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Contemporary Evolution of Plant Growth Rate Following Experimental Removal of Herbivores

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abstract: Herbivores are credited with driving the evolutionary diversification of plant defensive strategies over macroevolutionary time. For this to be true, herbivores must also cause short-term evolution within plant populations, but few studies have experimentally tested this prediction. We addressed this gap using a long-term manipulative field experiment where exclosures protected 22 plant populations from natural rabbit herbivory for <1 to 26 years. We collected seeds of *Rumex acetosa* L. (Polygonaceae) from our plots and grew them in a common greenhouse environment to quantify evolved differences among populations in individual plant growth rate, tolerance to herbivory, competitive ability, and the concentration of secondary metabolites (tannins and oxalate) implicated in defense against herbivores. In 26 years without rabbit herbivory, plant growth rate decreased linearly by 30%. We argue that plant growth rate has evolved as a defense against intense rabbit herbivory. In contrast, we found no change in tolerance to herbivory or concentrations of secondary metabolites. We also found no change in competitive ability, suggesting that contemporary evolution may not feed back to alter ecological interactions within this plant community. Our results combined with those of other studies show that the evolution of gross morphological traits such as growth rate in response to herbivory may be common, which calls into question assumptions about some of the most popular theories of plant defense.

Keywords: community genetics, eco-evolutionary interactions, herbivory, plant competition, plant defenses, *Rumex acetosa*.

Introduction

Herbivores have fed on plants for more than 400 million years (Labandeira 2007), and these interactions are believed to have driven the diversification of the myriad defense traits observed among plants (Fraenkel 1959; Ehrlich and Raven 1964; Becerra 1997; Agrawal 2007; Walters 2011). Plant defenses are often classified as either resistance traits or tolerance traits, where the former reduce herbivore damage and the latter limit the negative fitness consequences of damage (Karban and Baldwin 1997; Strauss and Agrawal 1999). For herbivores to drive the evolution of plant defenses over macroevolutionary timescales, they must also drive the evolution of defenses over microevolutionary timescales (one to hundreds of generations). Numerous studies show that the ingredients necessary for this short-term adaptive evolution are ubiquitous: plants contain heritable variation for both resistance and tolerance (Berenbaum et al. 1986; Maddox and Root 1987; Núñez-Farfán et al. 2007), and herbivores impose selection on these traits (Rausher and Simms 1989; Marquis 1992; Stinchcombe et al. 2002; Agrawal 2005). Despite these important results, few studies have explicitly linked the presence or absence of herbivores to actual evolutionary responses within plant populations, and these studies are typically observational (Zangerl and Berenbaum 2005; Stenberg et al. 2006). Thus, our understanding of which plant traits will evolve due to the direct or indirect consequences of herbivory are limited. This represents a large gap in our understanding of the evolutionary ecology of plant-herbivore interactions (Agrawal 2011; Johnson 2011).

Several observational studies provide strong evidence that natural selection imposed by the presence or absence of herbivores drives the microevolution of plant defensive traits. Both Stenberg et al. (2006) and Zangerl and Berenbaum (2005) found a positive correlation between herbivory pressure and the level of plant defenses among plant populations, suggesting that the absence of herbivores drives the evolution of decreased defenses and the presence

of herbivores drives the evolution of increased defenses. Lennartsson (1997) found similar results with respect to tolerance to herbivory. The results from these observational studies are consistent with the prediction that the presence and absence of herbivores can impose natural selection on plant defense traits that cause evolutionary change over tens to hundreds of years. However, these results are not sufficient to conclusively implicate a role of herbivores in driving evolution as recent theory shows that such correlations based on observational data can result from multiple evolutionary processes, including those that do not involve selection from biotic agents (Nuismer et al. 2010).

Herbivores can drive the evolution of plant traits through the direct effects of consumption and through any indirect effects of herbivory that modify the abiotic or biotic environment (Miller and Travis 1996; Agrawal et al. 2006). This is especially true for interactions between plants and grazing vertebrate herbivores, which have large impacts on plant community structure and are in many ways fundamentally different from those involving arthropods (McNaughton 1984; Pollard 1992; Edwards and Crawley 1999). Grazing can impose selection directly on traits, such as plant palatability, morphology, and growth rate (McNaughton 1984), but also indirectly, for example, by influencing nutrient availability or by altering competitive hierarchies among plant species (Crawley 1983; Agrawal et al. 2006). Thus, predicting what traits will evolve in response to the presence or absence of herbivores is difficult. Studies that quantify the evolution of plant traits after experimental manipulation of herbivores in the field are our best hope in understanding what traits will actually evolve due to the direct and indirect effects of herbivory.

Evolution of plant traits in response to herbivores could feed back to affect the ecology of herbivores or other plants and animals in the community. While the link between contemporary evolution and ecological interactions and processes is an old one (Pimentel 1961, 1968; Ford 1964), recently there has been increasing recognition that evolution of one population can have cascading effects throughout communities and entire ecosystems (Abrams 2000; Whitham et al. 2006; Fussmann et al. 2007; Bassar et al. 2010). However, there are few experimental tests of whether evolution driven by herbivores causes changes to the outcome of ecological interactions among other members of the community (Agrawal et al. 2012). For example, if intense grazing by mammalian herbivores drives adaptive evolution for high growth rate, this evolutionary change could then influence the performance of other plant species in the community and potentially their ability to coexist with the evolving population.

The lowland acidic grasslands of Silwood Park in England are an excellent setting to test the evolutionary impacts of herbivores. In this system, rabbits crop plants closely to the ground, which prevents flowering and causes individual plants to be much smaller than when rabbits are absent (fig. 1). On average, grazing directly reduces average plant height in communities by 56% (ungrazed$_{mean}$ = 18 cm, grazed$_{mean}$ = 8 cm) and standing aboveground biomass by 25% (ungrazed$_{mean}$ = 80 g, grazed$_{mean}$ = 60 g dry weight per 0.125 m^2; M. J. Crawley, unpublished data). Excluding rabbit herbivores also has cascading effects on net primary productivity (measured as total aboveground biomass produced in a growing season) and nutrient availability in soil, which increase by ~60% and ~180%, respectively, following 14 years of rabbit exclusion (Olofsson et al. 2007). Furthermore, rabbits alter the composition of plant communities over time due to the direct effects of grazing and indirect effects mediated through competitors (Farrow 1917; Watt 1962; Crawley 1990). Specifically, some species (e.g., *Holcus lanatus*, *Senecio jacobaea*, and *Trifolium repens*) increase in relative abundance and other species (e.g., *Achillea millefolium*, *Vicia sativa*, and *Festuca rubra*) decrease in relative abundance in the presence of rabbit grazing (Crawley 2005). Experimental evidence suggests that increasing species are faster growing and more tolerant to repeated damage than decreasing species (Del-Val and Crawley 2005a). A third class of species (e.g., *Rumex acetosa*, *Stellaria graminea*, and *Veronica chamaedrys*) does not significantly change in abundance in the presence or absence of rabbit grazing (Crawley 2005). One possible explanation for why the abundance of these species remains stable through time is that they adapt to the altered environmental conditions (i.e., reduced herbivory, altered competitive environment, and increased nutrients) in the absence of rabbits.

We conducted a 26-year manipulative field experiment to test whether the presence/absence of rabbits drives evolutionary change in plant populations. Specifically, we asked two questions. First, we asked, Does the removal of herbivores lead to directional evolutionary change in plant growth, chemical defenses, or tolerance to herbivory? We expected that defensive traits (chemistry and tolerance) would be highest in populations that continually experienced intense rabbit herbivory and would steadily decline following the removal of rabbits, as would be predicted if defense were costly (Strauss et al. 2002; Núñez-Farfán et al. 2007). With respect to the evolution of growth rate, there are several alternative predictions. Classic plant defense theory predicts that growth rate and investment in chemical and physical defenses trade off (Coley et al. 1985; Herms and Mattson 1992). Thus, if populations evolve high levels of constitutive resistance in the presence of herbivores, we expect plants that experienced recent grazing pressure to exhibit the slowest growth rates. Similarly,

Figure 1: *A*, Rabbits feeding at Silwood Park, England. Photo credit: M. Johnson. *B*, Image of rabbit exclosure at Silwood Park. Rabbits are excluded from the left side of the fence, and plant populations are heavily grazed by rabbits on the right. Photo credit: N. Turley.

observed increases in soil nutrients, net primary productivity, and competition for light associated with rabbit removal (Olofsson et al. 2007) are predicted to select for increased growth (Grime and Hunt 1975). By contrast, if adaptation follows a similar pattern seen in community-level responses of species (Del-Val and Crawley 2005*a*), we expect fast-growing plant genotypes to decline following removal of rabbits and slow-growing genotypes to increase. Second, we asked, Does evolution of plant traits feed back to affect interspecific ecological interactions between plant competitors? We expected that the competitive ability of plants would increase following the removal of rabbits, as predicted by a trade-off between defenses and competitive ability (Herms and Mattson 1992), which could influence the performance and coexistence of competing plant species.

Methods

Study System

We conducted our experiment in old fields and meadows at Silwood Park, Imperial College, England (51.409°N, 0.642°W; fig. 1). Our study sites are species-poor grasslands on sandy, acidic soil with a mixture of monocots and eudicots (Crawley 1990). Rabbits (*Oryctolagus cuniculus*) have been abundant at Silwood Park since the 1950s but have been present in England for thousands of years (Farrow 1917; Edwards and Crawley 1999). Rabbits act as a keystone species that plays a dominant role in shaping vegetation dynamics of herbaceous and woody plant species, most dramatically by inhibiting the succession of grasslands into forest (Crawley 1997).

Between 1984 and 2009, 22 rabbit exclosures were

erected around local plant populations and maintained for 4 months to 26 years, including plots of intermediate age (detailed information about plots is provided in table A1, available online). The only manipulation made to exclosures was the exclusion of rabbits. To sample the evolutionary consequences of excluding rabbit herbivores, we collected seeds in 2009 from a haphazard selection of all fruiting plants of the perennial herb *Rumex acetosa* L. (Polygonaceae; common garden sorrel) within each exclosure. Despite being heavily grazed by rabbits, its relative abundance does not consistently change between grazed sites and ungrazed sites: inside rabbit exclosures, *R. acetosa* has a mean ground cover of 4.4% and outside exclosures 3.6% (SE = 3.0, $N = 1,002$; M. J. Crawley, unpublished data).

We define the plants from within each exclosure as a "population" even though gene flow could occur among plots as they all occur in an area of about 100 ha. This definition is justified by previous work in British grasslands and other systems that find evolutionary response and local adaptation at the scale of several meters (Antonovics and Bradshaw 1970; Snaydon and Davies 1972; Linhart and Grant 1996; Silvertown et al. 2006). The natural history of the system also suggests there would be limited gene flow among populations. In the Silwood rabbit-*Rumex* system and many other heavily grazed environments (Crawley et al. 2004), reproduction is largely due to clonal vegetative growth. Plants seldom if ever produce fruits in grazed populations, and although plants produce flowers and fruit in the absence of herbivores, there is limited opportunity for seed recruitment because of intense competition among perennials (Crawley 1990). Thus, evolution in this system is likely dominated by clonal lineage selection. We erected new exclusion plots in 2009 so that we could collect seeds to represent plants from the grazed treatment, because plants exposed to continuous grazing do not flower. These plots were smaller than the older plots and were placed around the grazed region to provide a random sample of the grazed population.

Greenhouse Experiment

In fall 2009, we germinated seeds collected from each rabbit exclosure and grew these plants (24 from each exclosure) in a greenhouse common environment at North Carolina State University. This cross-sectional design allowed us to test the evolutionary impacts of herbivores over time by comparing plants from exclosures of different ages in a common environment (Conner and Hartl 2004). In the field, most *R. acetosa* individuals would have originated from vegetative reproduction (Crawley 1990), and our study assumes that the phenotypic traits of our focal plants grown from seed would be representative of such plants.

Seeds were germinated on moist filter paper, which insured that all plants used in this experiment germinated at the same time and thus controlled for maternal effects mediated by differences in time to germination (Galloway 2001). After germination, seedlings were transferred to 1.3-L, 15.2-cm-diameter round pots containing a 50 : 50 sand : soil mixture (Fafard no. 2, Agawam, MA) supplemented with ~1.8 g slow-release fertilizer (Osmocote, N : P : K, 14 : 14 : 16, Scotts, Marysville, OH). Some plots were represented by fewer plants (range: 4–24; mean: 22) due to a limitation on the number of seeds available from exclosures.

Tolerance and Competitive Ability

To measure tolerance and competitive ability and to test whether the expression of plant traits was dependent upon environmental conditions, we manipulated damage and competition in a factorial design ([damaged vs. undamaged] × [with vs. without a competitor]) replicated six times for all 22 populations. Our damage treatment simulated natural rabbit grazing by removing all aboveground biomass with a razor blade to a height of 1 cm above the soil surface. This was done twice, 23 and 42 days after planting. Competition was manipulated by growing *R. acetosa* in the presence or absence of a single *Oenothera biennis* (Onagraceae) plant, planted in the same pot at a distance of 6.5 cm from the *R. acetosa* plant. *Oenothera biennis* was introduced to Europe from North America and commonly occurs at Silwood Park and the surrounding area. We used a single *O. biennis* genotype from North America as a phytometer to measure competitive ability. After 68 days, the above- and belowground biomass of all plants was harvested, dried, and weighed. We carefully rinsed the soil from the roots of both species; for *R. acetosa* we harvested all fine roots, and for *O. biennis* we harvested the main taproot. We measured tolerance using final plant biomass and calculated it as (damaged plant biomass − undamaged plant biomass)/undamaged plant biomass, according to the recommendations of Wise and Carr (2008). This relative measure of tolerance was preferred over an absolute measure of tolerance because plants varied in size, and simulated herbivory damage was manipulated in a relative fashion rather than an absolute amount removed for each plant (Wise and Carr 2008). Similarly, competitive ability was calculated as (plant biomass with competitor − plant biomass without competitor)/plant biomass without competitor. We also tested for competitive effects of *R. acetosa* on *O. biennis* by comparing the biomass of *O. biennis* plants grown with and without *R. acetosa*.

Chemical Defenses

To test for evolution in constitutive expression of defensive chemical compounds, we conducted a second greenhouse experiment in which we grew *R. acetosa* from seed in the same soil and greenhouse conditions as above. We harvested plants when they had ~12–14 true leaves and freeze-dried all leaf tissue (VirTis Genesis freeze dryer, SP Industries, Warminster, PA) prior to chemical extraction.

We measured concentrations of two chemicals predicted to play an important role in resistance: oxalates and soluble protein-binding tannins. Oxalate is known to be a dominant secondary metabolite in *Rumex* leaves (Libert and Franceschi 1987) that binds calcium ions to form indigestible crystals that serve as a defense against vertebrate and invertebrate herbivores (Franceschi and Nakata 2005). Tannins are also an abundant secondary metabolite in plants that bind to proteins (Feeny 1970; Hagerman 1987) and reduce the digestibility of plant matter, especially in mammals (Robbins et al. 1987; Salminen and Karonen 2011).

We measured leaf tannin concentration using a radial diffusion assay that quantifies the concentration of tannins according to the protein-binding activity of extracted soluble tannins from leaves (Hagerman 1987). We weighed 100 mg of tissue per plant, ground it into a fine powder using a tissue homogenizer, and extracted the sample three times in 70% acetone at room temperature. We then evaporated the acetone using a vacuum centrifuge and redissolved the extract in deionized water. The subsequent methods followed Hagerman (1987). Briefly, we made a 1% agarose solution with 0.1% bovine serum albumin and added 10 mL to a 9-cm petri dish. Once the gel set we punched a 4-mm hole into the center of the gel and slowly added 8 μL of tannin extract. These assays were then incubated at 30°C for 2 days, at which time we measured the diameter of precipitate rings using digital calipers.

We measured oxalates according to the methods of Chamjangali et al. (2009) by combining 20 mg of dried homogenized tissue and 1.5 mL of deionized water in a microcentrifuge tube that was then heated in a water bath at 85°C for 50 min. The sample was then spun down in a centrifuge and a small amount of the supernatant was removed to make a 0.3% dilution with deionized water. In a 96-well plate, 240 μL of 2.125×10^{-5} M crystal violet solution was added to all wells, followed by 30 μL of diluted oxalate samples. To start the reaction, we added 30 μL of a 0.40-M H_2SO_4 and 0.004-M $K_2Cr_2O_7$ solution and measured absorbance at 630 nm after 4 min (PowerWave X model UPRWIE, BioTek, Winooski, VT).

Maternal Effects

We tested for differences in maternal investment in seed resources by weighing 50 seeds from each exclosure. We saw no relationship between seed mass and age of exclosure ($t = -1.1$, df $= 12$, $P = .3$).

Statistical Analyses

We used regression analyses to test how plant traits evolved through time following the removal of rabbits. These models, one for each of the five traits measured, allowed us to test whether exclosure age (i.e., time since herbivory) was associated with variation in growth rate (calculated as total final biomass accumulation divided by days of growth), tolerance to herbivory, competitive ability, and concentration of tannins and oxalate. In these analyses, a significant relationship between exclosure age and a plant trait provides evidence that the absence of rabbit grazing, either directly or indirectly, was associated with the evolution of the plant trait.

For *Rumex acetosa* growth rate data, we also fitted a linear mixed-effects model in R using the nlme package (R Development Core Team 2009). The full details and results of the model are provided in appendix A, available online. Briefly, we tested the main effects of exclosure age, damage treatment, competition treatment, and evolution of tolerance to competitors and damage using the interactions between exclosure age and damage treatment, as well as exclosure age and competition treatment. We tested the significance of parameters using likelihood ratio tests (LRTs) between alternate models, following Crawley (2007). Because the results of these models were in concordance with simpler linear regression analyses, we focus on the results from the latter but also provide results to the former (table A2, available online). For all models we analyzed both above- and belowground biomass, but because these two responses showed qualitatively similar results (data not shown), we present only results of total biomass here. We tested for trade-offs among plant traits using pairwise correlations between traits and estimating Pearson's product moment correlation coefficient (*r*). Individual data points, as with previous analyses, were averages of multiple plants collected from a single exclosure.

We calculated evolutionary rates of phenotypic change following the methods of Kinnison and Hendry (2001). Specifically, evolutionary rates were estimated as Haldanes, which quantifies mean phenotypic change in a population per year in standard deviation units. These calculations used predicted means for the oldest and youngest plots, estimated from the linear regression models, pooled standard deviation across all plot means, and a generation time of 1 year because *R. acetosa* are capable of reproducing by
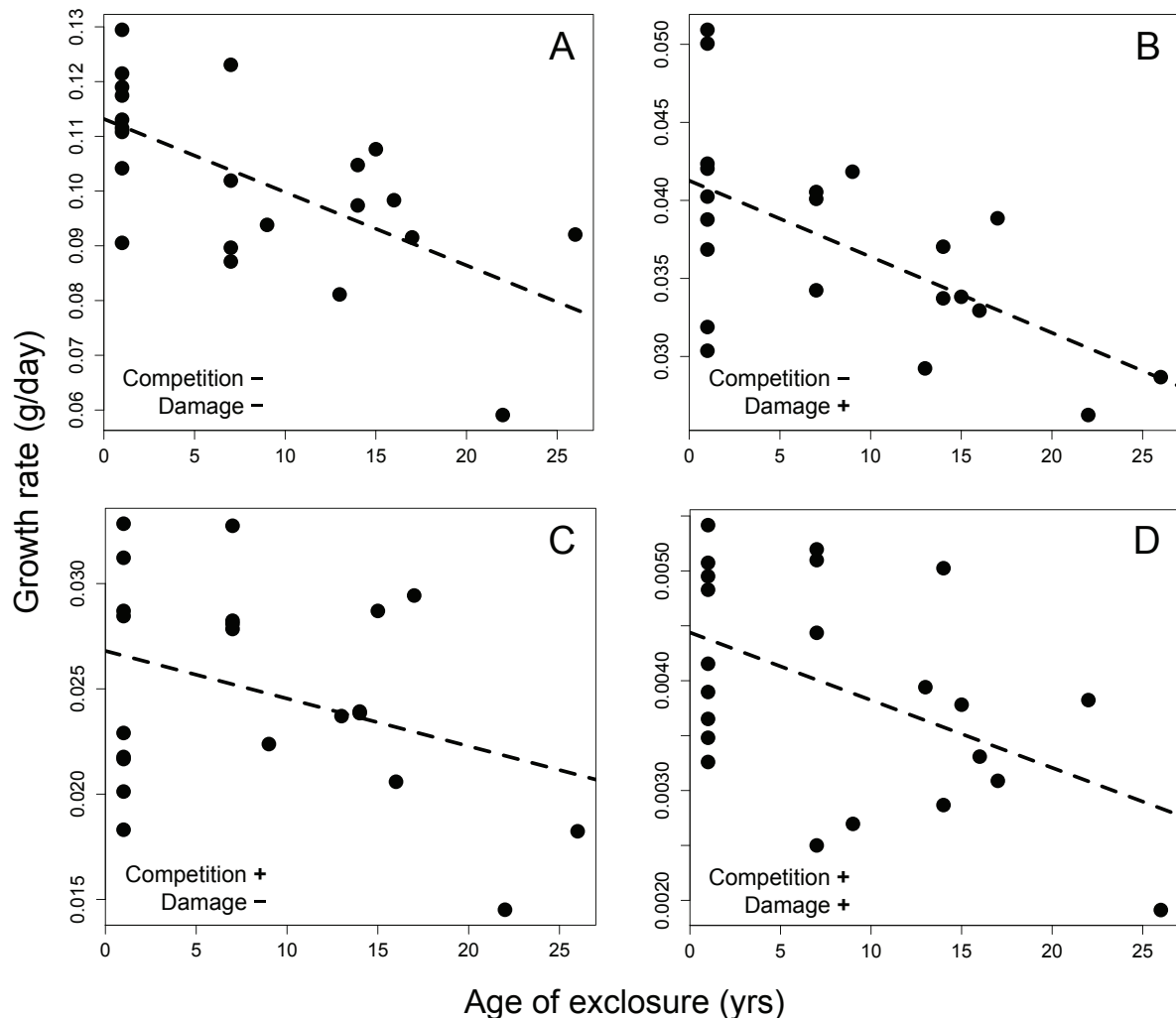
Figure 2: Growth rate (total above- and belowground biomass accumulation divided by 68 days) of *Rumex acetosa* populations grown in a common environment. Each panel represents a unique treatment combination: no competitor and no damage (*A*), no competitor and damage (*B*), competitor and no damage (*C*), and competitor and damage (*D*). Each dot is an average of one to six plants.

seed and vegetatively within one growing season. In cases where *R. acetosa* individuals persist for multiple years, our estimates of evolutionary rates are conservative. We also estimated evolutionary rates in Darwins, which quantifies proportional change in phenotype per millions of years. Evolutionary rates for all traits measured are presented in appendix A.

Results

When populations were compared in a greenhouse common environment, our results clearly show that the absence of intense rabbit grazing leads to evolution of decreased plant growth rate. Specifically, exclosure age was negatively related to intrinsic plant growth rate, and this result was consistent across damage and competition treatments (fig. 2). In 26 years without rabbit herbivory, growth rate declined linearly by 30% in undamaged plants with no competitors ($t = -3.7$, df $= 20$, $P = .001$), 30% in damaged plants with no competitors ($t = -3.3$, df $= 19$, $P = .004$), and 35% in damaged plants with competitors ($t = -2.5$, df $= 20$, $P = .02$). There was also a 21% decline in growth rate of undamaged plants grown with competitors, although this trend was not significant ($t = -1.66$, df $= 20$, $P = .1$). Across treatments, the maximum rate of evolutionary change in growth rate was 0.08 Haldanes. In other words, growth rate decreased by at least 0.08 SD per year during the quarter-century length

of our manipulations. Our fastest observed rates of evolution were not above average when compared to studies that measure evolutionary rates in plants (Bone and Farres 2001).

Surprisingly, the exclusion of herbivores did not result in directional evolution of chemical defense, tolerance to herbivory, or competitive ability (fig. 3). Specifically, the concentration of protein-binding tannins ($t = -0.8$, df $= 13$, $P = .4$) and oxalate ($t = 0.3$, df $= 11$, $P = .8$) in leaf tissue were not related to the age of exclosures. Our damage treatment reduced plant biomass by 68% (LRT, $\chi^2 = 669.1$, df $= 1$, $P < .001$, fig. A1, available online),

but the age of exclosures did not influence tolerance to herbivory ($t = 0.2$, df $= 19$, $P = .9$). Consistent with these results, the evolutionary rate of change in secondary chemistry and tolerance were near zero (table A4, available online).

Despite clear evolution in plant growth rate, there was also no evolutionary change in competitive ability. *Oenothera biennis* reduced *Rumex acetosa* biomass by 80% (LRT, $\chi^2 = 857.4$, df $= 1$, $P < .001$; fig. A1), but this competitive effect was not altered by the age of exclosures in which *R. acetosa* originated ($t = 0.7$, df $= 20$, $P = .5$). Competition was asymmetric, as the presence of *R. acetosa*
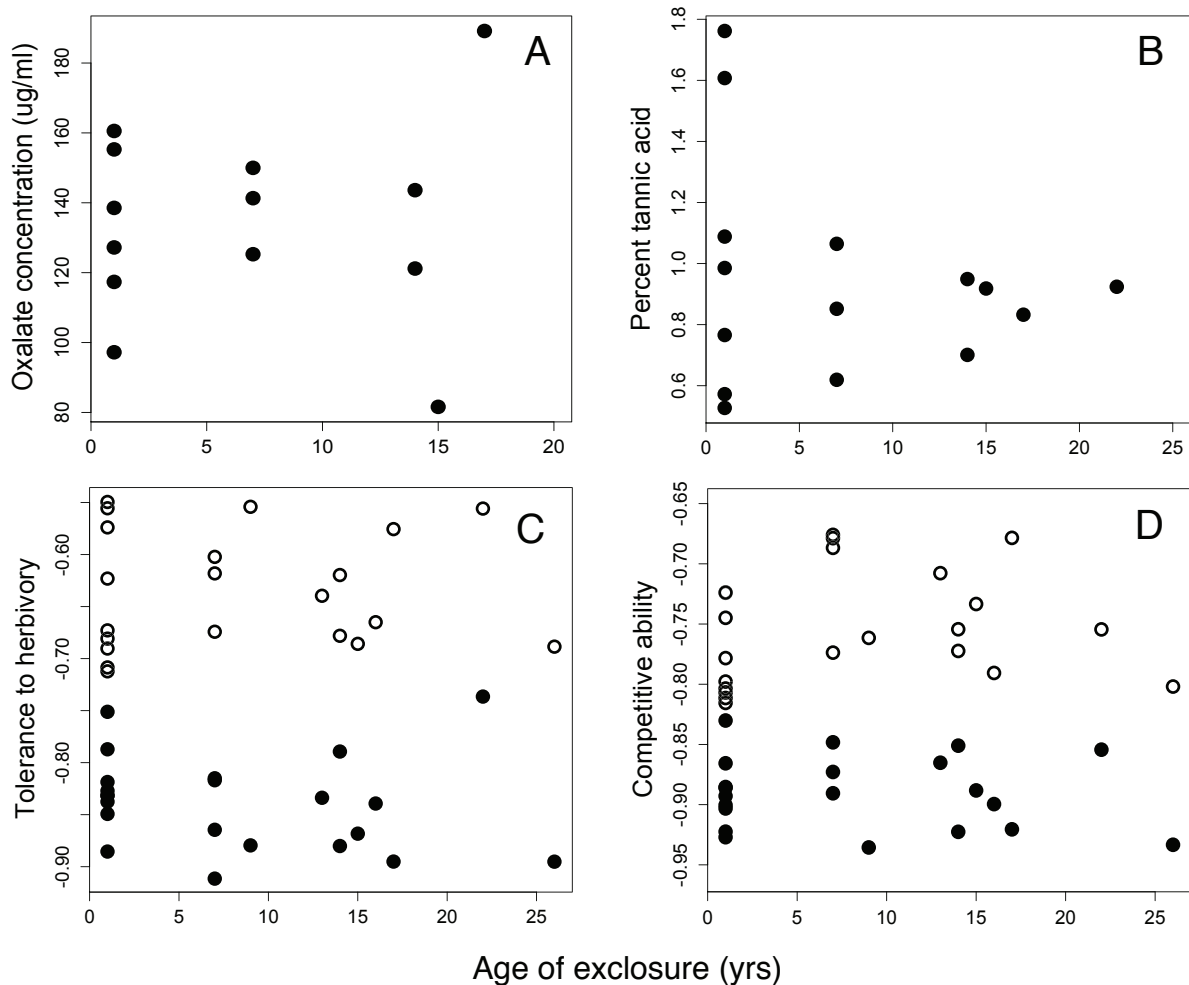

Figure 3: Traits of *Rumex acetosa* as a function of time since rabbit herbivory. Oxalate concentration is expressed as micrograms of oxalate per milligram of dried leaf tissue (*A*). Tannin concentration is the percent of protein-binding phenolics in extract (*B*). Tolerance to herbivory is defined as the relative difference in biomass between damaged and undamaged plants, where filled and open circles represent plants with and without competitors, respectively (*C*). Competitive ability is defined as the relative difference in biomass between plants with and without competitors, with filled and open circles showing damaged and undamaged plants, respectively (*D*). No significant relationships with age of exclosures were found among plots on all traits shown.

did not affect the biomass of *O. biennis* (*t*-test, $t = 0.4$, df $= 63$, $P = .7$). These results for tolerance are unchanged by the presence or absence of competitors, and similarly the results for competitive ability are the same in the presence or absence of damage (fig. 3). Also, we find the same patterns with the mixed-effects model; the interactions of exclosure age × damage treatment (LRT, $\chi^2 = 0.04$, df $= 1$, $P = .8$) and exclosure age × competition (LRT, $\chi^2 = 1.2$, df $= 1$, $P = .3$) were not significant. The full results of the mixed-effects model are available in table A2.

We tested for predicted trade-offs in defensive strategies (Coley et al. 1985; Van Der Meijden et al. 1988; Herms and Mattson 1992) by correlating population mean values for the traits measured. We observed a negative correlation between plant intrinsic growth rate and tolerance to herbivory (Pearson's correlation: $r = -0.44$, df $= 19$, $P = .04$; fig. A2, available online). By contrast, neither the correlations between growth rate and secondary metabolites (tannins and oxalate concentrations) nor the correlations between competitive ability and tolerance to herbivory were significant ($P > .1$). The results for all trait correlations are available in table A3, available online.

Discussion

Our long-term field experiment clearly shows that plant populations evolve following the exclusion of rabbit grazing. Our results are surprising because only intrinsic plant growth rate responded to the removal of herbivores, whereas traditional plant defenses such as the expression of secondary metabolites and tolerance to damage remained unchanged. These results support three hypotheses on the evolutionary ecology of plant-herbivore interactions: (1) gross morphological and life-history plant traits can evolve in response to selection imposed by herbivores (McNaughton 1984), (2) the evolutionary link between herbivores and plant chemical resistance traits is not always strong (Carmona et al. 2011), and (3) plants can adapt over short time periods in response to the presence or absence of herbivores (Bossdorf et al. 2005; Zangerl and Berenbaum 2005; Thompson 1998; Stenberg et al. 2006). In examining whether evolution influences ecological dynamics with other species (Abrams and Matsuda 1997; Fussmann et al. 2007; Pelletier et al. 2009), we found no evidence in support of the prediction that evolution in plant populations feeds back to affect the ecology of competitive interactions between plant species.

Evolution of Plant Growth Rate

The observed decline in growth rate following the removal of rabbits (fig. 2) suggests that high intrinsic plant growth rate is adaptive in grazed populations and decreased growth rate is adaptive in the environmental conditions without rabbits. One reason why high growth could be adaptive in grazed populations is that growth is a defensive strategy to repeated damage. Intrinsic growth rate is not traditionally classified as a defensive trait, defined here as any trait that increases plant fitness in the presence of herbivores (Karban and Baldwin 1997). Growth rate is sometimes equated with tolerance, but this is typically in the context of an induced compensatory growth response following herbivory (Rosenthal and Kotanen 1994; Strauss and Agrawal 1999), whereas we observed an evolutionary change in the intrinsic (noninduced) growth rate of plants. Thus, we suggest that high growth rate is serving as a defensive trait itself but not by means of increasing tolerance to herbivory. With intense and repeated grazing, rapid growth enables plants to continually replace removed tissue. By countering the continuous loss of tissue to herbivores with growth, fast-growing genotypes would maintain sufficient biomass to prevent being driven to local extinction (Farrow 1917; Hilbert et al. 1981; Jefferies 1988). Intense grazing would cause slow-growing genotypes to become less common while fast-growing genotypes would persist, thus maintaining increased average growth rates in grazed populations, as we observed.

The evolution of decreased growth rate following removal of herbivores implies a cost of rapid growth in environments without rabbit grazing. One prediction is that the increased nutrient availability and primary productivity in environments without rabbits (Olofsson et al. 2007) selects for increased growth; however, our results are opposite of this prediction. Following the exclusion of rabbits, we believe that selection shifted to favor slow-growing genotypes, which subsequently increased in frequency. The reason for this shift in selection within the exclosures is unclear, but the simplest explanation is that rapid growth was costly in the absence of rabbits, as observed with many other plant defenses (Strauss et al. 2002; Núñez-Farfán et al. 2007). Alternatively, a nonmutually exclusive explanation is that changes in competitive interactions among plants or, in other words, indirect effects of rabbit exclusion on the competitive environment, selected for decreased growth rate. Competition is an important factor shaping plant community composition in this system, and the nature of competitive interactions depends on herbivore pressure (Crawley 1990; Del-Val and Crawley 2005b).

The observed evolution in *Rumex acetosa* occurred at small spatial scales, implying that selection by herbivores (or, rather, their absence) was very localized. Because most reproduction by *Rumex* in this system is through clonal vegetative propagation (Crawley 1990), gene flow among exclosures would be minimal, allowing local adaptation to

occur at a very local scale with minimal counteracting effects of gene flow through pollen or seeds. Local adaptation of plant populations at small spatial scales and over short timescales has been observed in many British grassland communities (Antonovics and Bradshaw 1970; Snaydon and Davies 1972; Silvertown et al. 2006) and in plant systems more generally (Linhart and Grant 1996; Bone and Farres 2001) but more typically in response to variation in the abiotic environment.

Among studies that manipulate the presence of vertebrate herbivores, evolution of growth rate appears to be common, but the direction of the response is inconsistent. Through an extensive literature search, we found 12 studies that excluded vertebrate herbivores and measured the phenotypes of grasses (Poaceae) from grazed and ungrazed populations in common gardens (table 1). The direction of change in growth rate following removal of herbivores was mixed, with 40% increasing, 40% decreasing, and 20% showing no change. Increased growth rate in the presence of grazers and subsequent decline after their exclusion could be adaptive in the ways described above. The reverse pattern also has a number of possible explanations. For example, if herbivores drive evolution of increased defenses, a trade-off between chemical defense and growth could lead to the evolution of decreased growth rate in the presence of herbivores (Herms and Mattson 1992; Stenberg et al. 2006). Increased growth rate following herbivore exclusion could also be due to a trade-off between defense and competitive ability, if the competitive environment changes in the absence of herbivores. It should be noted that predicting evolutionary effects of herbivory in a community context becomes difficult, and this is es-

pecially true when the herbivores are generalist grazers (Crawley 1983; Del-Val and Crawley 2005b; Agrawal et al. 2006). For example, the effects of herbivory on the competitive interactions between plants can strengthen, reverse, or have no effect on competitive hierarchies depending on herbivore preference, plant tolerance, and density-dependent effects (Crawley 1983; Agrawal et al. 2006). Therefore, the complexity of direct and indirect effects in plant-herbivore and plant-plant interactions provides an abundance of mechanisms for herbivores driving both increased and decreased growth rate, which manifest themselves in empirical results (table 1).

Chemical Resistance Traits and Tolerance

We found no evidence that the presence or absence of grazing herbivores causes evolution in chemical defenses or tolerance to herbivory (fig. 3). There are several possible reasons why there was no evolutionary response. First, there could have been a lack of genetic variation in chemical resistance and tolerance traits (Westoby 1989), although the large variation in these traits within (data not shown) and among (fig. 3) populations suggests that this is not the case. Second, there was no selection on these traits. For example, rabbits may not be affected by variation in oxalate concentration. Alternatively, selection might have been too weak to counteract the effects of genetic drift or gene flow within the small population or to cause measurable evolutionary change in the time frame of this experiment.

The evolution of tolerance could also be limited by constraints caused by evolution of growth rate. Intrinsic

Table 1: Results from a literature review of studies that use common garden techniques to measure evolutionary change in plant populations following the experimental removal of vertebrate herbivores

Reference	Herbivore	Growth rate	Competitive ability	Tolerance
Carman and Briske 1985	Cattle	0	NA	NA
Polley and Detling 1990	Prairie dog	0	NA	0
Tomas et al. 2000	Cattle	0	NA	NA
This study	Rabbit	−	0	0
Briske and Anderson 1992	Cattle	−	−	NA
Jefferies 1988	Goose	−	NA	NA
Loreti et al. 2001	Cattle	−	NA	0
Polley and Detling 1988	Prairie dog	−	NA	NA
Detling and Painter 1983	Prairie dog	+	NA	−
Jaindl et al. 1994	Cattle	+	NA	0
Jaramillo and Detling 1988	Prairie dog	+	0	0
Painter et al. 1989	Prairie dog	+	+	−
Painter et al. 1989	Prairie dog	+	0	−

Note: All studies (except our own) used grasses (Poaceae). The table indicates the direction of trait change following the removal of herbivores, where 0 indicates no change, plus and minus indicate increases and decreases in traits, respectively, and NA denotes no data.

growth rate does not necessarily correlate with a plant's tolerance as commonly defined in the evolutionary ecology literature (Strauss and Agrawal 1999), and paradoxically, faster-growing plants can be less tolerant (Weis et al. 2000). Indeed, we found a significant negative correlation between growth rate and tolerance to herbivory (fig. A2, available online). Therefore, evolution of tolerance could be constrained by changes in growth rate, and this may be one reason why there was no evolution in tolerance (Weis et al. 2000). In this system, Del-Val and Crawley (2005*a*) found that species that increase in abundance in the presence of rabbits were faster growing and also more tolerant to multiple clippings. Thus, our intraspecific result of fast-growing genotypes increasing in the presence of grazing matches the interspecific results in the system.

Maternal Effects

While our experimental design does not allow us to completely rule out a role of maternal effects in shaping the observed pattern in growth, this explanation is unlikely for two reasons. First, maternal effects on plant growth and defense are typically short-lived, having notable effects only on early seedling stages of growth (Stanton 1984; Gross and Kromer 1986; Agrawal 2002). Second, long-term maternal effects are often mediated through germination timing (Gross and Kromer 1986; Galloway 2001), which we controlled for to preclude such effects. Therefore, we argue that evolution is the best plausible explanation for the observed change in growth rate (fig. 2).

Implications for Plant Defense Theory

If plant growth rate does commonly act as a defense against herbivores, as suggested by our data and accompanying review of the literature, this calls into question a fundamental assumption of the most popular plant defense theory, the resource availability hypothesis (RAH; Coley et al. 1985; Endara and Coley 2011). RAH predicts that plant populations evolve to optimize individual plant growth rate according to the availability of resources in the environment, which then constrains allocation to plant defenses, affecting the types and levels of optimal defense investment. The simplest predicted pattern from this model is a negative relationship between plant growth rate and investment to constitutively expressed defenses, which we did not observe (table A3, available online). However, RAH and its predictions are based on the assumption that growth rate is an adaptation to the availability of resources in the physical environment and does not serve as a defense itself. Although recent experimental and meta-analysis studies find support for RAH (Fine et al. 2004; Van Zandt 2007; Endara and Coley 2011), perhaps systems that violate

this assumption (such as our study) could explain why some recent comparative phylogenetic studies find patterns that are inconsistent with RAH (Agrawal and Fishbein 2008; Kempel et al. 2011). If grazing herbivores select for increased growth rate, RAH would paradoxically predict that herbivores would drive the evolution of decreased resistance. Whether this is a common pattern is yet to be seen.

Ecological Consequences of Microevolution

Interest in the ecological importance of evolutionary dynamics has exploded in recent years, yet there exist few empirical studies that follow microevolutionary changes within natural populations and attempt to determine their ecological effects (Strauss et al. 2008; Bassar et al. 2010; Hersch-Green et al. 2011; Schoener 2011). Despite the observed contemporary evolution in plant growth rate, we found no evidence for evolution of competitive ability in *R. acetosa* (fig. 3). Specifically, evolution of *R. acetosa* did not affect its ability to tolerate the presence of a heterospecific competitor, nor did evolution in growth rate affect the performance of the competing plant species. Therefore, we find no evidence that evolution in *R. acetosa* influences its ability to coexist with other members in the community. It is possible that differences in competitive ability would manifest themselves when in competition with a plant genotype or species that is a weaker competitor than the genotype of *O. biennis* we used or, in longer experiments, where crowding and light competition might play a larger role. In the few studies that have measured the evolution of plant competitive ability in response to vertebrate herbivores, the results are variable, but there are too few studies to make clear generalizations (table 1). While it is possible that selection from herbivores could drive plant evolution that would then have cascading effects on a larger community (Lankau and Strauss 2007; Johnson et al. 2009; Genung et al. 2011), there remains limited empirical evidence for such processes.

Our understanding of when microevolution will influence ecological processes more generally is very limited (Hairston et al. 2005; Thompson 2009; Hersch-Green et al. 2011). It is predicted that evolution in dominant species and ecosystem engineers will be of greatest ecological importance (Whitham et al. 2006; Post and Palkovacs 2009). But, predicting what species will play important roles in shaping communities and ecosystems is notoriously difficult (Power et al. 1996), and it is currently unclear whether the ecological effect size resulting from adding or removing a species is correlated with the ecological effect size resulting from evolution in that species. The best evidence for rapid evolution impacting ecological processes in communities comes from microcosm and chemostat

experiments involving very simple communities with specialized species interactions (Pimentel 1968; Bohannan and Lenski 2000; Yoshida et al. 2003). However, in communities where niches are occupied by many similar species, theory predicts that environmental changes are more likely to cause shifts in community composition than evolution within species (de Mazancourt et al. 2008). In our system, for example, different ecological manipulations cause dramatic shifts in plant community composition (Crawley 2005; Allan and Crawley 2011). However, evolution may still play a role in this context by broadening the niche space of each species, resulting in less community change in response to changing environmental conditions than would be expected without genetic variation and adaptation. A similar idea is supported in a grassland mesocosm experiment where genetic diversity and evolution within multiple co-occurring species helped maintain species diversity over time (Booth and Grime 2003; Whitlock et al. 2007).

Conclusions

We show that the removal of rabbits drives the evolution of plants over short timescales. These results suggest that plant growth rate can be an important defensive strategy against grazing herbivores. When growth rate itself evolves as an adaptive defensive trait, a key assumption of the resource availability hypothesis (Coley et al. 1985) is violated, and this observation may explain why there has been contradictory support for this hypothesis. Despite contemporary evolution in plant growth rate, this evolution has no clear effect on the performance of plant competitors and the ability of our focal species to coexist with competing plant species.

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