) annual plant completes its growth from seed to seed within a few months permitting me to quantify the impact of the aphids on its host.

The experiments focus on a single population of aphids collected at the University of California Motte-Rimrock Reserve near Perris, California (MRR, http://nrs.ucop.edu/reserves/motte/motte_rimrock.htm). The UC Natural Reserve System provides accessible yet protected natural sites that are excellent locations to study non-agricultural species interactions in natural settings. The MRR is particularly good location to study this insect-plant system since it is very close to the UCR campus, which reduces travel, facilitates experimental execution, and permits more thorough sampling. The MRR is a mix of Riversidean sage scrub habitat, coastal-desert grassland, and willow riparian thickets (Minnich and Dezzani 1998). All collections of aphids and host seeds were made within the reserve and the field experiment (Chapter 4) was conducted here. I focused on a single population of aphids as opposed to

sampling multiple populations because I wanted to assess how local, i.e. available, genetic variation might lead to eco-evolutionary interactions. Artificially increasing genetic variation by combining genotypes from multiple populations that would never interact in nature would limit my ability to understand the importance of eco-evolutionary interactions in natural populations (for an example see Agashe 2009).

Population Sampling

In March 2008, I collected 22 adult female apterous (non-winged) *M. persicae* feeding on *Hirschfeldia incana* from the Motte-Rimrock Reserve. I sampled early in the season because this is period when the population should contain the highest number of clonal lineages from sexual reproduction and migration (Vorburger 2006). These asexually reproducing females were used to create isofemale colonies that were maintained in the greenhouse on individually caged *H. incana* seedlings under conditions that maintain asexual reproduction (16hrs light / 8hrs dark) (Blackman 1974). Given that the clonal composition of aphid population in future experiments was to be manipulated I genotyped the isofemale colonies to determine whether they differed genetically.

Clone Molecular Identification

I identified clonal lineages using six published microsatellite markers (Sloane et al. 2001, Wilson et al. 2004). Microsatellites are short repeated

genomic sequences (usually 1-5 base pairs) with highly variable number of repeats and can be used to identify aphid clones (Wilson et al. 2003). I selected these microsatellite markers over other neutral genetic markers because they are highly reliable, can be genotyped cheaply because they do not require sequencing, are co-dominant and highly polymorphic, and can be multiplexed, meaning that multiple loci can be genotyped with a single PCR. Because they are used commonly, the methods for *M. persicae* were readily available (Sloane et al. 2001, Vorburger et al. 2003, Wilson and Swenson 2003, Wilson et al. 2004).

Three individuals from each isofemale colony were genotyped. PCR methods were modified from previous studies (Sloane et al. 2001, Wilson et al. 2004). One microsatellite primer from each pair was 5' labeled with fluorescent dyes. Loci with overlapping lengths were labeled with dyes of different wavelengths (loci-dye: myz2-6FAM, myz3-6FAM, M40-6FAM, M86-HEX [Sigma-Aldrich], M49-PET [Applied Biosystems], and myz9-HEX [Invitrogen]). All six microsatellite were composed of dinucleotide repeats. DNA from a single aphid was extracted using 5% Chelex 100 resin (Bio-Rad) and incubated for 35min at 56°C and for 15min at 95°C. Samples were then centrifuged at 12000rpm for 2min and the supernatant retained. PCR reactions were 10µL in volume that included 0.6 units of *Taq* polymerase (New England Biolabs), 1µL of 10x Mg-free standard reaction buffer, 0.2mM dNTP, 1µM of forward and reverse primer, 1.5mM $MgCl_2$, and 0.8µL of DNA extract. Touchdown PCR was used to amplify

these loci. Two different PCR cycling programs were used that follow those of Sloane et al. (2001) except that one additional amplification cycle was added to the last annealing temperature. Loci myz2, myz9, M40, and M86 used PCR program PMS1 and myz3 and M49 used PMS2. The lengths of PCR products were determined using an ABI 3100 Genetic Analyzer, using the GeneScan 500 LIZ size standard, and *GeneMapper* software (Applied Biosystems).

Our genotyping identified 10 genetically unique clonal lineages. Table 1.1 presents the each clones genotype at these loci.

Clone Maintenance

Multiple clonal populations were maintained in the greenhouse throughout the dissertation research. Populations were always kept on *H. incana* grown from seeds collected at the Motte-Rimrock Reserve. Initially, in 2008-2009, colonies were maintained on seedlings that were contained in large plastic jars with screen windows. Every three or four weeks approximately two dozen aphids were transfer to a fresh seedling. This was repeated for each clonal colony. Older colonies were kept as backups. In 2010-2011, I switched to using larger plants (at the end of the rosette stage) within large cages. These populations were larger and the plants survived longer. Transfers were still conducted every three to four weeks but consisted of hundreds of aphids. The three focal clones (813, 815, and 828) were maintained in duplicate. All colonies were kept within a partially temperature controlled greenhouse without humidity control under

natural lighting as well as metal halide lights that extended daylight to 16hrs a day. These conditions maintained asexual reproduction (Blackman 1974).

Every 4-6 weeks a group of 3-5 aphids from each clonal population was tested for clonal contamination. If this was detected individual aphids were used to establish new isofemale colonies and these were tested until the original clone was rescued. In the hundreds of tests I never found new alleles/genotypes in the microsattelite loci tested. In other words, all contaminating aphids were from a known aphid clonal lineage and none of these had mutations at microsatellite loci. Due to contamination and greenhouse cooling failures I did, however, permanently lose some clones.

Population Sampling: Clone Identification and Characterization

For adaptive evolution to occur within a population composed of different clones these clones must differ in relative fitness. Given that these aphids grow and reproduce very quickly I was able to assess fitness by measuring their intrinsic growth rate. The intrinsic growth rate (r_m) is a good index of fitness in this system because populations typically grow exponentially, then crash as their host plant senesces (Wallner 1987, Karley et al. 2003, Karley et al. 2004). Intrinsic growth rate is known to vary greatly between aphid clones within species and between species (Weber 1985a, Vorburger 2005). The r_m of a given clone is sensitive to the plant species or genotype on which it grows, so it is commonly used to measure host suitability (Wyatt and White 1977). Intrinsic growth rate,

and how it relates to temperature, is also a key component in predictive models used in integrative pest management (Guldemond et al. 1998, Ro and Long 1999).

Intrinsic growth rate is commonly estimated in aphids using full or truncated life table analyses (Birch 1948, Wyatt and White 1977, Le Roux et al. 2004). These methods however have their limitations in that they isolate individual adults in small clip-cages. This procedure can cause stress to the insect, harm the plant, alter the microclimate, and restricts selection of feeding sites (Guldemond et al. 1998). I instead extracted r_m from observed population growth rates on whole caged plants using population growth models (Vehrs et al. 1992, Guldemond et al. 1998). These conditions mimic future experimental conditions and are more informative than life table approaches.

EXPERIMENT 1: ECOLOGICAL CHARACTERIZATION OF *M. PERSICAE* CLONES

The objective of this experiment is quantifying intrinsic growth rates for the unique clonal lineages collected from the Motte-Rimrock population. *H. incana,* seeds collected at the Motte-Rimrock Reserve were grown in small pots (~500mL) using UC soil Mix III, a sand/peat moss mix supplemented with micronutrients. Once the seedlings, still in the rosette stage, reached approximately 25cm wide they were placed in cages within the greenhouse. Cages were constructed to individually house each plant. A cage consisted of an

eight liter pot with a wire frame creating a 75cm high dome that held up thin transparent mesh (Bridal Organza, #664-7242, Jo-Ann). Aphid clones were cleared of plant-viruses by using the approach suggested in Raybould et al. (1999). On day 0 of the experiment each plant (the unit of replication) received 12 apterous third instar *M. persicae* from a single clonal lineage. I attempted to test all lineages: however, clone 831 did not have enough aphids to initiate the experiment and clone 820 was contaminated with another clone. Thus I tested 8 different clonal treatments and each was replicated three times. This experiment was conducted in a partially temperature controlled greenhouse (mean daytime temperature = 26.8°C, range = 19°C to 32°C, mean nighttime = 21°C, range = 14°C to 29°C). Additional lighting to extend light to 16hrs / 8hrs day as a way of maintaining asexual reproduction (Blackman 1974).

Population size was measured, by counting all aphids, on days 0, 3, 6, 9, 12, and 23. Because it took longer than a full day to count all the aphids (there were other treatments not presented here), certain treatments were counted on day 13 and others on days 24 and 25. I tested for differences in growth rate by fitting an exponential growth model to the population dynamics observed on days 0 to 13 because after this day population growth declined. I fit a linear mixed-effect model (LME) with a linear exponential growth equation. I set a common intercept (mean density of aphids on day 0) across treatments. Thus the dependent variable was LN(x) transformed number of aphids, the fixed effect was aphid treatments and day (as the main covariate). Given that plants were

repeatedly counted violating the assumption of independent observations I set unique plant identity as a random effect on population growth rate and used an autoregressive correlation error structure (Pinheiro and Bates 2000). Increasing variance through time was modeled by using a variance function within the LME that increases with the power of the variance covariate (varPower). All analyses were implement in R (v. 2.11.1; R Development Core Team 2009) using the nlme package (Pinheiro et al. 2009).

RESULTS

This first experiment revealed significant clonal variation in intrinsic growth rate (Fig. 2.1, LME, $p = 0.002$). Clonal lineages differed by as much as 17%, which causes a range in doubling time of 41 to 48 hours. Over a period of 10 days of exponential growth the fastest clone should reach 85% higher population size than the slowest clone. I thus identified clonal lineages that differed in fitness. However, this experiment had limitations that I wanted to correct.

EXPERIMENT 2: FOCUSED CHARACTERIZATION

Experiment 1 identified clones that differed greatly in fitness but it had limitations. I repeated the above experiment but improved upon it by using more replicates (5), by counting more often, and initializing the population with a stable age distribution. I decided to focus on a subset of clones that had different growth rates (813, 815, and 828) to confirm their differences. Clone 820 was selected

also since it was contaminated in the previous experiment and thus its growth rate was not determined. I was able to re-isolate this clone before experiment 2.

The methods were very similar to experiment 1 and I here focus on the differences. *H. incana*, were grown in larger four liter pots. Once the seedlings, still in the rosette stage, reached approximately 20cm wide they were placed in cages within the greenhouse. On day 0 of the experiment each plant received 10 apterous *M. persicae* from a single clonal lineage. From Experiment 1 I estimated a stable age structure after a few generations. I estimated the mean proportion of aphids in each growth stage during the last days of exponential growth. I replicated this stable age distribution by introducing two adults, one 4th, two 3rd, and five 1st or 2nd instars to each plant. Instar stages were distinguished by size. These treatments were replicated five times and the position of plants randomized within the partially temperature controlled greenhouse (mean daytime temperature = 23°C, range = 13°C to 37°C, mean nighttime = 14°C, range = 8°C to 21°C).

Population size was measured by counting all aphids on days 0, 3, 6, 9, 12, 15, 18, 21, 25, 29, and 36. Some treatments were counted one day later on days 19, 22, 26, and 30 because of time limitations. I fit a LME exponential growth model to the population dynamics but excluded day 36 since the plants started senescing. The analysis mirrored that of experiment 1.

RESULTS

Clones grew exponentially until day 30, reaching densities between 10 to 20 thousand aphids per plant (Fig. 2.2). Exponential growth lasted much longer than in the first experiment because the plants were larger and could grow faster as they had more soil. Also, aphids grew more slowly probably due to lower temperatures during the experiment. *M. persicae* grows faster at a mean fluctuating temperature of 24°C (Exp. 1) than 18.5°C (Exp. 2) (Davis et al. 2007). Clonal differences in the second experiment were smaller than the first but were still highly significantly different among clones. Analysis of the population dynamics from days 0 to 30 showed that clone 813 grew fastest (daily r_m mean ± 1 SE: 0.268 ± 0.002) which was 2.2% faster (planned contrast, $p<0.001$) than clone 815 (0.263 ± 0.004). In turn, clone 815 grew 4.6% faster ($p<0.001$) than clone 828 (0.251 ± 0.004). Thus clone 813 grew 6.9% faster ($p<0.001$) than the clone 828. Only three clonal lineages were required for future experiments and I decided not to use clone 820 (which had a growth rate of 0.251 ± 0.004) since it had a similar value to clone 828.

An analysis restricted to the first 19 days of the experiment revealed the same pattern of growth rates but differences between clones were even larger. Clone 813 grew 9% faster ($p<0.001$) than the 815 that in grew 8.9% faster than 828 ($p=0.005$). This implies that 813 grew 18.9% faster ($p<0.001$) than the 828. These growth rates predict population densities that differ by as much as 2.3 fold by day 19. Comparing the analyses of days 0-30 with 0-19 suggest that fitness

differences between clones changed slightly with density.

DISCUSSION

My experiments identified significant genetically based variation among clones in fitness quantified as exponential growth rate. I identified up to 17-18.9% variation in fitness depending on the dataset and analysis. Because these clones grew under controlled environmental conditions, differences between clones are genetically based (Via and Shaw 1996). Such intraspecific variation between clones is not uncommon in *M. persicae* and even larger differences have been observed if clones are collected from different host plant species. Weber (1985a) found up to 8 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. My sampling of the Motte-Rimrock Reserve was not extensive; I began with 22 females, representing 10 clones, all of which were feeding on the host plant species used in the future experiments.

One surprising result from my experiments is that clone 813 in the first experiment was one of the slower genotypes (Fig. 2.1). However, in the second experiment it was the fastest growing clone. This could be explained by a variety of causes such as the lack of proper temporal sampling and replication in the first experiment and potentially human error (I was still improving the counting technique). It is also possible that given differences in temperature between experiments, this clone could simply grow relatively more quickly at lower

temperatures. These results highlight that experiments need to be self-contained i.e., having all controls needed for the analysis concurrently being studied.

Given the variation I identified in the clones I assigned the three focal clones a letter for easier reference. Clone 813 is henceforth clone A, 815 is clone B, and 828 is clone C. Each of the clones was selected because they differed in growth but also because their genotypes at three of their microsatellite markers were unique. These markers had PCR conditions that permitted multiplexing (using one PCR reaction to amplify all three), alleles were very diverse, and thus one genetic analysis provided information on 3 loci that could each identify the clones in case some loci did not amplify in the sample (loci myz2, M40, M86 in Table. 1.1). This streamlined genotyping in the large experiments presented in chapters 2, 3, and 4.

CONCLUSIONS

I identified unique clonal lineages from a natural population, characterized them genetically, identifying markers for easy genotyping, and quantified how they differed in fitness. The differences in intrinsic growth suggest that clonal frequency could rapidly evolve in a mixed clone population. Because aphids undergo multiple generations within a growing season, changes in frequency should be measurable within the time course of short-term population dynamics and could potentially have an impact on population growth rate thereby linking evolution with concurrent population dynamics.

Table 2.1: Aphid Microsatellite Genotypes

The multilocus microsatellite genotypes of the 10 clonal *M. persicae* lineages collected at the reserve. Numbers represent the length of each allele at each locus. Unique clones have alleles of unique length or unique combinations of alleles. The three bolded clonal lines will be used in future experiment. Their alphabetical coding is listed for reference. The bolded loci are used to quickly genotype aphids in those experiments. The number of unique alleles and unique genotypes are listed for each locus.

Clonal Lineage	Microsatellite Loci					
	myz2	myz3	myz9	**M40**	M49	**M86**
813- Clone A	**188/202**	119	203/209	**125**	156/171	**98/135**
815 – Clone B	**186/196**	115/121	195/207	**121/125**	201/203	**110/112**
820	162/186	105/115	207/209	125/131	166	117/133
822	186/198	117/119	195/223	121/131	138/156	123
825	186/198	117/ 119*	221/223	119/125	152/166	117/140*
828 – Clone C	**186/188**	117	195/209	**121**	154	**135**
831	186/188	117	195/209	119/121	154	117/135
834	174	117	195/223	121/133	138/ *	98/100
836	174/188	117	203	121/133	136/156	110/123
M1	172/186	113/119	207 / 238*	121/125	143/179	108/112
# Alleles	8	6	7	5	11	9 or 10
# Genotypes	8	5	8	8	8 or 9	10

** alleles where microsatellite lengths were inconsistent due to bad amplification.*

Figure 2.1: Aphid Clonal Variation in Intrinsic Growth Rate

Daily intrinsic growth rates, from experiment 1, of the eight clonal lineages estimated during population exponential growth in pure (single clone) populations.

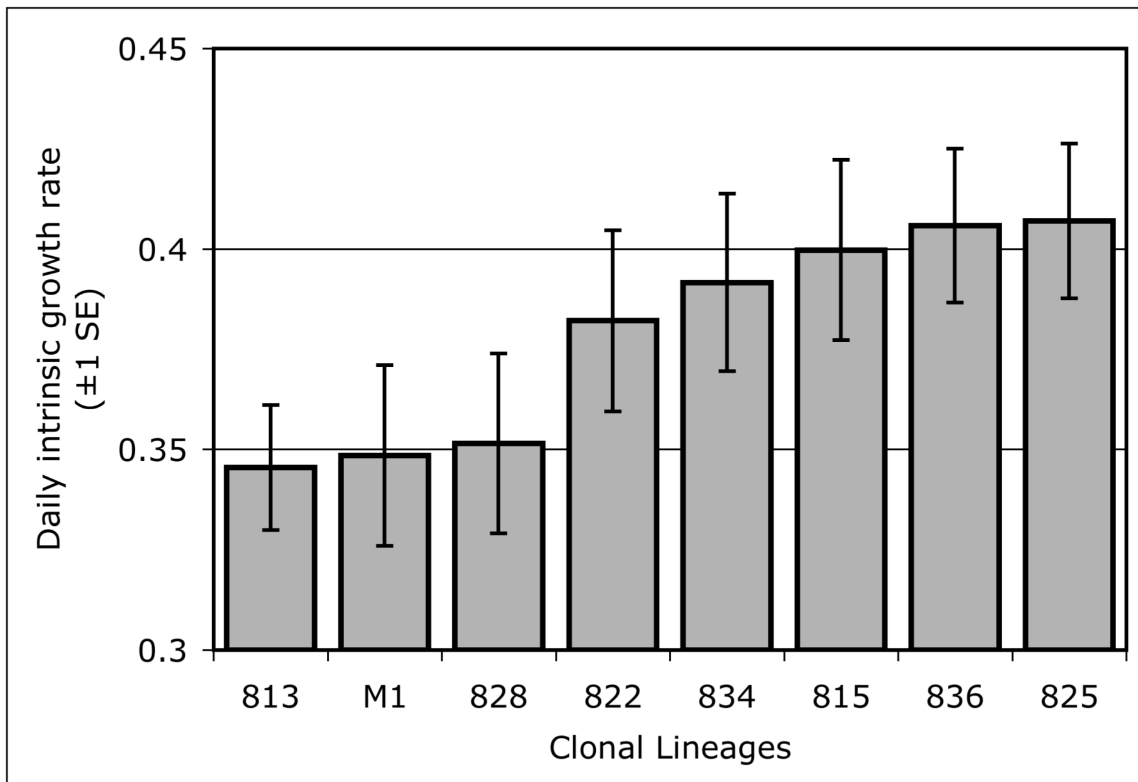
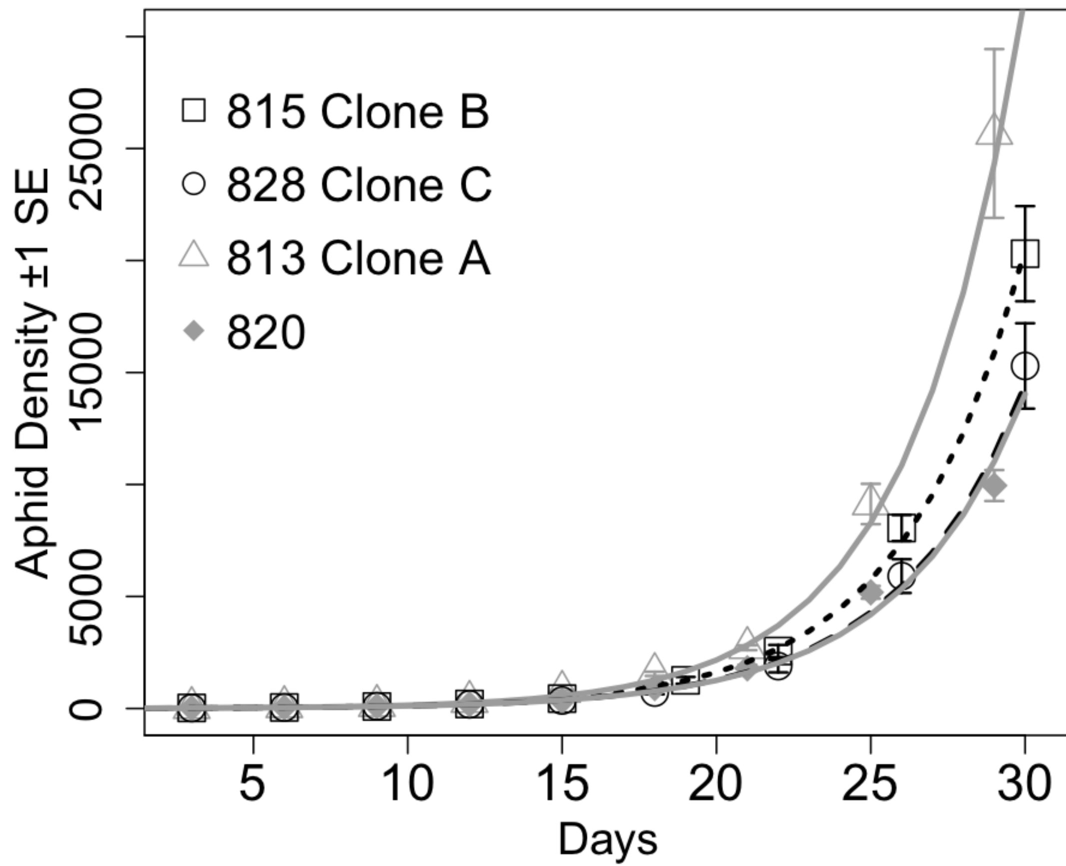


Figure 2.2: Population Dynamics of Pure Clone Populations

Population dynamics of the clonal lineages in experiment 2. Values represent mean number of aphids (±1 SE) and the lines are the best model fit more predictions from the analysis. The three clones that are used in subsequent experiment have their alphabetical code listed in the legend. Populations crashed after day 30.



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CHAPTER THREE: EXPERIMENTAL ASSESSMENT OF THE IMPACT OF RAPID EVOLUTION ON POPULATION DYNAMICS IN THE GREENHOUSE

ABSTRACT

Most short-term population dynamic models and studies assume that evolution occurs on slower timescales and thus do not allow for parameter values to evolve during their study. Yet multiple recent examples of rapid evolution in many systems could invalidate this approach if evolution impacts short-term ecological dynamics. The objective of this study is to quantify the impact of rapid evolution on short-term population dynamics using an aphid (*Myzus persicae*) and an undomesticated host (*Hirschfeldia incana*). This is the first experimental Eco-Evolutionary Dynamics study system using an insect-herbivore to my knowledge. I manipulated the amount of genetic variation in intrinsic growth rate within replicated aphid populations by altering the clonal composition. Aphid populations evolved rapidly changing significantly from their initial frequency within four weeks, well within a growing season and approximately four or five aphid generations. As populations were evolving I quantified their population dynamics. Evolving populations grew significantly faster, between 28% and 34%,

and reached higher densities, compared to non-evolving control populations. Evolving aphid populations did not cause increased damage to their host plant. My results countermand to prevailing approach that assumes that short-term population dynamics are too fast for evolution to have an influence.

INTRODUCTION

Few studies or models concerning short-term population dynamics consider the possibility that evolution could alter population parameters (e.g. intrinsic growth rates, carrying capacity, interspecific interactions) during the study period (Thompson 1998, Levins et al. 2003). Although studies take into account changes in population parameters, due to changes in age structure or spatial distribution, they rarely consider genetic changes (Cappuccino and Price 1995, Sibly and Hone 2002) even though certain ecologists have advocated such a consideration for decades (Pimentel 1961, Chitty 1967, Anderson and King 1970, Berry et al. 1978). This view, however, seems to be changing (Thompson 1998, Hairston et al. 2005, Saccheri and Hanski 2006, Hughes et al. 2008, Pelletier et al. 2009).

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). Alternatively, models are fit to historical density data to forecast future population density (Onstad et al. 2005). These approaches however assume that genotypic frequencies and hence

parameter values do not evolve. The justification for ignoring evolution in short-term ecological studies is based on two assumptions. Firstly, populations are often assumed to be genetically homogeneous, at least in traits that impact population dynamics (Roughgarden 1979, Cappuccino and Price 1995). Yet, population genetic variation in ecologically important traits has been demonstrated repeatedly since the 1960s reviewed in (Ayala 1968, Berry et al. 1978). Moreover, such variation can significantly influence population dynamics in the laboratory (Schlager 1963, Leips et al. 2000) and in nature (Hazell et al. 2006, Saccheri and Hanski 2006). Secondly, evolution is assumed to occur on a much slower (and thus separate) timescale, over hundreds or thousands of generations, than short-term ecological processes, occurring over a dozen or fewer generations (Slobodkin 1980, Thompson 1998, Hairston et al. 2005). Thus even if genetic variation is considered, evolution is ignored because it is perceived to be too slow to have an ecological effect (Endler 1991). 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 very common (see reviews in Dyer 1968, Thompson 1998, Hendry and Kinnison 1999, Bone and Farres 2001, Reznick and Ghalambor 2001, Ashley et al. 2003) but what remains unclear is whether such rapid evolution actually impacts short-term ecological dynamics (Pelletier et al. 2009).

Theoretical models have shown that rapid evolution in population parameters and genotypic frequencies can significantly alter population growth trajectories (Pimentel 1961, Anderson and King 1970, 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 possible. If this common genotype's relative fitness advantage decreases with increasing density and frequency it might slow the growth of the evolving population (Agrawal 2004). Because of such potentially non-intuitive interactions it is important to experimentally assess the impact of rapid evolution.

Whether the effect of evolution is simple or complex does not imply that evolution should be considered in all population dynamic studies. It is important to quantify how strongly evolution can impact concurrent population dynamics in different contexts (Hairston et al. 2005). The strength of this effect might depend on 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). For example, Yoshida et al. (2003) showed that rapid evolution within their rotifer-algal chemostat system causes the predator-prey cycles to become almost perfectly out-of-phase and not one quarter out-of-phase as predicted by 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 under differ conditions are required to quantify the importance of this process.

A small but growing number of experimental studies have begun quantifying the impact of rapid evolution on population dynamics using different methods (Fussmann et al. 2007). Bohannan and Lenski (2000) observed changes in mean density, and the fluctuation in density in both bacteria and phage, as the bacteria evolved resistance to this phage. Fussmann et al. (2003) used a combination of modeling and an experimental verification to show that rapid evolution of asexual reproduction qualitatively changed populations dynamics within 30 days. Without evolution the populations has a single peak in density and then crashes, however, with evolution a second peak in density occurs. Other studies experimentally compare the population dynamics of evolving populations to those that cannot evolve due to replacement of the population with unselected individuals (Pimentel et al. 1963, Pimentel and Al-Hafidh 1965, Pimentel 1968), a lack of genetic variation (Yoshida et al. 2003, Fussmann et al. 2007, Agashe 2009), or 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 how evolutionary dynamics influence the dynamics and outcome of short-term ecological phenomena and argue convincingly for causality.

Plant-herbivore interactions are thought to be one of the most common and important ecological interactions in natural populations, generating much of

the species and phenotypic diversity in nature as well as having immense economic importance (Ehrlich and Raven 1964). Surprisingly, there have been no experimental studies in which evolution was manipulated and its impact on population dynamics quantified in plant-herbivore interactions (except spider mites in Agrawal 2000). Yet a growing body of research in eco-evolutionary dynamics is focused on how plant genetics impacts insect communities ('community genetics' reviewed in Whitham et al. 2006, Hughes et al. 2008). This 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 in such outbreaks. The ecological effects of rapid evolution in a plant-herbivore system 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 outbreaks and crashes (reviewed in Wallner 1987, Karley et al. 2004) as opposed to equilibrium or predator-prey cycles observed in the rapid evolution studies listed previously. Second, herbivores might have a weaker affect 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 support for this process (Maron and Crone 2006).

My goal was thus to develop a model plant-herbivore system to study how rapid evolution through natural selection acting on genetic variation present within natural populations impacts concurrent population dynamics. To do so, I developed a study system wherein evolution can easily be manipulated. I selected the green peach aphid (*Myzus persicae*) and a local wild invasive annual mustard, *Hirschfeldia incana*, as a host. In this aphid species populations are replete with clonal variation at the beginning of the season, resulting from immigration and sexual reproduction, which permits rapid evolution at the beginning of the growing season (Vorburger 2006). Variation declines throughout the growing season leading to the evolutionary changes in population mean trait values (Via and Shaw 1996). To study the impact of such rapid evolution on population dynamics I experimentally manipulated aphid populations' genetic composition and evolutionary potential by controlling which clones were present in replicated populations. By selecting different pairings of clones I altered the level of genetic variation in an ecologically important trait and could thus test how the evolutionary context might alter the impact of rapid evolution on concurrent population dynamics. I specifically tested the following hypotheses-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 aphids*, 2) *If evolutionary context is important, then the impact of rapid evolution on population dynamics will differ between the different evolution treatments*, and 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

I- Experimental Design

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°C to 47°C, mean nighttime = 19°C, range = 13°C to 30°C). Asexual reproduction was maintained by using addition lighting providing 16hrs light/ 8hrs dark (Blackman 1974). To minimize variation in this primarily outcrossing plant, plants used in the experiment were grown from the seeds of a single *H. incana* plant collected in 2008 at the Motte-Rimrock Reserve. These seeds were planted in four liter pots, using UCR soil mix III, a sand/peat moss mix supplemented with micronutrients, and watered every three days. Cages were constructed to individually house each plant. Cages consisted of an 8 liter pot with a wire frame creating a 75cm high dome that held up thin transparent mesh (Bridal Organza, #664-7242, Jo-Ann). On day 0 of the experiment, the six week old seedlings in the rosette stage, approximately 10-15cm wide, were inoculated with seven different aphid treatments by placing 20 third instar aphids onto each plant. These aphids came from stock greenhouse clonal populations, regularly tested for contamination. Replicates were initiated on three consecutive days starting October 1st 2009. On day 1 missing aphids were replaced with fourth instar

aphids. Each treatment combination was assigned in a randomized block design and replicated 10 times.

I used three aphid clonal lineages, identified as 'A', 'B', and 'C', that were collected in 2008 from the University of California Motte-Rimrock Reserve near Perris, California (see Chapter 1). These clones differ in microsatellite markers (Appendix 3.1). A preliminary greenhouse experiment revealed that they also differ in exponential growth rates when grown in pure cultures (Chapter 2, daily growth rate of clone A = 0.268 ± 0.002, B = 0.263 ± 0.004, and C = 0.251 ± 0.004). I used these three clones to establish seven aphid treatments. Three evolution treatments consisted of aphid populations (on a single plant) that have two different clones. I established evolution treatments that consisted of all three two-way combinations (A-B, B-C, and A-C). Based on the ranking of growth rate in the preliminary experiments (Chapter 2), I 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 populations have genetic variation in fitness (e.g. A clone's rm is greater than that of the C clone) 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, thus preventing evolution. Finally, a ‘no-aphid control’ treatment did not receive any aphids.

II- Rates of Evolution

On day 28 I collected 100 aphids from every population to track changes in clonal frequencies (evolution). Between 16 and 24 aphids from each sample were genotyped (for a total of 497 aphids) at three microsatellite loci using a multiplex approach (see Appendix 3.1 for detailed genetic methods). I calculated the frequency of the faster clone for each treatment and replicate separately. For each evolution treatment I then determined whether the mean frequency of the faster clone differed significantly from the initial clonal frequency of 25% using one-sample t-tests.

III- Aphid Population Dynamics

Aphid population dynamics were quantified by counting all aphids on days 3, 7, 10, 14, 17, 21, 24, 28, and 33. When populations rose above 2000 aphids per plant I sub-sampled by counting one half of every leaf. Plant senescence caused the aphid populations to crash after day 28 so I excluded the census taken on day 33. I fit an exponential population growth model and I found, by looking at the residuals of every treatment, 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 to 28.

The exponential growth phase, days 0-14, was analyzed with a linear mixed-effect model (LME) where the dependent variable was LN(x) transformed number of aphids, the fixed effect was aphid treatments and day (as the main covariate). Because the repeated aphid counts on the same plant violated the assumption of independent observations, I 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). I modeled increasing variance through time by using a variance function within the LME 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 size 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 implement in R (v. 2.11.1; R Development Core Team 2009) using the nlme package (Pinheiro et al. 2009).

IV- The Impact of Evolution on Population Dynamics

My objective is not to predict which clone will out-compete the other but 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 those 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. I

thus generated the expected population growth parameters of such a non-evolving population by using the pure aphid treatments. I 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. I did so with the use of planned contrasts that are orthogonal comparisons between a subset of the aphid treatment levels within the LME analysis. Different hypotheses are tested by assigning weights to treatments levels. I 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 are compared to the AC evolution treatment= 1). My three hypotheses were tested using the following simplified contrast matrix:

Aphid Treatments	**Hypothesis 1:** AB vs Pure A and Pure B	**Hypothesis 2:** BC vs Pure B and Pure C	**Hypothesis 3:** AC vs Pure A and Pure C
Pure clone A	-0.25	0	-0.25
Pure clone B	-0.75	-0.25	0
Pure clone C	0	-0.75	-0.75
Evolution AB	+1	0	0
Evolution BC	0	+1	0
Evolution AC	0	0	+1

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.

V - Host Plant Fitness

Finally, to quantify the impact of aphid rapid evolution on its host's fitness I 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). I fit a general linear model on LN(x) transformed plant weight measurements. The factors were aphid treatment, block and initial plant size (width of rosette on day 3). The interactions between these factors were non-significant and thus removed from the final model. I again used planned contrasts to determine whether plants with evolving aphid populations were smaller than expected from no-evolution treatments.

RESULTS

I - Pure Clone Treatments

Pure clone treatments differed in their population dynamics as the rank order of growth rates changed throughout the experiment (Fig. 3.1). In the exponential phase (day 0- to 14) the B clone grew fastest (5.6% faster than A), the A clone was second fastest (4.8% faster than the C clone; Fig. 3.1.a). In the linear growth phase (day 14 to 28) the B clone grew significantly slower than the other two clones. The A clone grew 80% and the C clone grew 37% faster than the B clone respectively and the A clone grew 12% faster than clone C (Fig. 3.1.b).

II - Evolution Treatments - Genetic Analyses

On day 28 I tested for changes in the frequency of clones in the evolution treatments away from the initial frequency of 25% : 75%. In the A-B evolution treatment clonal frequency did not change (frequency of A clone = 23%, one sample t-test, p = 0.63; Fig. 3.2). The B-C and A-C evolution treatments did significantly evolve 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. 3.2).

III - Impact of Aphid Evolution on Aphid Population Dynamics

To test the impact of rapid evolution on concurrent population dynamics I compared the observed population dynamics in evolving 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). 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 (non-evolving) frequency of 25% (for clone A with a growth rate of 0.0363) 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 to 14), the only evolution treatment that differed in daily growth rate from its no-evolution expectation was the A-C treatment (Fig. 3.3 a-b-e, Table 3.1). Oddly, although the faster growing A clone

increased in frequency in this treatment, the evolution treatment grew 8.5% slower (LME, $p = 0.0015$) than the no-evolution expectation (Fig. 3.3.e). In the second growth phase, days 14 to 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. 3.3.b, Table 3.1). Evolution in the B-C treatment did not alter density on day 14 but significantly accelerated population growth rate compared to the no-evolution expectation afterwards (+28.2%, $p = 0.008$, Fig. 3.3.d, Table 3.1). 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. 3.3.f, Table 3.1).

IV - 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 ($p < 0.001$). Although in certain treatments evolution led to higher aphid densities, this did not magnify the impact of aphids on the host plants' above ground biomass. Final plant weight did not differ significantly between the three evolution treatments and their corresponding no-evolution expectations (ANOVA, all p-values > 0.1).

DISCUSSION

I experimentally assessed the impact of rapid evolution on concurrent ecological dynamics in a plant-herbivore system. I found that rapid evolution, occurring within weeks, significantly accelerated population growth rates and density as clonal frequencies changed. Yet, this rapid evolution of aphids did not have a detectable effect on the plant host. 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 the wild populations (de Barro et al. 1995, Vorburger 2006). In the A-C evolution treatment, the A clone almost doubled its initial frequency, which is expected given that the A clone grew faster than the C clone in pure treatments in both growth phases (Fig 3.1). Yet, the evolutionary outcome was not always predictable from the difference in the growth rates of single clone cultures. The A-B treatment did not evolve (Fig. 3.2), even though on day 28 the pure A clone was 41% more dense than the pure B clone (Fig. 3.1). This might be explained by the fact that the pure B clone grew faster than the A during the first growth phase but slower during the second growth phase, reversing the evolution that

occurred during the first phase. This reversal may in turn suggest that the fitness difference between the clones is density dependent. Also, the B clone in the B-C treatment reached a frequency of 44% on day 28, even though its density on that day was 30% lower than the pure C clone. This is surprising since in the early phase pure B grew faster than C but the opposite occurs in the later growth phase. One possible explanation for these two unpredictable results is that one clone reduces the others' growth (Rochat et al. 1999). In my experiment it is possible that the B clone interferences with the C and A clones' feeding, thus lowering their relative fitness when mixed, resulting in higher clone B frequencies than expected on day 28. Also, it is possible that the early growth phase determines the evolutionary outcome since populations expand approximately 170 fold during the early phase, as opposed to only 4.5 fold in the later phase.

The novelty of my study is not to determine the exact process leading to these evolutionary changes but to assess the impact of these changes on concurrent population dynamics. I observed strong impacts of rapid evolution on population dynamics in both treatments that evolved. Rapid evolution accelerated population growth rate by 28% and 33% in the two mixed treatments where evolution occurred, supporting my first hypothesis (Fig. 3.3, Table 3.1). Evolution increased population density by as much as 17% for the B-C and 19% for the A-C treatments compared to 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 as important.

For example increasing temperature from 20-25ºC and 25-30ºC causes *M. persicae*'s intrinsic growth rate to increase by 14% and 3.6% respectively (Davis et al. 2007). Gurevitch et al (2000)'s factorial meta-analysis of dozens of experiments manipulating predation and competition revealed impact sizes similar to those reported here. The strong effects I observed suggest that population density and growth rate might not be predictable by simply averaging the demographic parameters of a mixed genotype population (Wallner 1987, Endler 1991).

Rapid evolution could have even stronger effects if I had used clones with larger fitness differences. Such variation would be likely if I had 1) sampled more than a dozen clones from a natural population, 2) collected aphids from different host species, or 3) conducted the experiment in an agricultural setting with pesticide application that would select for *M. persicae* clones that differ in resistance. Other studies in *M. persicae* have reported higher levels of genetic variation between clones. Weber (1985a) found up to 8 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. In another study, Weber (1985b) found 3000 fold variation in resistance to parathion. Also, the acceleration in growth rate due to evolution that I observed only occurred in the latter half of the experiment (Table 3.1). This delay is likely a consequence of a lag of at least a few generations before evolution can change the population's growth parameters. The fact that such a change occurred within

only 30 days directly challenges the assumption that evolutionary change happens on a much longer time scale than the change wrought by ecological interactions. These results suggest that if the experiment had lasted longer, either by having a longer-lived host or by having new host plants, the effect of evolution could have been magnified. 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 timescales'.

It is difficult to compare the effect of rapid evolution on population dynamics between very 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 both species 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 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.

My second hypothesis tests whether the evolutionary context (identity of genotypes and rate of evolution) influences the impact of rapid evolution on population dynamics. Given that one evolution treatment did not evolve and the other two did so at similar rates nullifies my ability to quantify the relationship between rate of evolution and impact size since I have dichotomous treatments (rapid and no evolution). These results suggest that, in these experimental conditions, genotypic identity of clones, as long as they evolve at similar rates, does not change the magnitude of the impact of rapid evolution on population growth rate. Yet a closer examination of my results suggests that clonal identity might have an important impact. In the A-C evolution treatment it is clear why the growth rate accelerates; the faster growing A clone becomes more common. For the B-C treatment however, the B clone increases in frequency but the B clone grows more slowly in the second growth phase (by 37%) than the C clone did in pure treatments (Fig. 3.1). Why exactly this evolutionary change accelerates the growth of the evolving population remains unresolved. One possibility is that the B clone experiences more severe density-dependent growth when at high frequency which only occurs in the pure B treatment. Reduced growth rate does not occur in the B-C evolution treatment because the B clone only reaches 44% frequency. Experiments at different initial clone frequencies could help resolve this issue. Such intraspecific variation in the strength of density dependence has

been document in other aphid species (Agrawal et al. 2004) and is obvious from the pure clone treatments (Fig. 3.1). I have also observed that the B aphids become smaller in body size and are more likely to produced winged aphids but only at very high densities and only when it represents a dominant frequency of the population (M. M. Turcotte pers. observation in multiple experiments). Aphid clones are known to differ in their propensity to create winged individuals during crowding (Muller et al. 2001, Hazell et al. 2005).

An obstacle I 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. My aim was to assess the impact of evolution itself and not the former factors. Hence I created planned contrasts that compare 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 interclonal interactions are equivalent to intraclonal interactions. This caveat might explain why the A-C evolution treatment is initially grows 8.4% slower (Fig. 3.3.E, Table 3.1). This result suggests that one clone, probably A, is interfering with the other. This decreases total population growth rate and only once the faster growing A clone has increased significantly in frequency, days 14-28, can evolution compensate for this effect and accelerate population growth rate.

Alternatively, interclonal interference might be density- and/or frequency-dependent and thus change throughout the experiment. Clones with aphid populations are known to vary genetically in competitive ability and this variation can also change with ecological context (Hazell et al. 2006).

This study quantified one half of the 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 opened question is whether changes density reciprocally influences future bouts of evolution. If this is the case it would complete the eco-evolutionary feedback cycle (Fussmann et al. 2007, Kokko and Lopez-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 dynamic studies. My experimental results strongly countermands 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 (Hufbauer and Roderick 2005, Duffy and Sivars-Becker 2007).

Table 3.1: Analysis of Population Dynamics Comparing Evolving and Non-Evolving Populations

Planned contrasts from a linear mixed-effect model, comparing each type of evolving population to its corresponding no-evolution expectation, generated from the pure aphid treatments following the initial frequency of clones (see Methods for details). 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 increases due to evolution. 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 2-tailed tests. Significant results were bolded for easier identification.

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**

Figure 3.1: Population Dynamics of Pure Clone Treatments

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

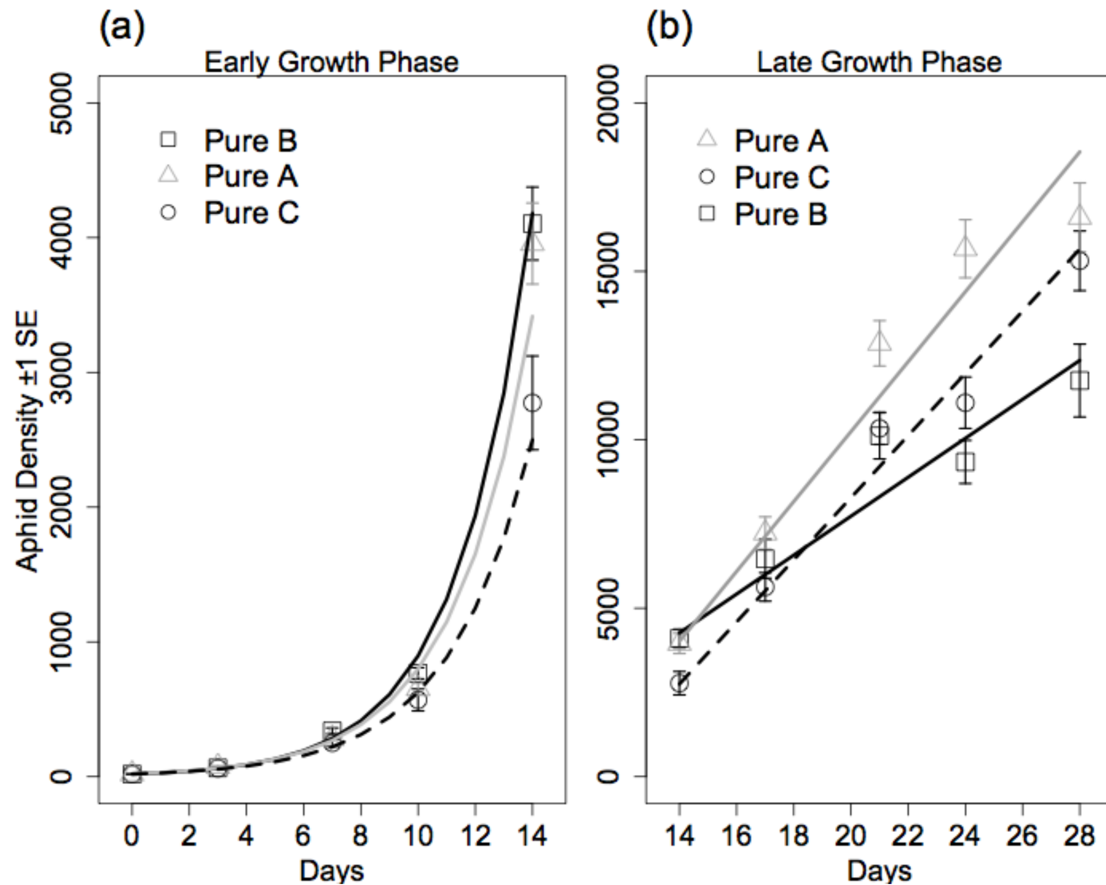


Figure 3.2: Rapid Experimental Evolution of Clonal Frequencies

Rapid clonal evolution as shown by the mean frequency of the faster growing aphid clones in each evolution treatment (±1 SE). X-axis shows which clone's frequency is being tested in each evolution treatment. Dashed horizontal bar indicates initial clonal frequency of 25% and (*) indicate significant divergence from initial frequency. A, B, and C are the aphid clones.

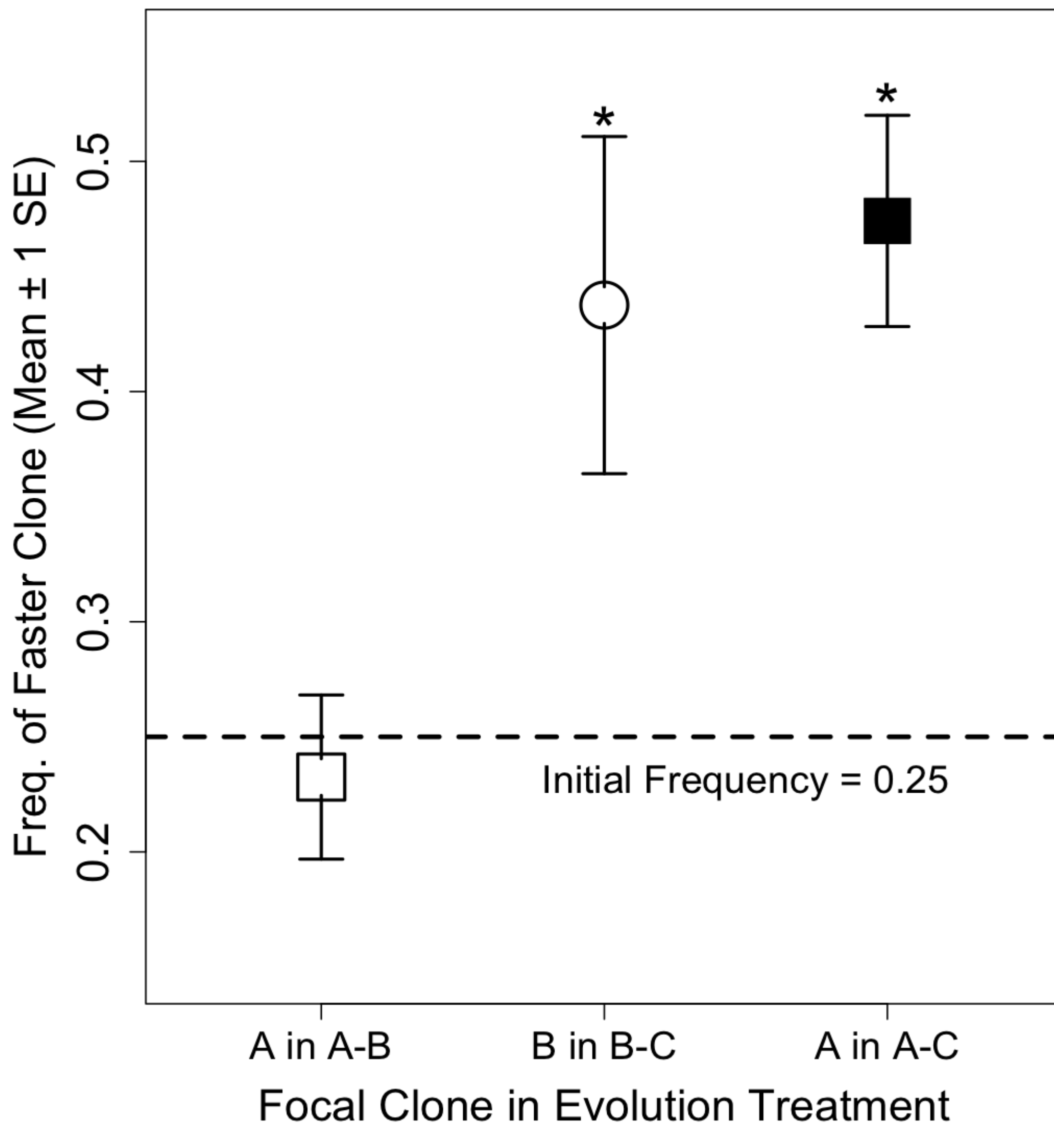
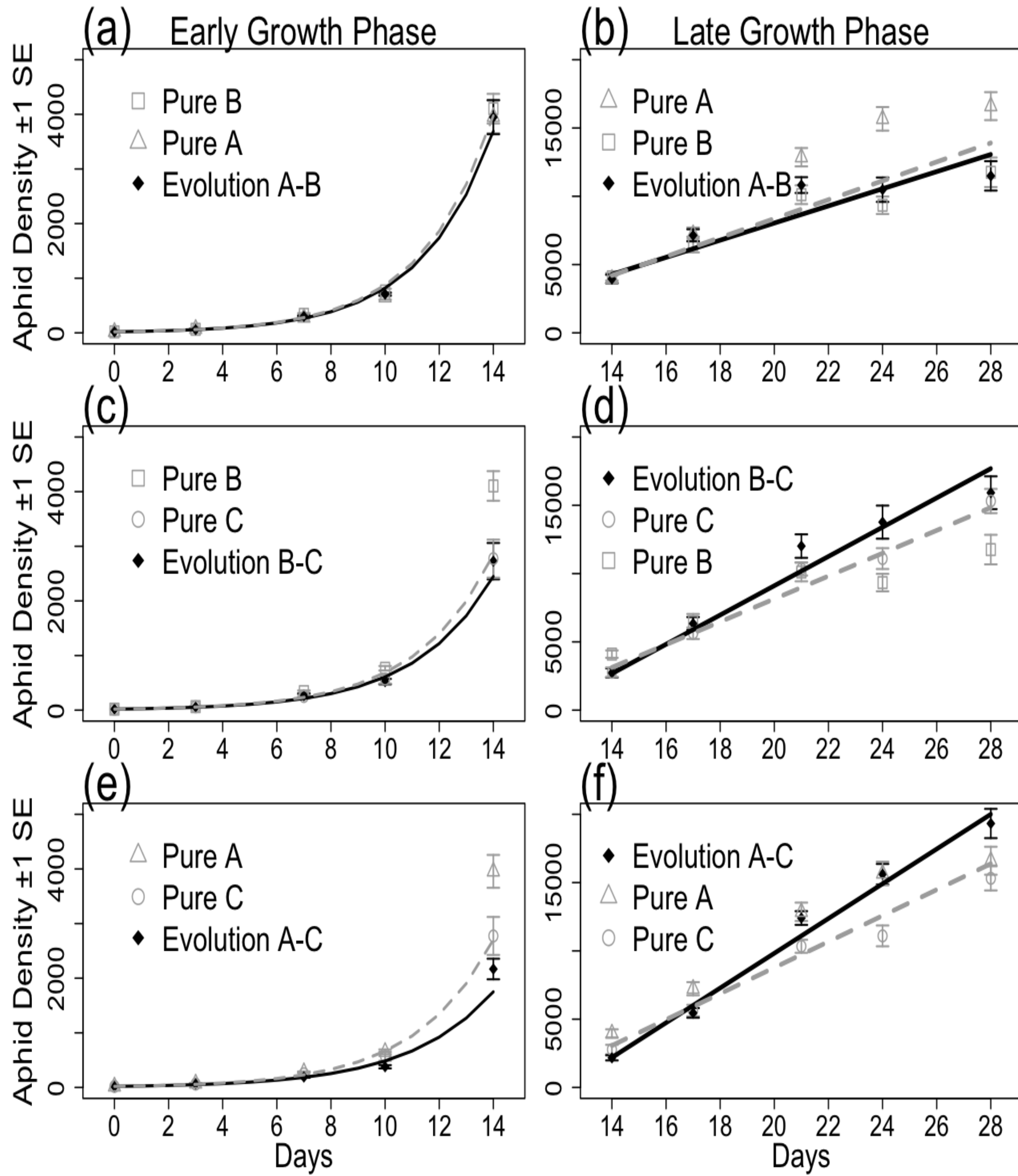


Figure 3.3: Populations Dynamics of Evolving and Non-Evolving Aphids

Population dynamics of the three observed evolution treatments (black diamonds) with the best fit model from LME analysis (black line). Left panels are for the early growth phase and panels on the right for the late growth phase for easy visualization. The dashed gray line represents the best fit model that combines both pure treatments using the constant (non-evolving) frequency of clones (25:75). For each treatment I added the corresponding pure clone treatments (grey symbols) used to generate the no-evolution expectation. Values represent mean number of aphids (±1 SE) and the y-axes differ between left and right panels. Evolution treatments have two letters.

Figure 3.3



APPENDIX 3.1: DETAILS OF MULTIPLEX GENETIC ANALYSIS

Methods for Multiplex Genotyping used during Experiment

During the experiment I genotyped aphids using three microsatellite loci for which the three clones had unique genotypes (Appendix Table 3.1). This reduced cost and workload while providing robust genotyping. A single multiplex PCR reaction amplified three microsatellite loci at once (myz2, M40, and M86) using the PMS1 program. The PCR reactions were identical to those used to genetically characterize all clones in Chapter 1 except for the use of a mixed primer solution that contained three forward and three reverse-labeled primers. To normalize signal strength the concentrations of the primer pairs were: 1, 3, and 5µM for loci myz2, M40, and M86 respectively.

Appendix Table 3.1: Microsatellite Genotype of Focal Aphid Clones

Microsatellite genotypes of the three clonal lineages of green peach aphid (*Myzus persicae*) used in this experiment. These were collected from the Motte-Rimrock Reserve in spring 2008 from the mustard plant *Hirschfeldia incana*. Numbers represent the length of the each allele at each locus. The three bold loci were used to identify clones during the experiment.

Clonal Lineage	**Microsatellite Loci**					
	myz2	myz3	**myz9**	**M40**	M49	**M86**
A	186 202	119 119	203 209	125 125	156 171	98 135
B	186 196	115 121	195 207	121 125	201 203	110 112
C	186 188	117 117	195 209	121 121	154 154	135 135

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Chapter Four: The Impact of Rapid Evolution on Population Dynamics in the Wild: Experimental Test of Eco-Evolutionary Dynamics

Abstract

Rapid evolution challenges the assumption that evolution is too slow to impact short-term ecological dynamics. This has led to a push to empirically study how evolution and ecological processes reciprocally impact each other on short time scales termed '*Eco-Evolutionary Dynamics*'. In this study I tested how rapid evolution impacts concurrent population dynamics using an aphid (*Myzus persicae*) and an undomesticated host (*Hirschfeldia incana*) in replicated wild populations. I manipulated the amount of genetic variation in intrinsic growth rate within aphid populations, which altered rates of evolution (changing clonal, or gene, frequencies) in both caged and uncaged populations. Evolving populations grew significantly faster, up to 42%, and reached higher densities, up to 67% higher, compared to non-evolving control populations. Moreover, the magnitude of the impact of evolution on population growth rate increased with observed rates of evolution. Yet this effect only occurred in uncaged treatments that were open to a natural spectrum of herbivores and predators. Also, the relative fitness of competing clones changed with density. This suggests that as evolution

changes density, density feeds back changes the selective environment leading to reciprocal eco-evolutionary dynamics at the same timescale. Finally, aphid evolution did not significantly influence their host's fitness.

INTRODUCTION

Ecological and evolutionary forces are usually thought to influence each other asymmetrically, i.e. ecology shapes evolution (Levins and Lewontin 1980, Hairston et al. 2005, Kokko and Lopez-Sepulcre 2007). Ecological changes are often assumed to occur independently of evolution, e.g. an organism's population size is reduced due to a drought. Evolutionary changes, however, are usually a consequence of the ecological 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 because evolution is perceived to act on a much slower time scale relative to ecological interactions (Slobodkin 1980, Endler 1991, Thompson 1998, Hairston et al. 2005, Pelletier et al. 2009). This assumption has now been challenged by dozens of studies documenting rapid evolutionary changes in nature occurring on 'ecological time scales', sometimes within a few generations (Thompson 1998, Hendry and Kinnison 1999, Reznick and Ghalambor 2001, Ashley et al. 2003). Given the convergence of time scales, the next step is to assess whether rapid evolution and concurrent ecological dynamics influence each other reciprocally (Pelletier et al. 2009). This reciprocal interaction defines

eco-evolutionary dynamics (Hairston et al. 2005, Bull et al. 2006, Kokko and Lopez-Sepulcre 2007). This process could have important implications for fisheries, pest, and infectious diseases management where accurate evolutionary and population dynamic predictions are required (Hufbauer and Roderick 2005).

A growing body of eco-evolutionary dynamic theory, based on very different biological assumptions, generally 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 and King 1970), the density and stability of victim-exploiter systems (Pimentel 1961, Fussmann et al. 2003, Bull et al. 2006, Duffy and Sivars-Becker 2007), the structure of multi-species communities (Loeuille and Leibold 2008), and even ecosystem processes (Loeuille et al. 2002).

I 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, Ezard et al. 2009, Pelletier et al. 2009). One fruitful empirical approach consists of using models to assess the influence of ecological and evolutionary processes on observed ecological dynamics (Hairston et al. 2005, Duffy and Sivars-Becker 2007, Ezard et al. 2009). In such studies, rapid evolution is usually strongly

correlated with ecological dynamics and ecological predictions are usually significantly improved by including evolutionary dynamics. A second approach consists of *post-hoc* comparisons of the ecological properties of ancestral populations versus populations that have undergone evolution. Such studies assess how evolution has altered life history traits (Reznick and Bryga 1996), population dynamics (Hanski and Saccheri 2006), community structure (Post et al. 2008) and ecosystem processes (Bassar et al. 2010). These field studies demonstrate the strength and generality of rapid evolution's ecological effects.

Other empirical studies can quantify the impact of rapid evolution as populations evolve. These powerful studies test the causal impact of rapid evolution on population dynamics by experimentally manipulating the occurrence of evolution itself thus directly comparing evolving and non-evolving populations. Pimentel first used this approach to show how the population dynamics of a parasitoid wasp were changed as its housefly host evolved resistance compared to a non-evolving control (Pimentel et al. 1963, Pimentel and Al-Hafidh 1965, Pimentel 1968). By replacing the control housefly population every generation, he prevented the evolution of resistance. Rapid evolution within a few years in the host reduced the parasitoid's population size and variance even though host population size was held constant. This experimental approach has only been attempted a handful of times (Tuda 1998, Bohannan and Lenski 1999, Yoshida et al. 2003, Fussmann et al. 2007, Agashe 2009, Terhorst et al. 2010). In my previous study (Chapter 3) I experimentally quantified the impact of aphid rapid

evolution on population growth rate compared to non-evolving control populations in the greenhouse. I found that certain rapid evolution treatments significantly accelerated population growth rate by as much as 34%. These studies demonstrate how evolutionary dynamics influence the dynamics and outcome of short-term ecological phenomena and argue convincingly for causality, but only under carefully controlled laboratory conditions.

Here I assess whether and how strongly rapid evolution impacts concurrent ecological dynamics in natural populations. While recognizing the value of lab studies, field experiments are crucial because ecological context can influence both ecological and evolutionary processes (Holt 2005). Experiments conducted in the wild within realistic communities encompass more realistic levels of biotic and abiotic variation as well as gene flow. These confounding factors could impose different selective pressures, altering the rate or direction of evolution itself, or they could interfere with the manner in which rapid evolution impacts population dynamics, e.g. by altering the strength of density regulation. All of these problems imply that eco-evolutionary dynamics should be studied in the wild since non-intuitive results could occur that differ significantly from predictions based only upon laboratory experiments.

Most eco-evolutionary dynamics studies focus on interspecific interactions and all experimental systems to my knowledge utilize predator-prey or host-parasitoid model systems. Whether short-term interspecific eco-evolutionary dynamics are important in plant-herbivore systems remains an open question.

Although many studies show that plant genotypes can influence the composition of arthropod communities (reviewed in Whitham et al. 2006, Hughes et al. 2008) they have yet to document these effects of rapid evolution itself. In my previous greenhouse experiment I found that aphids significantly harm their host plant, reducing above ground biomass by a factor of five (Chapter 3). Yet aphid evolution, although accelerating population growth rate, did not alter damage to the host. Field populations permit better quantification of host plant fitness differences because pollination can occur. Thus in this experiment I more thoroughly quantify host fitness by investigating host characteristics that are more tightly linked to fitness such as flower and seed production in order to explore interspecific eco-evolutionary dynamic effects.

My specific objective was to experimentally assess the impact of rapid evolution on concurrent population dynamics in the wild. To do so, I used my study system wherein evolution can easily be manipulated. I selected the green peach aphid (*Myzus persicae*) and a local wild invasive annual mustard, *Hirschfeldia incana,* as a host. I manipulated aphid populations' genetic composition and evolutionary potential by controlling which clones were present within each treatment. In order to explore the importance of ecological context I conducted this study in caged and uncaged populations in the wild; the difference is that uncaged populations admit a natural spectrum of herbivores and predators. I specifically tested the following hypotheses-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 aphids, 2) *If ecological context is important, then the impact of rapid evolution on population dynamics will differ between caged and uncaged treatments,* and *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

I- Study System

Myzus persicae is considered the world's most important crop pest and thus its life-history and ecology are well-studied (Mackauer and Way 1976). It is highly amenable to experimental evolution because of its short generation time (6-10 days), ease of culture, and the large magnitude of genetic variation identified in multiple traits (Chapter 1; Vorburger 2005). This cyclically parthenogenetic aphid undergoes sexual reproduction to survive cold winters. In the 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 permitted the experimental manipulation of the level of genetic variation within a population by controlling the initial frequency of clones therein.

In 2008, I collected multiple clonal lineages from a single wild population from the Motte-Rimrock Reserve in Perris California. I identified clones using 6 microsatellite markers and characterized their intrinsic per capita growth rates experimentally (Chapter 2; Sloane et al. 2001, Wilson et al. 2004). I selected three of these clones for this and previous experiment (Chapters 2 & 3) that differ in intrinsic growth rate (detailed below).

II- Field Experiment Design

The focal experiment was conducted in a wash area (20m by 12m), which was cleared of vegetation, at the Reserve where the aphids were collected. A wire fence was erected to keep out large vertebrate herbivores. Clonal aphid reproduction was maintained by the long days and high temperature during the month of July (Blackman 1974). To minimize variation in this primarily outcrossing plant, plants used in the experiment were grown from the seeds of a single *H. incana* plant collected in 2008 at the Reserve and germinated in the greenhouse. 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) to prevent insect damage and permit aphid populations to become established. On day 0 of the experiment, seven different aphid treatments were initiated by placing 20 third instar aphids onto each plant. These aphids came from stock greenhouse clonal populations, regularly tested

for contamination, that were maintained on *H. incana*. Replicates were initiated on three consecutive days starting June 30th 2009. On day 1 missing aphids were replaced with fourth instar aphids. On day 13, for half of the plants, the cage mesh was lifted but tied to the top of the wire frame. This maintained consistent shade between treatments but allowed full access to the arthropod community. 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.

I used three aphid clonal lineages, identified as *'A', 'B', and 'C',* that differ in microsatellite markers (Appendix 3.1). A preliminary greenhouse experiment revealed that they also differ in exponential growth rates when grown in pure cultures (Chapter 2, daily per capita growth rate of clone A = 0.268 ± 0.002, B = 0.263 ± 0.004, and C = 0.251 ± 0.004). Using these clones, seven aphid treatments were established as follows: 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). These populations have genetic variation in fitness (e.g. A clone's r_m is greater than that of the C clone) and thus could evolve by changing in clonal frequency (away from the initial ratio). To explore how the rate of evolution might alter the magnitude of evolution's impact on population dynamics, I established evolution treatments that consisted of all three two-way combinations (A-B, B-C, and A-C). Three different non-evolution (pure clone) treatments received 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, thus preventing evolution. Finally, ‘no- aphid control’ treatments did not receive any aphids. Each treatment combination was assigned in a randomized block design and replicated 8 times for a total of 112 plants.

III - Rates of Evolution

On days 13, 20, and 31 I collected 20, 50, and 70 aphids respectively from every population in order 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 Appendix 3.1). I calculated the frequency of the faster clone for each treatment, sampling day and replicate separately. For each treatment I then determined whether the mean frequency of the faster clone differed significantly from the initial clonal frequency of 50% using one-sample t-tests. I also tested how caging, evolution treatment, and their interaction impacts the final frequency of clones using a general linear model. All analyses were implement in R (v. 2.11.1; R Development Core Team 2009) using the *nlme* package (Pinheiro et al. 2009).

IV - 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. When populations rose above 2000

aphids per plant I sub-sampled by counting one half of every leaf. The removal of cages on day 13 for half the treatments qualitatively altered population dynamics. Because of this I analyzed caged and uncaged treatments separately. Treatments that remained caged for the entire experiment grew exponentially until day 27 (Fig. 4.1.a). On day 31, population growth started to slow and on day 36 populations crashed due to plant senescence. The uncaged treatments were initially caged for 13 days during which the aphids grew exponentially (Fig. 4.1.b). Once cages were removed many lower leaves were damaged or consumed by vertebrate herbivores which reduced growth rate. However, after this reduction populations once again grew exponentially until day 31 before crashing on day 36 (Fig. 4.1.c). Thus I analyzed these time periods separately (days 0 to 13 and then starting day 13 to 31) for the uncaged treatments, this greatly improved residuals normality for all treatments.

I thus had three separate analyses; caged from days 0 to 27, uncaged days 0 to 13, and uncaged 13 to 31. For each of these I tested for differences in population growth rate between treatments using the following exponential growth model:

$$N_t = N_0 * e^{(rm *day)}$$

where N_t is the number of aphids on day t, N_0 is the number of aphids on day 0, and *rm* is the intrinsic growth rate. Models were analyzed using linear mixed-effect models (LME) where the dependent variable was LN(x) transformed number of aphids and the fixed effects were aphid treatment, day (as the main

covariate), and their interaction. Because the repeated aphid counts on the same plant violated the assumption of independent observations, I set unique plant identity, nested within block, as a random effect on population growth rate and intercept and used an autoregressive correlation error structure (Pinheiro and Bates 2000). I modeled increasing variance through time by using a variance function that increases with the power of the variance covariate (varPower). Because plants differed slightly in size and stage of development at the start of the experiment I explore whether the inclusion of these covariates improved model fit. Initial plant size was measured as the number of true leaves and rosette width and combined into a principal component score. Only the first principal component was used since it explained 73% of the variation. Stage of development was quantified into an ordinal scales (1= rosette, 2 = low bolt, 3= bolt). Only in the caged treatments did the covariates improve fit and were kept in the final model. All analyses were implement in R (v. 2.11.1 R Development Core Team 2009) using the *nlme* package (Pinheiro et al. 2009).

IV - The Impact of Evolution on Population Dynamics

My objective is not to predict which clone will out compete the others but to statistically test the impact of changes in clonal frequency on concurrent population dynamics (*Hypothesis 1)*. Ideally one would compare the observed aphid population dynamics in the evolution treatment to those of a mixed population, containing the same clones, but cannot evolve (remains at a

frequency of 50:50 even though clones differ in fitness). This is not possible in my field experiment. I thus generated the *expected* population growth parameters of a non-evolving population (that remains at constant clonal frequency) by using the pure aphid treatments. I 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. I did so with the use of planned contrasts that are orthogonal comparisons between a subset of the aphid treatment levels within the LME analysis. I set the 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 to the AC evolution treatment= 1). The contrast matrix is found in Chapter 3. 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. I present the results of planned contrasts for the slope and intercept estimates of these comparisons.

V - The Effect of Density on Natural Selection

I also investigated whether competing clones reacted differently to changes in density in order to explore whether more complex eco-evolutionary dynamics are occurring such as density-dependent selection. To test this possibility I compared how the relative fitness of competing clones, in each

evolution treatment, depends on density. For each population I calculated the quantity of each clone on days 0, 13, 20, and 31 by multiplying their genotypic frequencies by total population size. Next I calculated per capita growth rate in each time period (days 0-13, 13-20, and 20-31) using $\ln(N_2)$- $\ln(N_1)$ / (day_2-day_1), where N= number of aphids of this clone (Agrawal et al. 2004). This was done for each clone in each evolution treatment. I then fitted a linear mixed-effect model on these growth rates. I fit 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) and initial density in that time period and their interaction. I included plant identity and block as a random effect as well as autocorrelation error. With the use of planned contrasts I determined if density differentially reduced growth rate between competing clones.

VI - Host Plant Fitness

I first determine whether aphid treatments impact their host's growth by measuring final above ground dry biomass as done in previous experiments. I fit a general linear model with plant weight as the response variable, the fixed effect was aphid treatment, including the 7th treatment which did not receive any aphids, the covariates were initial plant stage of development and the first principal component of plant size. Planned contrasts were used to determine whether aphid herbivory in general influence plant final weight. Other contrasts,

mimicking those used in the population dynamic models, were used to determine whether plants with evolving aphid populations differed in weight compared to plants with non-evolving populations.

I also quantified plant fitness traits. I first calculating flower-days by summing my counts of the number of flowers present on every sampling day multiplied by the length of that counting period. When harvesting the plants I estimated the total number of seeds and mean seed dry mass by sub-sampling. I performed a similar analysis as described above but given correlation among traits I did so in a MANCOVA framework.

Finally I determined whether final plant dry biomass predicted plant fitness by fitting three separate linear models one focusing on flower days, one on seed number, and one on mean seed weight. The dependent variable was final plant weight, and the covariates were stage and size of the plant at the start of the experiment. In all three analyses these covariates were non-significant and did not improve model fit and were thus removed. I thus present results from Pearson correlations without the covariates.

RESULTS:

I - Pure Clone Treatments

Pure clone treatments differed greatly in their population dynamics in the field (Fig. 4.1) as expected from greenhouse studies. In the caged treatments, the exponential population growth rate (slope) of the A clone was fastest. It grew

9.4% faster than the B clone and 14.5% faster than the C clone (Fig. 4.1.a). The B clone in turn grew 4.7% faster than the C clone. The caged treatments reached very high densities approximately ten times higher than the uncaged treatments (see y-axis scales in Figs. 4.1.a & c). This difference occurred because of strong vertebrate herbivory I observed when the cages were removed from the uncaged treatments on day 13. This herbivory reduced plant biomass and in addition to predation on the aphids slow the uncaged aphid's population growth. The pattern of growth rate between the pure clone populations was similar in the uncaged treatments. Within the first 13 days, clones with higher expected r_m grew at faster rate. The A clone grew 9.8% faster than the B clone and 19.3% faster than the C clone (Fig. 4.1.b). The B clone in turn grew 8.6% faster than the C clone. After cages were removed, the A clone still grew fastest, 12.1% faster than the B clone and 19.3% faster than the C clone and finally the B clone grew 6.4% faster than the C clone (Fig. 4.1.c).

II - Evolution Treatments - Genetic Analyses

Within only 20 days, in caged and uncaged populations, the faster growing clones in the A-C and in the B-C evolution populations significantly increased in frequency compared to their initial frequency of 50% (one sample t-tests, all p-values < 0.03; Fig. 4.2). By day 31 all evolution treatments showed significant evolution in the expected direction (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, which is significantly less than the uncaged population reaching 79% ($p = 0.04$, Fig. 4.2). This implies that caged treatments evolved 38% slower than in uncaged (29% increase in frequency divided by 21%). Aphid treatments differed significantly in their rate of evolution ($p < 0.001$) the A-C and B-C treatments differed significantly from A-B but not between each other (Fig. 4.2). Caging did not interact with evolution treatment ($p = 0.46$, Fig. 4.2). Thus the A clone in the A-C evolution treatments reached on average 85%, the B clone in the B-C treatments reached 80%, and the A clone in the A-B treatments reached 62% (Fig. 4.2). Thus evolution treatments that differ in their rate of evolution were successfully created.

III - Impact of Aphid Evolution on Aphid Population Dynamics

To test the impact of rapid evolution on concurrent population dynamics I compared the observed population dynamics in evolving 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). In the caged treatments, although rapid evolution occurred leading to an increase in the frequency of the faster growing clone this did not alter the evolving populations growth rate compared to their no-evolution expectations (Table 4.1, Fig. 4.3). The B-C evolution treatment, even though it grew 4.2% faster than its no-evolution expectation this differences was not significant ($p = 0.09$). As for the uncaged treatments, in the early growth phase, none of the

evolution treatments differed in growth rate or density from their no-evolution expectations (Table 4.1, Figs. 4.4.a, b, c). After day 13, in the uncaged treatments all evolution treatments grew at a significantly faster rate than their corresponding no-evolution expectations (between 33% and 42% faster, Table 4.1, Figs. 4.4.d, e, f). I also created predicted population densities for each treatment based on the model fitted parameters. This approach is more reliable than simply comparing densities on day 31 because the fitted values incorporate all the time series data. Thus predicted population sizes on day 31 in the evolution treatments reached much higher densities than expected without evolution (A-B treatment +13.7%, B-C +67%, and A-C +17.5%, Table 4.1). Although evolution in the A-B and B-C treatments have similarly accelerated population growth rate (by 33% and 35%) their predicted densities on day 31 differed greatly because the fitted models incorporate differences in intercept. Moreover, the evolution treatment with the slowest rate of evolution (A-B) showed the smallest effect of rapid evolution on population growth rate. The faster evolving treatments (A-C and B-C), which do not differ in their rate of evolution, showed a stronger positive impact on population dynamics.

IV -The Effect of Density on Natural Selection

I tested for density-dependent natural selection by fitting a linear mixed-effect models that compared how strongly density reduces the growth rate of each clone in the evolution treatments. Increases in density more severely

reduced the growth of clone C than clone A (caged $p<0.001$; uncaged $p < 0.001$, Table 4.2) and also compared to clone B (caged $p= 0.065$; uncaged $p < 0.001$, Table 4.2). Thus the relative fitness of clone C decreases in response to increased population density when competing with another clone.

V - Impact of Aphid Evolution on Host Fitness

Because rapid evolution did not impact aphid dynamics in the caged treatments I only present results for the uncaged treatments. Firstly, my analysis revealed that aphid herbivory significantly reduced final host biomass (mean ± SE weight with aphids = 15.3 g. ± 2.6, weight without aphids = 20.8 g. ±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 no-evolving aphid populations (GLM, all three p-values > 0.11). My 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 = 0.57, $F= 1.1$, overall treatment p-value = 0.37, planned contrasts p-values all > 0.12). Although no-aphid treatments had higher plant fitness means there was a lot of variation that overwhelmed any trends (e.g. mean ± SE for flower-days for no-aphid treatment = 1683 ± 373, mean for aphid treatments = 1542 ± 135).

I also documented significant correlations between the above ground dry biomass of the plants and the number of seeds produced and total flower-days. Mean seed weight, however, was marginally non-significant ($p=0.084$, Table 4.3).

Discussion

I experimentally assessed the impact of rapid evolution on concurrent ecological dynamics in the wild for the first time. I found that rapid evolution, occurring within weeks, significantly accelerated population growth rates and density as the frequency of faster growing clones increased (*Hypothesis 1*), yet this change in aphid density and growth rate did not impact host plant fitness (*Hypothesis 3*). I also found that the magnitude of the impact of rapid evolution on population dynamics depended positively on the rapidity of evolution and on ecological context (*Hypothesis 2*). Unexpectedly, in caged populations, although rapid evolution occurred this had no impact on population dynamics. This highlights the important affect that ecological context can have the strength of eco-evolutionary dynamics. My results have important implications for basic as well as applied ecological and evolutionary biology.

In my field experiment, pure aphid treatments grew at significantly different rates (Fig. 4.1). As expected, when populations contained two clones their genetic composition quickly changed as the faster clones increased in frequency (Fig. 4.2). For example the frequency of the C clone in the uncaged A-C evolution populations was reduced by more than four fold within only 31 days.

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 my study lies in quantifying the effect of this evolutionary change on the population's growth rate within that short time period. *Hypothesis 1* was supported but only in the natural uncaged treatments. Focusing on these results, I saw that evolving populations grew at a significantly faster rate than the expected rate if evolution is not taken into account (Fig. 4.4, Table 4.1). 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."* Moreover, the magnitude of this effect was dependent on the observed rate of evolution *(Hypothesis 2)*. The more quickly a population evolves the strong impact of that rapid evolution on population growth rate (Figs. 4.2 and 4.4, Table 4.1).

Even more complex and unexpected qualitative changes in population dynamics have been observed in some laboratory studies of rapid evolution. 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. These systems might

inherently have more opportunity for qualitative changes in population dynamics since both species 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 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.

All three uncaged evolution treatments accelerated population growth rate. However evolution seems to have had a more pronounced impact on population dynamics in the B-C evolution treatment (Fig 4.e). This occurred because the A-B and A-C evolution treatments had lower day 13 densities than their corresponding no-evolution expectations (Fig. 4.d & f), although these differences were not significant (Table 4.1). Evolution in these two treatments compensated for this effect and eventually overtook the no-evolution expectations. On the other hand the B-C evolution treatment has similar intercept values thus a 35% increase in growth has a very large effect on final density. Given these differences it is clear that the increase in growth rate in the evolution treatments is not simply due to some evolution treatments having lower day 13 densities. Overall my results, in addition to my previous greenhouse experiment (Chapter 3 and those of Pimentel 1968, Fussmann et al. 2003, Yoshida et al.

2003) argue that evolution should no longer be assumed to be too slow to impact short-term ecological dynamics (Slobodkin 1980, Endler 1991, Thompson 1998, Hairston et al. 2005). Rapid evolution can be a strong driver of population dynamics.

My experiment, however, demonstrated that ecological context can have an important impact on rapid evolution's ecological effects. When I designed the experiment I created caged and uncaged treatments because I hypothesized that environmental biotic variation might overwhelm any effects of evolution. The impact of rapid evolution in the uncaged treatments was evident even in the face of potentially confounding factors. First, when cages were opened on day 13, vertebrate herbivores chewed off many large leaves causing a reduction in aphid growth. Second, a competing aphid species (*Brevicoryne brassicae*) colonized some plants (although remained at low density) as did aphid predators (e.g. spiders, coccinellid larvae and adults) that seemingly influenced the treatments randomly, adding to variation between replicates. Finally, on average, 10% of the aphids genotyped were immigrants. I did not remove the effect of these ecological processes because they occur in natural populations. These confounding factors and environmental variation strengthen the importance of my results because the impact of rapid evolution on population dynamics was still measurable and large.

I expected a stronger impact of rapid evolution on population dynamics in the caged treatments because migration, interspecific competition, predation,

and non-aphid herbivory were prevented. Surprisingly, even though populations rapidly evolved in the caged treatments this did not significantly impact population dynamics (Fig. 4.3, Table 4.1). I posit that my ability to detect an effect of evolution might have been reduced by the large population sizes reached in the caged treatments (up to 50 000 aphids) because counts become less accurate at such densities. The coefficient of variation for triplicate counts of the same plant at a density of 6000 aphids was 0.02 whereas it was 0.13 for a plant with 26000 aphids. Moreover caging significantly reduced the rate of evolution by 38% (Fig. 4.2) thus potentially reducing the effect of evolution. Aphids clones might differ in susceptibility to predation (Muller 1983, Pilson 1992) a selective force not present in cages thus potentially slowing the rate of evolution cages. Another possibility is that the rate of evolution was reduced because populations were less density-regulated than the uncaged populations. Stronger competition between clones could magnify selective advantages and change the selection environment (Table 4.2, and see text below). Aphid clones are known to differ in how their growth rates respond to increased population density (Agrawal et al. 2004). I 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 to uncaged populations in the second growth phase (r_m = 0.08). This difference can be attributed to the fact that the uncaged host plants were 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 caged treatments, and hence a stronger impact of evolution on population growth rate. A greenhouse experiment using the same aphid clones supports these findings in that aphid density can alter not only the clonal selection environment but also the impact of rapid evolution on population dynamics (Chapter 5). Saccheri & Hanski (2006) proposed that population density is less likely to be influences by natural selection or evolution if that population is under strong density regulation, which should overwhelm for eco-evolutionary effects. The population dynamics in my system do not behave as though they are tightly regulated yet my results still suggest that density affects the selective environment leading to non-intuitive outcomes.

Conducting studies in this emerging field in realistic ecological conditions is crucial. The choice of genotypes are studied can also alter the rate of evolution, which can change the impact strength of rapid evolution on population dynamics. Together my results emphasize that to properly assess the relative importance of evolution on population dynamics requires experiments under natural conditions. These should manipulate ecological variables in a realistic manner, e.g. mimicking natural levels of variation in competition, and also use genetic variation present within populations (i.e. not artificially magnifying variation by using non-local genotypes).

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). I investigated whether this is occurring in my system by assessing host plant fitness. Aphid rapid evolution could impact its host's fitness if one clone is more damaging per capita, and thus an increase in its frequency would correspondingly reduce plant fitness. More directly an increase in aphid density through rapid evolution could also increase damage. Although aphid evolution increased aphid population growth rate and density, by up to 42%, this did not impact plant fitness or plant final biomass (*Hypothesis* 3). One possibility is that non-aphid herbivore damage determined plant fitness, overwhelming the aphid's impact. This suggestion is supported by the result that plants being consumed 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 (coefficients of variation among replicates: range of 0.1-1.9 with a mean of 0.6). To properly quantify the interspecific effects of rapid evolution in natural populations one must not experimentally exclude such confounding ecological processes.

My experiment focused on the less-studied half of the eco-evolutionary dynamics cycle. Rapid evolution impacts concurrent population dynamics and its host fitness but is the reciprocal causal process also occurring? I found that the competitive advantage of the A and the B clones over C clone significantly increases at higher density, in both caged and uncaged treatments. 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 and Lopez-Sepulcre 2007). As rapid evolution leads to higher densities this alters natural selection thus potentially modifying future bouts of evolution. Comparing my caged and uncaged genetic results the strength of density regulation might be more important than the absolute density in influencing the selective environment. I limit my interpretation of these results since my experimental design cannot disentangle the effect of density and time. The C clone might simply have a fitness disadvantage on mature plants, which is confound with density in this experiment.

In many applied fields such as fisheries, pest management, conservation biology, invasion biology, and epidemiology, accurate predictions of population dynamics are crucial. Rapid evolution in many traits has been observed in these systems (Ashley et al. 2003). My results suggest that considering rapid evolution as a force impacting concurrent population dynamics might provide important insight into many of these applied issues (Hufbauer and Roderick 2005, Duffy and Sivars-Becker 2007). For example in pest management, models are used to establish guidelines for the economic use of pesticides yet these almost always assume non-evolving population growth parameters. My results argue that this oversight might be problematic. Even in a small population, where drift might be stronger, within only 31 days, predictions made without considering rapid evolution could underestimate actual population growth rate by up to 42% and

population size by 67%. This could significantly alter optimal control strategies. My novel study suggests predictions of population dynamics and rapid evolution will be more accurate if they explicitly consider these processes and their interactions at all timescales.

Table 4.1: Analysis of Population Dynamics Comparing Evolving Aphids to Non-Evolving Controls for Caged and Uncaged Treatments

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 to 27), for the early growth phase (days 0 to 13) and the late phase (days 13 to 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 represent 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. Significant results were bolded for easier identification.

Table 4.1

Evolution Treatment (Clones)		Caged (days 0 to 27				Uncaged (days 0 to 13)				Uncaged (days 13 to 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 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 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 Density				0.1%				-21.5%				17.5%

Table 4.2: Analysis of Density-Dependent Clonal Selection

Summary of density-dependent clonal selection analysis. Results of planned contrasts comparing how strongly density reduces the growth rate of each clone in the evolution treatments. Slopes represent daily growth rate as a function of initial density in each time period (see methods for details). All slope values (means and SE) should be multiplied by 10^{-4}. All *p-values* are for 2-tailed tests.

	Treatments	Slope of Faster Clone (SE)	Slope of Slower Clone (SE)	% Difference in Slope	d.f.	t-value	*p-value*
Caged	*A-B*	-0.35 (0.14)	-0.27 (0.12)	30.1%	85	-0.8	0.4284
	B-C	-0.22 (0.11)	-0.59 (0.2)	-62.7%	85	1.9	0.0653
	A-C	-0.29 (0.13)	-0.66 (0.14)	-56.6%	85	3.5	0.0008
Uncaged	*A-B*	-4.2 (0.7)	-3.2 (0.7)	32.7%	78	-1.5	0.1497
	B-C	-2.8 (0.5)	-7.2 (1.1)	-61.3%	78	4.1	0.0001
	A-C	-2.7 (0.7)	-7.8 (1.2)	-65.0%	78	4.3	0.0001

Table 4.3: Correlation Between Final Plant Weight and Plant Fitness Traits Summary of Pearson correlation analyses between final plant dry above ground biomass and three fitness traits. All correlations were positive. All *p-values* are for 2-tailed tests.

Fitness Trait	d.f.	t-value	*p-value*	Pearson Correlation
Total Seed Number	49	5.19	0.0000	0.60
Mean Seed Weight	40	1.77	0.0838	0.27
Flower-Days	49	3.62	0.0007	0.46

Figure 4.1: Population Dynamics of Pure Clone Populations for Caged and Uncaged Treatments
Partial residual plots of population dynamics, over the time periods used in the analyses, of pure clonal treatments in field experiment. 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 to 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. Notice that panels differ in y-axis scales. Functions represent the best fit exponential model for each treatment (clone A is gray, clone B is the full black line, and clone C is the dashed black line).

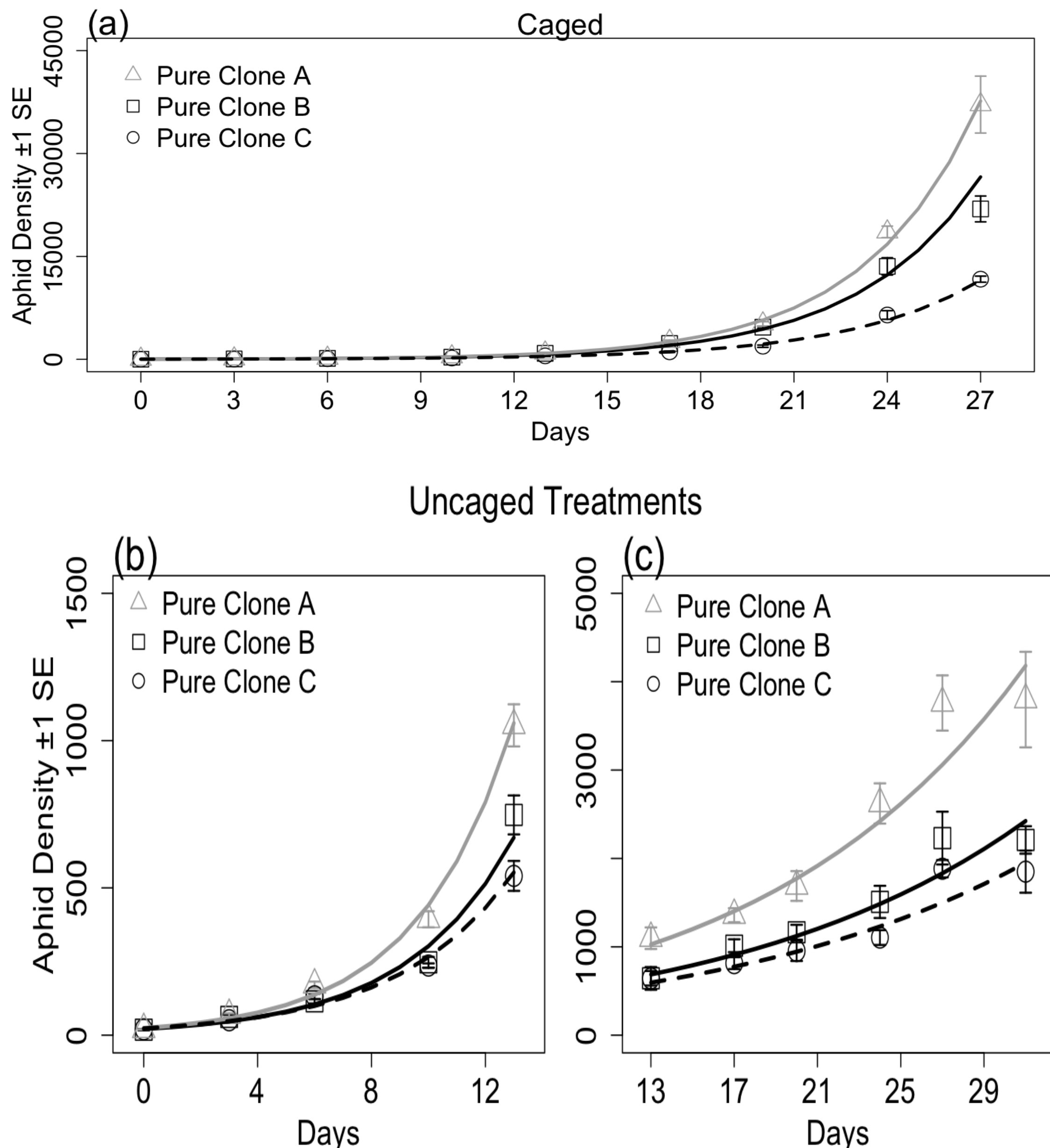


Figure 4.2: Temporal Changes in Clonal Frequency During the Experiment
Rapid clonal evolution as shown by the mean frequency of the faster growing aphid clones in each evolution treatment (±1 SE). Panels separate caged (a) and uncaged (b) treatments Horizontal bar indicates initial clonal frequencies of 0.5.

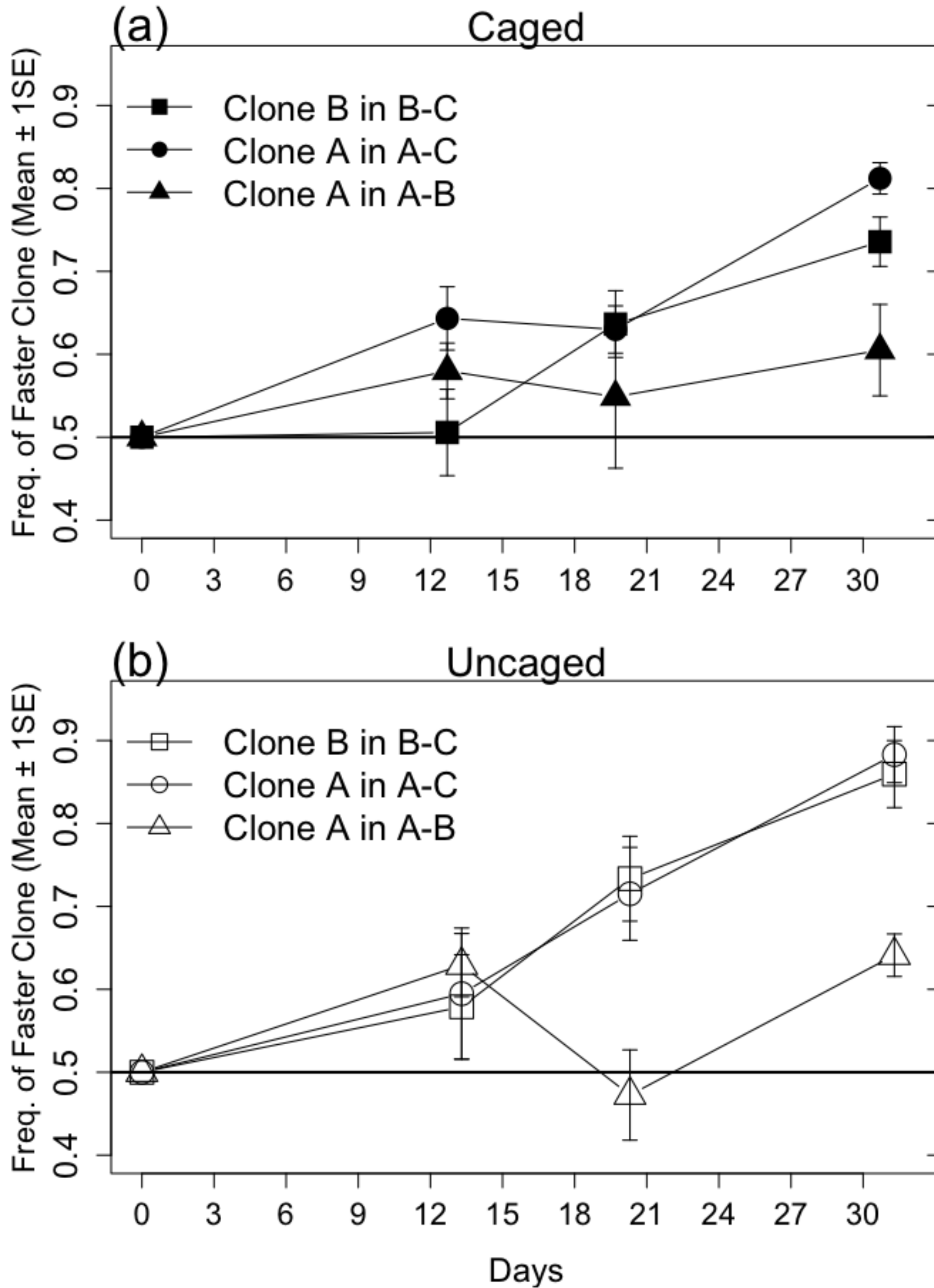


Figure 4.3: Population Dynamics Comparing Evolving Aphids to Non-Evolving Controls in Caged Treatments
Partial residual plot of population dynamics of the three observed evolution treatments (black diamonds) with the best fit model from LME analysis (black line) for caged treatments. The dashed gray line represents the best fit model that combines both pure treatments using the constant (non-evolving) frequency of clones (50:50). For each treatment I 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.

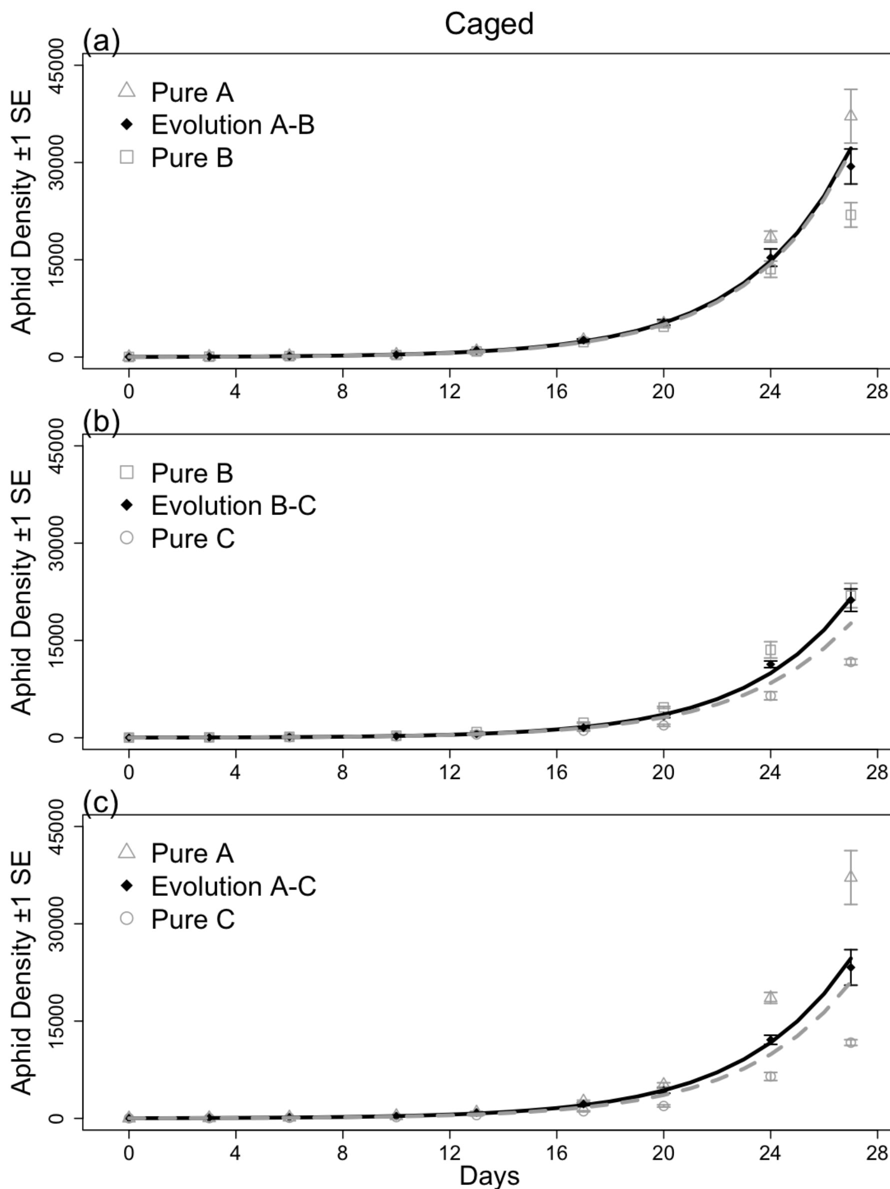
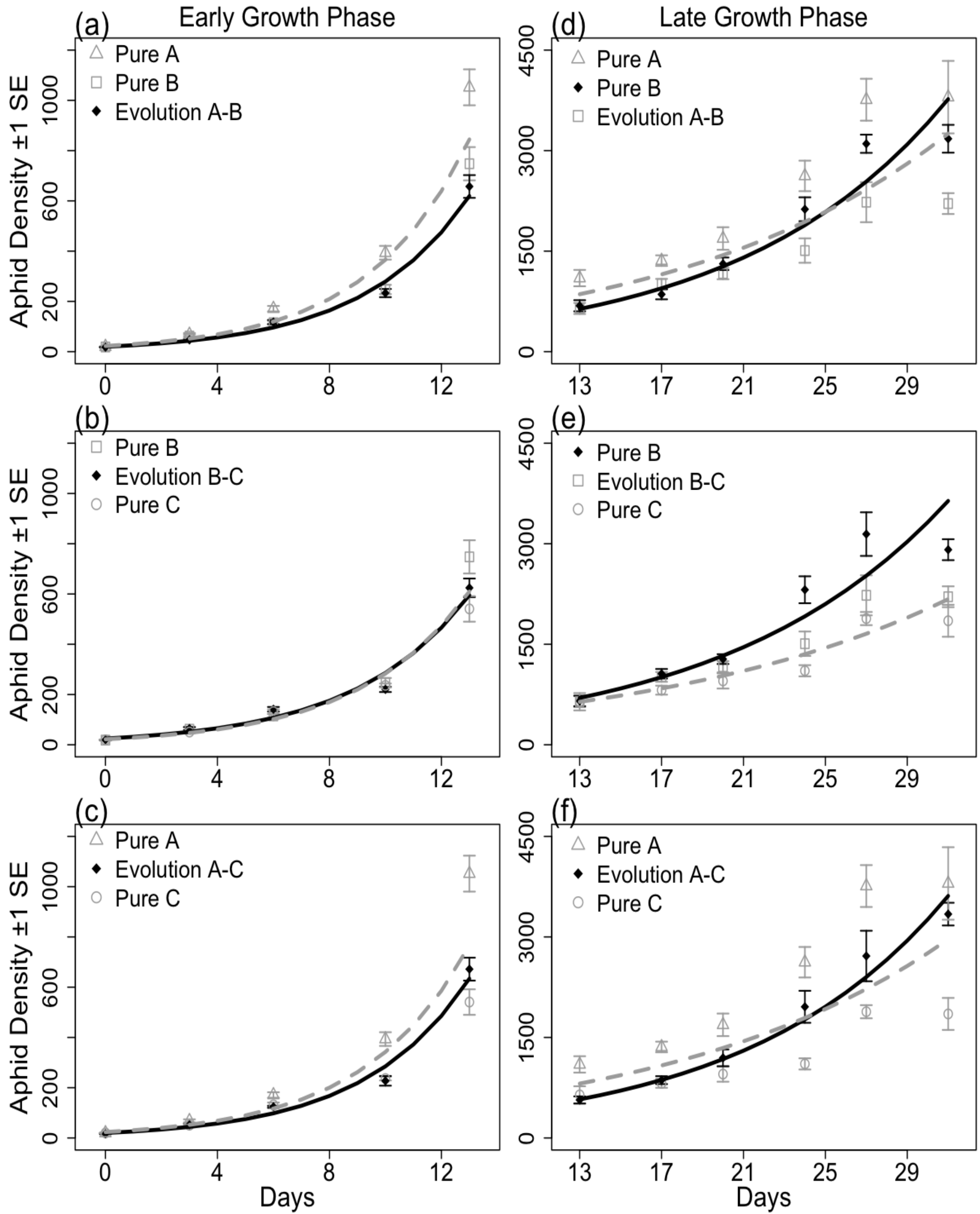


Figure 4.4: Population Dynamics Comparing Evolving Aphids to Non-Evolving Controls in Caged Treatments

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 (caged results can be seen in Appendix 3.1). The dashed gray line represents the best fit model that combines both pure treatments using the constant (non-evolving) frequency of clones (50:50). For each treatment I 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 to 13) and right panels are for the late growth analysis (days 13 to 31).

Figure 4.4



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CHAPTER FIVE: AN EXPERIMENTAL ASSESSMENT OF THE FULL ECO-EVOLUTIONARY DYNAMICS CYCLE BETWEEN RAPID EVOLUTION AND POPULATION DENSITY AND THEIR RELATIVE IMPACTS

ABSTRACT

This study builds on previous findings that aphid rapid evolution impacts concurrent population dynamics by experimentally testing whether such changes in density alter the course of evolution. This represents the first explicit experimental test of the full eco-evolutionary dynamic cycle (two-way causality between ecological and rapid evolutionary dynamics) in a plant-herbivore system. Using aphids and mustard, I manipulated aphid initial density and aphid rapid evolution. I found strong evidence for density-dependent selection. Initial aphid density altered the rate and outcome of evolution, as measured by changes in clonal frequency. Density also quantitatively and qualitatively altered how rapid evolution impacts concurrent population growth rate. Aphid evolution, within only 16 days, significantly accelerated population growth rate (by up to 10.3%) compared to non-evolving controls in some combinations of clones. Yet in one treatment combination, rapid evolution reduced population growth by 6.4%. These effects were similar in magnitude to the reduction in population

growth rate caused by a three-fold increase in initial density. These results suggest that the full eco-evolutionary dynamic cycle is occurring in this system and can have relatively strong effects on both population dynamics and rapid evolution. I also identified one treatment where aphid evolution significantly augmented the damage caused to the host plant. These results have important implications for the study of population dynamics and pest management.

INTRODUCTION

The reciprocal causal influences between evolution, genotypic variation, and ecological dynamics termed '*Eco-Evolutionary Dynamics'* (Fig. 1.1) is currently receiving much attention because such dynamics can alter the predicted outcome of ecological interactions (reviewed in Fussmann et al. 2007, Johnson and Stinchcombe 2007, Pelletier et al. 2009, Schoener 2011). This renewed interested emanates from recent empirical studies of eco-evolutionary dynamics occurring on short-time scales, i.e. within a few dozen generations (Fussmann et al. 2003, Yoshida et al. 2003, Hairston et al. 2005). 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, Fussmann et al. 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) cause selection and evolution (focused on arrow

1 in Fig. 1.1; Levins et al. 2003, Johnson and Stinchcombe 2007). The reciprocal arrow of causality was rarely studied but important exceptions do exist (Pimentel 1961, Chitty 1967, Pimentel 1968).

Many reviews listing examples of rapid evolution have recently challenged the assumption that evolution is too slow to have an impact on contemporary ecological interactions (Thompson 1998, Hendry and Kinnison 1999, Bone and Farres 2001, Reznick and Ghalambor 2001). Such examples of rapid evolution inspired a few but growing number of empirical studies that quantify how rapid evolution alters ecological dynamics (Bohannan and Lenski 2000, Fussmann et al. 2003, Yoshida et al. 2003, Terhorst et al. 2010). For example, in my previous studies using the green peach aphid, *Myzus persicae,* I experimentally quantified that aphid rapid evolution significantly accelerates population growth rate within a few weeks compared to non-evolving control populations in the greenhouse (Chapter 3) and in the field (Chapter 4). Yet, my previous experiments focused on one arrow of causality in the eco-evolutionary dynamics cycle, which is how rapid evolution might alter ecological dynamics (arrow 2 in Fig. 1.1). Eco-evolutionary dynamics can potentially be more complex in this system if both processes are linked through cyclical causality (Fig. 1.1). Ecological changes induced by rapid evolution could alter future bouts of evolution by changing the selection experienced by the target organism. Full eco-evolutionary dynamics seem to be occurring in a side-blotched lizards field system (Sinervo et al. 2000, Svensson and Sinervo 2000) and in rotifer-algae chemostat experiments

(Yoshida et al. 2003). In both cases density cycles lead to, and are caused by, cycles in phenotypic composition thus linking ecological and evolutionary dynamics. In most studies in this emerging field however, only parts of the cyclical pathway are investigated.

My previous studies have shown that rapid evolution can increase population growth rate and density (arrow 2 in Fig. 1.1; Chapters 3 & 4) but it remains unknown whether the full eco-evolutionary dynamic cycle is occurring. The ecological change that I observed in the first bout of eco-evolutionary dynamics (altered population growth rate) could alter the affect of evolution on population dynamics in the second bout in at least two ways. First, changes in density might alter the selective environment directly through density-dependent aphid clonal selection (thus modifying arrow 1 in Fig. 1.1). Aphid clones are known to differ in how their growth rates respond to increased population density (Agrawal et al. 2004). My field experiment (Chapter 4) found correlerative evidence for density-depdendent clonal selection but more robust experiments are required that do not confound host plant age with density. If the higher population densities, caused by aphid evolution, feed back and alters the selective environment then the aphids might not evolve at the rate or even direction as predicted within a constant selective environment. Second, increased density could also affect the strength of population regulation, which might interfere with the ability of rapid evolution to impact population growth rate

or density (altering arrow 2 in Fig. 1.1). Strong population regulation could overwhelm any effect of rapid evolution (Saccheri and Hanski 2006).

Even if rapid evolution occurs within a system and has an ecological impact, this does not guarantee a full eco-evolutionary dynamic cycle. This could be the case if the ecological change does not change the selective environment and does not feedback to influence future evolution. Theoretical studies suggest that a break in the feedback cycle could lead to very different ecological and evolutionary outcomes (Anderson 1971, Abrams and Matsuda 1997, Shertzer et al. 2002, Day 2005). Thus the objective of this study is to explicitly assess whether the full eco-evolutionary dynamic feedback cycle is occurring in my study system. I will extend previous studies by testing ***1)*** *whether aphid clonal selection is density-dependent by comparing the final aphid clonal frequency in replicated populations initiated at different densities.* I am thus testing whether ecological context impacts the selective environment (if arrow 2 impacts arrow 1 in Figure 1.1). Given these density treatments I will also test ***2)*** *whether the impact of rapid evolution on concurrent population dynamics depends on initial density* (if arrow 1 influences arrow 2 in Fig. 1.1) *by comparing whether treatments initiated at different densities differ in how strongly evolution alters population growth rate.* Also by conducting these experiments with different aphid clones I will test ***3)*** *whether the evolutionary context (genotypic composition of the population) affects the results.* Finally, simply showing that eco-evolutionary dynamics occur fails in telling us how important this process is. It is thus

imperative to compare its effect size with other commonly studied ecological forces (Hairston et al. 2005, Johnson and Stinchcombe 2007, Pelletier et al. 2009). Thus I will also ***4)*** *tests the relative impact of rapid evolution on population growth rate compared to the effect of manipulating density.*

METHODS

I- Study System

The system used in this study is the green peach aphid, *Myzus persicae*, and one of its many hosts the annual mustard, *Hirschfeldia incana*. *M. persicae* is a globally distributed species best know for its tremendous impact as an agricultural pest (Blackman 1974, Mackauer and Way 1976). I developed this system to study eco-evolutionary dynamics because the aphid naturally evolves rapidly, through changes in clonal frequency, during its asexual growth phase in the spring-summer months (Foster et al. 2002, Vorburger 2006). In 2008, I collected multiple clonal lineages from a single wild population from the Motte-Rimrock Reserve in Perris California. I identified clones using 6 microsatellite markers (Chapter 2; Sloane et al. 2001, Wilson et al. 2004) and characterized their intrinsic per capita growth rates experimentally (Chapter 2). I selected three of these clones for this and previous experiment (Chapters 2, 3, & 4) that differ in intrinsic growth rate (detailed below).

II- Experimental Design

Evolving and non-evolving *M. persicae* populations on *H. incana* plants were studied in a partly cooled greenhouse (mean daytime temperature = 33°C, range = 21°C to 47°C, mean nighttime = 21.5°C, range = 16°C to 28.5°C). Asexual reproduction was maintained by using addition lighting providing 16hrs light/ 8hrs dark (Blackman 1974). To minimize variation in this primarily outcrossing plant, plants used in the experiment were grown from the seeds of a single *H. incana* plant collected in 2008 at the Motte-Rimrock Reserve. These seeds were planted in four liter pots, using UCR soil mix III, a sand/peat moss mix supplemented with micronutrients, and watered every three days. Cages were constructed to individually house each plant. Cages consisted of an 8 liter pot with a wire frame creating a 75cm high dome that held up thin transparent mesh (Bridal Organza, #664-7242, Jo-Ann). On day 0 of the experiment, the eight week old plants, were inoculated with 6 different aphid treatments by placing a given number of third instar aphids onto each plant. These aphids came from stock greenhouse clonal populations, regularly tested for contamination, that were maintained on *H. incana* (Chapter 2). Replicates were initiated on three consecutive days starting July 25th 2010. On day 1 missing aphids were replaced with fourth instar aphids.

I used three aphid clonal lineages, identified as *'A', 'B', and 'C',* that differ in microsatellite markers (Appendix 3.1). A preliminary greenhouse experiment revealed that they also differ in exponential growth rates when grown in pure

cultures (Chapter 2, daily per capita growth rate of clone A = 0.268 ± 0.002, B = 0.263 ± 0.004, and C = 0.251 ± 0.004). I used these three clones to establish 12 aphid treatments in a factorial design that manipulated density (low and high ~3x) and aphid evolution (possibility of evolution or not). Three evolution treatments consisted of aphid populations (on a single plant) that have two different clones at an initial frequency of 50% : 50%. I established evolution treatments that consisted of all three two-way combinations (A-B, B-C, and A-C). These populations have genetic variation in fitness (e.g. A clone's r_m is greater than that of the C clone) and thus could evolve by changing in clonal frequency (away from the initial ratio). Three different non-evolution (pure clone) treatments received 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, thus preventing evolution. I also manipulated initial density by initiating these six treatments with either 20 or 60 third instar aphids on each plant. I tripled the initial density because in my field collections I often observed such a range of density on single plants (*M. M. Turcotte personal observation*). Each treatment combination was assigned in a randomized block design and replicated 7 times for a total of 84 plants.

III- Rates of Evolution

On day 22 I 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 (see Appendix 3.1 for detailed genetic methods). I calculated the frequency of the faster clone for each treatment and replicate separately. For each treatment I then determined whether the mean frequency of the faster clone differed significantly from the initial clonal frequency of 50% using one-sample t-tests. I also tested whether the frequency of the faster clone in each evolution treatment differed between initial density using 2-sample t-tests.

IV- Aphid Population Dynamics

Aphid population dynamics were quantified by counting all aphids on days 3, 6, 10, 13, 16, 22, 25, and 29. When populations rose above 2000 aphids per plant I sub-sampled by counting one half of every leaf. By fitting different population growth models I found that the results were best described by fitting an exponential model from days 0 to 16. On day 22 plants were starting to senesce, having multiple yellow and/or drying leaves. When the logistic growth model was extended to include day 22 many treatments had severely overestimated population sizes on that day. On day 26, population size declined as the populations crashed. Given the crash of aphid density due to plant senescence, logistic growth models no longer yielded accurate estimates of carrying capacity.

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

$$N_t = N_0 * e^{(rm *day)}$$

where N_t is the number of aphids on day t, N_0 is the number of aphids on day 0, and *rm* is the intrinsic growth rate. This model was analyzed with a non-linear mixed-effect model (NLME). The fixed effect for N_0 was initial density (low or high) and the fixed effects for *rm* were initial density, aphid treatment, and their interaction. Because the repeated aphid counts on the same plant violated the assumption of independent observations, I 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). I modeled increasing variance through time by using a variance function that increases exponentially with the variance covariate (varExp) but that differs between both density treatments. 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. Because plants differed slightly in size and stage of development on day 0 these data were included as covariates for population growth rate. Initial plant size was measured as the number of true leaves and rosette width and combined into a principal component score. Only the first principal component was used since it explained 70% of the variation. Stage of development was quantified into an ordinal scales (1= low bolt, 2 = bolt, 3= flowers). All analyses were implement in

R (v. 2.11.1 R Development Core Team 2009) using the *nlme* package (Pinheiro et al. 2009).

V- The Impact of Evolution on Population Dynamics

My objective is not to predict which clone will out-compete the other but to assess 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 those of a non-evolving mixed population containing the same two clones (remains at a frequency of 50% : 50%). This is impossible since clones will change in frequency because of fitness differences. This is not possible in my field experiment. I thus generated the *expected* population growth parameters of a non-evolving population (that remains at constant clonal frequency) by using the pure aphid treatments. I tested three *a priori* null hypotheses that the population growth rate (slope) 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. I did so with the use of planned contrasts that are orthogonal comparisons between a subset of the aphid treatment levels within the NLME analysis. I set the 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 to the AC evolution treatment= 1). The contrast matrix is found in Chapter 3. Thus differences in population growth rate between the evolution treatment and the no-evolution

expectation represent the impact of changes in the frequency of clones (rapid evolution) on population dynamics. To enhance interpretation of the magnitude of differences in population growth rate I calculated expected densities on day 16 from the model parameters and set common covariate values (mean values).

VI - Host Plant Fitness

Finally, to quantify the impact of aphid rapid evolution on its host's fitness I measured the above ground dry biomass of the plants at the end of the experiment as a proxy for host fitness (Chapter 4, Mitchell-Olds and Bradley 1996). I fit a general linear model on LN(x) transformed plant weight measurements. The factors were treatment (combination of aphid treatment and density), initial plant size PCA score, and stage of development. The interactions between these factors were non-significant and thus removed from the final model. I again used planned contrasts to determine whether plants with evolving aphid populations were smaller than expected from no-evolution treatments.

RESULTS

I - Pure Clone Treatments

Pure clone treatments differed in their intrinsic growth rate and these differences changed in magnitude and rank with density (Fig. 5.1). At low initial density, differences between pure treatments were smaller between clones. Clone A grew fastest, its *rm* value was 2.4% higher than clone B and 5.3% higher

than clone C (Fig. 5.1.a). Clone B grew faster than clone C by 2.9%. At high density however there was a change in rank order (Fig. 5.1.b). Clone A grew 21% faster than clone B and 14 % faster than clone C, and clone B grew 5.7% slower than clone C. Expected density on day 16 based on the best fit growth estimates predict that pure population could differ by as much as 27% in low density and 117% at high density.

II - Genetic Analyses of Density-Dependent Selection

On day 22 I tested for changes in the deviation of the frequency of clones in the evolution treatments from the clonal initial frequency of 50% : 50%. All treatments rapidly evolved except for one within this period and clear evidence for density-dependent clonal evolution was observed (Fig. 5.2). The A-B evolution treatments significantly evolved but only at high density (frequency of A clone = 57%, one sample t-test, p = 0.02). In the B-C evolution treatments density altered the direction of evolution; at low density the B clone became more frequent reaching 69% (p=0.017) whereas at high density clone C reached 70% (p=0.004). Finally in the A-C evolution treatments the A clone became more common reaching 71% (p=0.002) and 65% (p=0.044) in the low and high density treatments respectively (Fig. 5.2).

III - Impact of Initial Aphid Density and Evolution on Aphid Population Dynamics

Although high initial density treatments reached higher densities on day 16 (see axis scale in Figs. 5.1 and 5.3) these treatments had significantly reduced population growth rates. Overall the high density treatments had 9.5% lower population growth rates (NLME, $F= 25$, $p < 0.0001$). To test the impact of rapid evolution on concurrent population dynamics I compared the observed population dynamics in evolving 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, $F= 4.8$, $p = 0.0003$) I conducted planned contrasts for each density separately (see Table 5.1). Evolution in the A-B evolution treatments did not significantly alter population dynamics at either density (Fig. 5.3.a & b, Table 5.1, both $p > 0.2$). The impact of evolution in the B-C treatments was highly influenced by initial density. At low density, rapid evolution (i.e., an increase in the frequency of clone B) slowed population growth by 6.4% ($p=0.0004$, Fig. 5.3.c) whereas at high density rapid evolution (i.e., an increase in clone C) accelerated population growth by 10.3% ($p=0.003$, Fig. 5.3.d, Table 5.1). Expected density on day 16 based on the best fit growth estimates predict that rapid evolution causes differences in population size of –25% and +48% respectively. Finally, in the A-C evolution treatments initial density magnified the effect of rapid evolution. At low density rapid evolution marginally accelerated

population growth rate by 1.1% (p=0.022, Fig. 5.2.e) yet at high density evolution accelerated population growth by 9.4% (p= 0.0001, Fig. 5.2.f, Table 5.1). 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.

IV - Impact of Aphid Evolution on Host Plant Fitness

My analysis of final plant weight did not reveal an overall effect of initial aphid density (p=0.25). Of the six comparisons between evolving populations and their corresponding no-evolution treatments only one revealed a significant difference. The evolution treatment with the largest effect on population growth, high density B-C, significantly reduced its host's biomass by 27% (GLM, p= 0.030).

DISCUSSION:

I experimentally assessed eco-evolutionary dynamics by manipulating both density and rapid evolution in a plant-herbivore system. I found that aphid populations rapidly evolved through changes in clonal frequencies but the magnitude and direction of evolution depended on initial density. Aphid evolution significantly altered population growth rate but the direction and magnitude depended upon initial density and genotypic composition. These results suggest that the full eco-evolutionary dynamics cycles are occurring in this system (Fig. 1.1) and can have relatively strong effects on both population dynamics and rapid

evolution. Rapid aphid evolution changed population growth rate and density influenced the course of evolution. I also identified one treatment where aphid rapid evolution significantly augmented the damage cause to the host plant. These results have important implications for the study of population dynamics and pest management because they suggest that neither short-term ecological or evolutionary dynamics should be studied separately.

My prior experiments in this system experimentally tested the less studied half of eco-evolutionary dynamics. I showed that over a period of 28 days rapid evolution significantly accelerated population growth rate in the greenhouse by 28% to 34% (Chapter 3). In my field experiment (Chapter 4), even in the presence of environmental variation, immigration, the presence of competitors, herbivores, predators, and parasitoids, rapid evolution still significantly accelerated population growth rate by 33% to 42% within 31 days. These experiments have thus revealed that evolution can affect concurrent ecological dynamics (arrow 2 in Fig. 1.1). The experiment presented here builds on the previous ones experimentally quantifying the full eco-evolutionary cycle by assessing changes in population density, which is the product of population growth and rapid evolution, can in turn feed back on and influence the outcome of interclonal competition.

My experiment revealed clear evidence of density-dependent selection. Initial density had multifarious effects on the pattern of evolution depending on genotypic composition (Fig. 5.2). Differences in density altered whether rapid

evolution occurred or not (A-B evolution treatments), it affected the direction/outcome of evolution (B-C evolution treatments, Fig. 5.2). My goal is not to predict the outcome of clonal selection but to document that changes in density alter the outcome. However, it remains true that differences in population growth rate in single clone cultures most often predicted the direction of evolution correctly (e.g. the change in outcome of B-C treatments) but not always the rate of evolution (e.g. when A and B were grown together at high density, A reached only 57% frequency even though the pure A clone grows 21% faster than pure B clone). Overall my experiment revealed density-dependent clonal evolution, which suggests that as density changes due to rapid evolution, the selective environment is also changing in a way that alters the evolutionary outcome, thus linking rapid evolution and population dynamics in a cyclical causal pathway.

Multiple traits might explain why one clone out-competes another. Aphid clones vary in many traits including life-history traits, competitive ability, predator and pathogen resistance, adult weight, body size, morphology, feeding rate, probing behavior, plant choice, alate production, tendency to drop from plants, response to alarm pheromones, photoperiod response, ability to transmit viruses, and temperature tolerance (Muller 1983, Pilson 1992, Via and Shaw 1996, Rochat et al. 1999, Hazell et al. 2005, Vorburger 2005, Hazell and Fellowes 2009), although some of these differences might be attributable to secondary endosymbionts (Oliver et al. 2010). I conducted a life-table experiment on the aphids clones used in this experiment and found that clones A, B, and C differ in

lifetime fecundity and survival rates (*M.M. Turcotte, unpublished data*). Changes in aphid density could interact with a number of these differences between clones thus leading to density-dependent selection. Aphid clonal variation in the strength of density-regulation as also been observed (Agrawal et al. 2004).

To further test the eco-evolutionary dynamics cycle, I also tested whether density would alter how rapid evolution affects population dynamics (how arrow 1 affects arrow 2 in Fig. 1.1). Initial density quantitatively and qualitatively altered how rapid evolution impacts population growth rate (Fig. 5.3, Table 5.1). In the A-C evolution treatments an increase in initial density led to a much stronger effect of evolution on population dynamics compared to non-evolving controls (+1.1% *versus* +9.4%). This magnification of the impact of evolution might be explained by the fact that the fitness differences between clones A and C are exaggerated at higher densities (Fig. 5.1). Thus an increase in frequency of clone A would more strongly accelerate population dynamics in the high density treatment than in the low density treatment. Overall, increased initial density led to stronger effects of rapid evolution on population dynamics (Table 5.1). This is consisted with my field experiment where higher levels of competition also led to stronger impacts (Chapter 4). The mechanism seems to be stronger clonal selection at higher densities. I still posit that this magnification of the effect of rapid evolution on population growth rate might be diminished if density regulation is strong enough to maintain the populations near a carrying capacity (Saccheri and

Hanski 2006). These conditions do not seem to apply to my aphid system where the population grows rapidly and crashes.

Initial density also qualitatively changed the affect of evolution in the B-C treatments. At high density the C clone becomes more frequent and since clone C's growth rate is higher than clone B it accelerates the evolution treatment's population growth rate by 10.3% above the non-evolving control (Table 5.1, Fig. 5.3.d). At low density the B clone becomes more dominant, reaching a frequency of 69%, but this evolutionary change slows down population growth rate by 6.4%, even though the B clone grows more quickly than the C clone at low initial density. I posit that the B clone interferes with its own growth and that of the C clone when it reaches a high frequency and density. Interference could occur through resource competition or competition for feeding site. Alternatively, if clones vary in their propensity to produce or respond to alarm pheromones emitted at high density (Muller 1983) this might alter the clones feeding and reproduction. Thus the evolving population's growth slows as it becomes dominated by the B clone. A similar hypothesis was proposed in Chapter 3, where I documented evidence that clone B has reduced growth at high density and frequency. Such intraspecific variation in the strength of density dependence has been document in other aphid species (Agrawal et al. 2004) as well as in this experiment (Fig. 5.1). Overall my results demonstrate the dual causal interplay between rapid evolution and concurrent ecological dynamics.

Study systems where the full eco-evolutionary dynamics have been shown are very rare (Svensson and Sinervo 2000, Yoshida et al. 2003). In some systems only a tentative hypothesis is suggested. For example in Palkovacs and Post's (2008) aquatic system, populations of alewifes (*Alosa pseudoharengus*) have become landlocked by dams and have evolved within 300 years. The ancestral anadromous type is only present in certain freshwater lakes in the spring and summer months. Because of year round foraging, the landlocked fish have altered the zooplankton community by favoring smaller species and smaller individuals within species. It is suggested that these ecological changes have fed back and caused the evolution of more efficient foraging morphology (smaller gill raker spacing and gape width) and higher prey selectivity in the landlocked alewifes compared to anadromous forms (Palkovacs and Post 2008). Thus eco-evolutionary dynamic cycles are inferred as a plausible explanation for existing patterns. Given that we are just looking at patterns without a clear establishment of cause and effect relationships, we must accept that alternative explanations may account for them. Thus clear advantage of my study system is that because of rapid evolution occurring within a few weeks I can dissect the eco-evolutionary dynamics as they occur and establish causality.

My experiment revealed that evolution altered population growth by 1.1% to 10.3%, which is smaller than the effect sizes I found in previous experiments (Chapters 3 & 4). I posit that is because I used more mature plants in the current study. This led to an earlier termination of the experiment as the plants

senesced, which in turn reduced the number of days (and hence generations) of usable data in the experiment from 28 and 30 days in Chapters 3 & 4 to 16 days in the current study. This reduced time period limits the extent of evolution and the time evolution can take to impact population dynamics. In the previous experiments I found that evolution had no significant effects in the early time periods. Moreover the reduced time implies smaller population sizes, which could minimize the potential impact of an acceleration of population growth rate. Yet, my current results are surprising in that they show a significant effect of evolution on population growth rate over only 3-4 generations. Most other studies of the ecological effects of rapid evolution study time periods representing many dozen generations (Tuda and Iwasa 1998, Fussmann et al. 2003, Yoshida et al. 2003, Terhorst et al. 2010).

Recent reviews of eco-evolutionary dynamics stress that it is of vital importance to test the relative impact of rapid evolution compared to other ecological forces on population dynamics compared to more commonly studied ecological processes (Hairston et al. 2005, Johnson and Stinchcombe 2007, Pelletier et al. 2009). My experimental design lends itself well to address this issue. I found that a three-fold increase in initial density reduced population growth rate by 9.5%. Rapid evolution in two different evolution treatments had similar effect sizes (Table 5.1) suggesting that rapid evolution and eco-evolutionary dynamics can be important forces in population dynamics on short-timescales.

Unlike any other experimental study system to my knowledge, I have shown eco-evolutionary dynamics within a single species. This suggests that short-term eco-evolutionary dynamics could be very common, even occurring in systems that do not show strong population dynamic coupling with other species (as opposed to predator-prey systems). I also observed that aphid rapid evolution, in the treatment with largest impact on aphid population dynamics, had an interspecific impact on its host. The evolving aphid population reduced plant biomass by 27% compared to non-evolving controls. This suggests that eco-evolutionary dynamics could extend beyond the aphids in this system. More experiments will be required to explore this issue.

My results have important implication for the study of both population dynamics and evolutionary dynamics. It suggests that even on very short timescales of a few generations rapid evolution can occur and can significantly alter population dynamics in complex ways. Moreover the rate and direction of such rapid evolution can be influenced by changes in population dynamics thus completing the eco-evolutionary dynamics cycle. These results suggest that in certain systems ecological and evolutionary predictions would be improved if eco-evolutionary dynamics were explicitly considered. Improving predicted population growth rates should enhance our ability to manage agricultural pests and exploited populations such as in fisheries.

Table 5.1: Analysis of Population Dynamics Comparing Evolving and Non-Evolving Populations in Low and High Density Treatments

The effect of rapid evolution on population growth rate compared to non-evolving controls. Results of planned contrasts from a non-linear 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 Methods for details). The percent change represents the change in slope from the non-evolving expectation to that of the observed evolution treatment. Thus positive changes represent increases due to evolution. Slope represents the rate of growth of aphid populations. All *p-values* are for 2-tailed tests. Significant results were bolded for easier identification.

Evolution Treatment (Clones)	Low Density				High Density			
	d.f.	*F*	*p*	% Change	d.f.	*F*	*p*	% Change
A-B	397	0.3	0.577	+1.0%	397	1.6	0.205	+3.6%
B-C	**397**	**12.8**	**0.0004**	**-6.4%**	**397**	**9.1**	**0.0028**	**+10.3%**
A-C	**397**	**5.3**	**0.022**	**+1.1%**	**397**	**15.2**	**0.0001**	**+9.4%**

Figure 5.1: Population Dynamics of Pure Clone Treatments at High and Low Densities
Partial residual plots of population dynamics of pure clonal treatments. Values represent mean number of aphids (±1 SE) through time, once all explained variation in the model has been removed, separated by a) low initial density treatments and b) high initial density treatment. Functions represent the best fit exponential model for each treatment (clone A is gray, clone B is the full black line, and clone C is the dashed black line). The y-axes differ between panels.

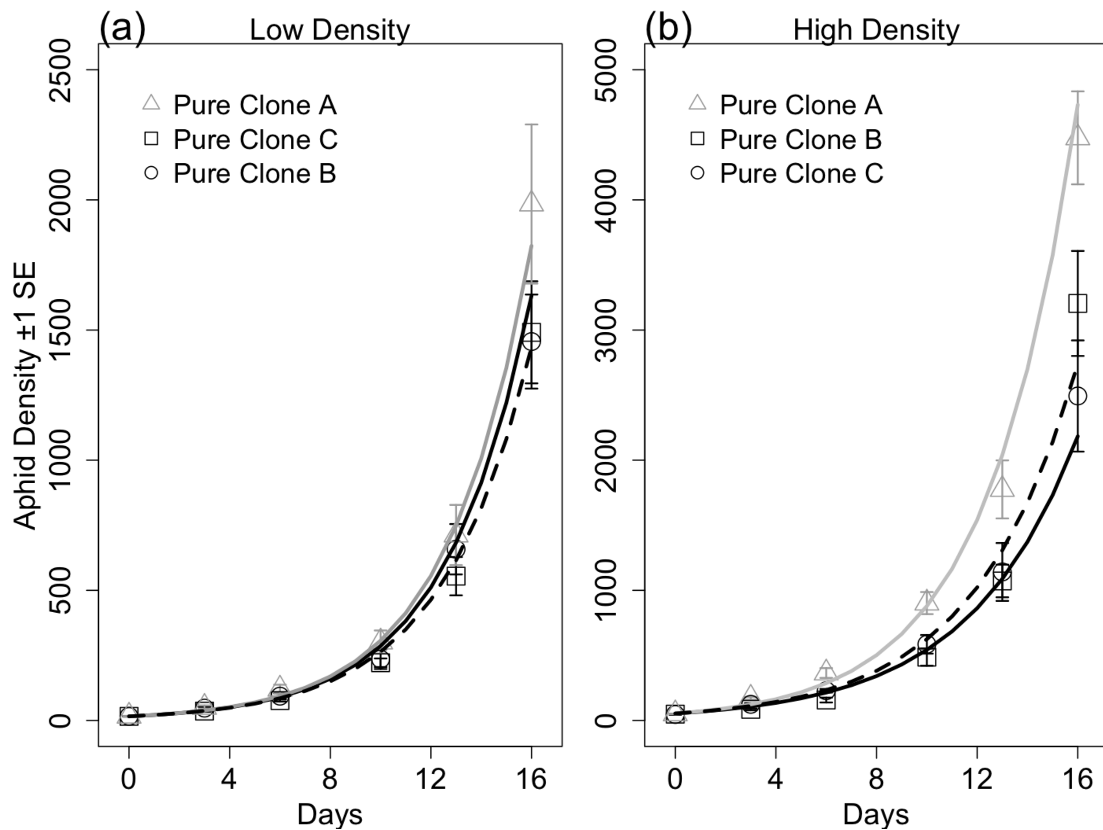


Figure 5.2: Rapid Evolution Through Changes in Clonal Frequency at Different Initial Aphid Densities
Rapid clonal evolution as shown by the mean frequency (±1 SE) of the faster growing aphid clones on day 22 of the experiment. The horizontal bar indicates the initial clonal frequency of 50% on day 0. The X-axis shows which clone's frequency is being tested in each evolution treatment. Dashed vertical lines separate the evolution treatments for which both initial density treatments are shown. The (*) indicate significant divergence from initial frequency and (**) indicates that clone frequency significantly differs between low and high initial density within an evolution treatment.

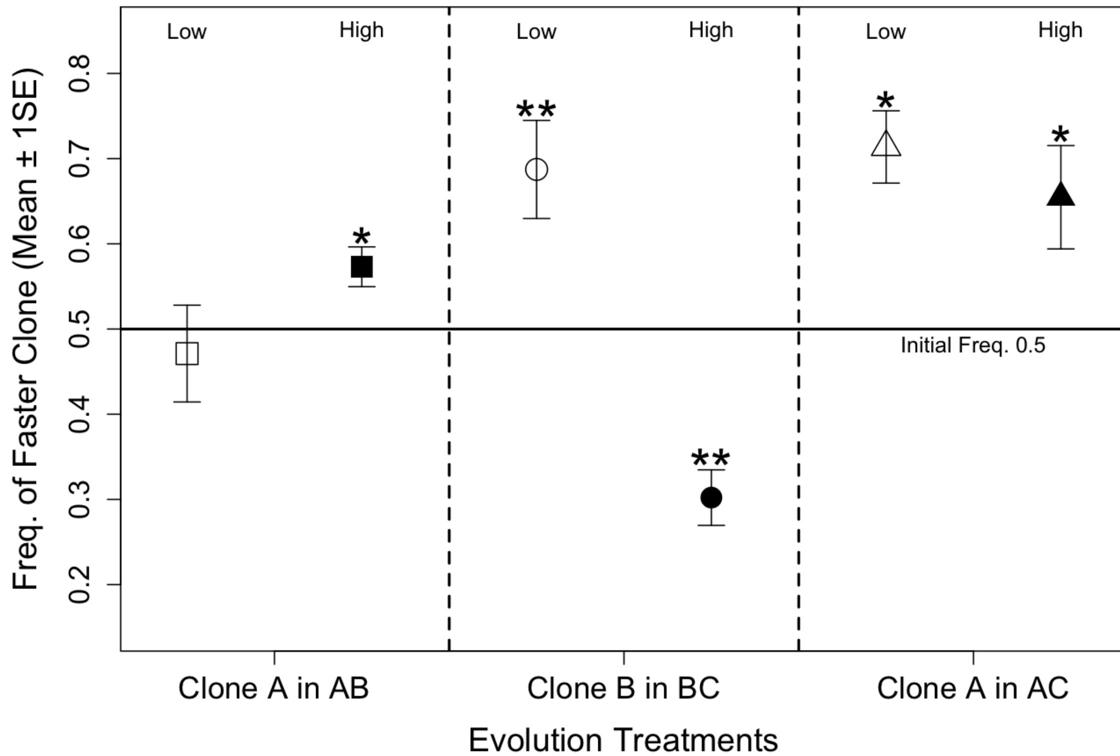
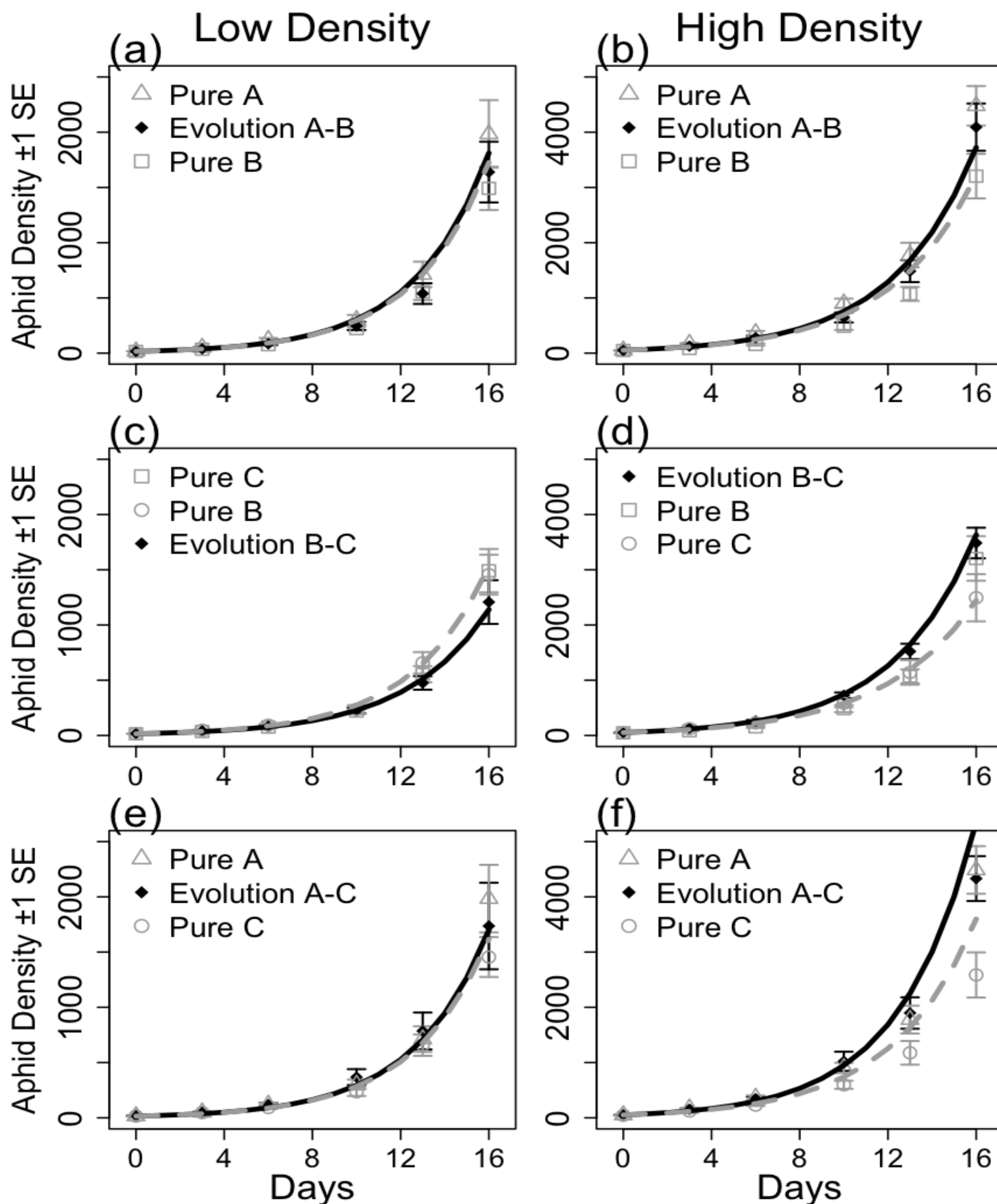


Figure 5.3: Population Dynamics of Evolving and Non-Evolving Aphids at Different Initial Densitites
Partial residual plot of population dynamics of the three observed evolution treatments (black diamonds) with the best fit model from LME analysis (black line). Left panels are for the low initial density treatments whereas the right panels are for the high initial density treatments. The dashed gray line represents the best fit model that combines both pure treatments using the constant (non-evolving) frequency of clones (50:50). Corresponding pure clone treatments (grey symbols) used to generate the no-evolution expectation. Values represent mean number of aphids (±1 SE) once explained variation in the model was removed. The y-axes differ between panels.



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CHAPTER SIX: CONCLUDING REMARKS ON EXPERIMENTAL TESTS OF RAPID ECO-EVOLUTIONARY DYNAMICS IN A PLANT-HERBIVORE SYSTEM

Summary

My dissertation research quantified short-term eco-evolutionary dynamics in an aphid-mustard study system. Chapter 1 introduced the sub-field of eco-evolutionary dynamics in a historical context, it then summarized previous studies, and highlighted outstanding questions. Chapter 2 described the study system consisting of a local population of the green peach aphid, *Myzus persicae*, and its host *Hirschfeldia incana.* I genetically identified many aphid clonal lineages and found that these differ greatly in fitness as measured by intrinsic growth rate. Chapters 3, 4 and 5 addressed one of the focal objectives, which was to experimentally quantify the impact of rapid evolution on concurrent population dynamics in different ecological contexts. More specifically, Chapter 3 first explored this possibility under controlled greenhouse conditions and found that rapid evolution occurred within a few weeks. This rapid evolution significantly accelerated population growth rate by a large quantity (+28-34%). Chapter 4, tested whether these eco-evolutionary dynamics occured in the wild in the face of environmental variation as well as other ecological forces not present in the greenhouse (e.g. predation, competition, and herbivory). I identified similar strong impacts of rapid evolution on population growth rate (up to +42%) but only in the

more natural uncaged treatments suggesting the importance of ecological context. Finally Chapter 5 explicitly tested for the full eco-evolutionary feedback cycle. I found that not only does rapid evolution influence population growth rate and density but that density alters aphid rapid evolution and that density impacts how strongly this evolution in turn impacts population growth rate, completing the cyclical causal interaction between ecological and evolutionary dynamics. I also found that both rapid evolution and initial density had similar magnitudes of effect on population growth rate. Overall my experiments reveal strong interactions between rapid evolution and populations within only a few weeks, however, the occurrence, magnitude, and direction of the affect of rapid evolution on population dynamics depends on the ecological (density, presence of predators...) and the evolutionary context (genotypes and amount of genetic variation in fitness used).

Other patterns emerged when looking across experiments. One pattern is that in general populations that evolved more rapidly showed a stronger affect of evolution on population dynamics. Although ecological context, such as the exclusion of predators and herbivores in the field caged treatments in Chapter 4, could make such a pattern less obvious. This general pattern makes sense in that more rapidly evolving populations will more quickly change in mean population growth parameters and should more quickly and strongly influence population growth rate.

Another pattern I observed is that rapid evolution seems to more strongly impact population growth rate when aphid competition is stronger. In the caged treatments in Chapter 4 and the low initial density treatments in Chapter 5 evolution occurred in most treatments but this evolution had no impact or a smaller average impact than the uncaged and the high initial density treatments. I argued that both the caged and low initial density treatments had reduced aphid competition, as was demonstrated by reduced population growth rates. Chapters 4 and 5 also revealed that stronger clonal competition leads to larger differences between clones in fitness. This could help explain why rapid evolution's impact is dependent on the ecological context.

Importance

My dissertation research has important implications for basic research in evolutionary-ecology and for many applied fields of biology. The potential importance of short-term eco-evolutionary dynamics is slowly being acknowledged. Although theoretical studies (see Chapter 1) have suggested such effects for years only recently has empirical support emerged. My dissertation pushes the boundaries of this nascent field in a number of ways and my results strongly support the integration of ecological and evolutionary dynamics on short timescales. I presented the first studies of eco-evolutionary dynamics using the experimental method, where evolution itself is manipulated, in the wild and also the first plant-herbivore study system (wild or greenhouse).

My novel studies have shown experimentally for the first time that rapid evolution significantly impacts concurrent population dynamics in the field in the face of environmental variation. I have also found large accelerations of population growth rate within species without strong selection impose by another species (no predator-prey dynamics). Rapid evolution thus significantly alters predicted population growth rate. This has important implications for our ability to predict population dynamics when ignoring evolution, which is the normative approach. My dissertation also found evidence that this ecological change caused by rapid evolution also impacts the selective environment thus leading to even more complex ecological and evolutionary outcomes. In many applied issues in biology, such as fisheries, pest management, invasive species and climate change, rapid evolution has been observed. My research suggests that these areas of research could potentially profit greatly from better understanding of how ecology and evolution interact on short time scales to better reach their objectives.