

LETTER

Herbivory enhances positive effects of plant genotypic diversity

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Abstract

Both plant diversity and vertebrate herbivores can impact plant fitness and ecosystem functioning, however their interactions have not been explicitly tested. We manipulated plant genotypic diversity of the native plant *Oenothera biennis* and monitored its survivorship and lifetime fitness with and without one of its major vertebrate consumers, white-tailed deer *Odocoileus virginianus*. Intense but unmanipulated herbivory by meadow voles *Microtus pennsylvanicus* killed over 70% of nearly 4000 experimental plants. However, plants grown in genotypically diverse patches suffered fewer vole attacks and had higher survival and reproductive output than plants in monoculture. Moreover, positive effects of genotypic diversity were enhanced by the presence of deer, indicating a non-additive interaction between diversity and trophic-level complexity. Genetic selection analyses showed that the selective value of ecologically important traits depended on plant diversity and exposure to deer, demonstrating that community complexity can promote fitness through multiple ecologically and evolutionarily important feedbacks.

Keywords

Biodiversity and ecosystem functioning, community genetics, diffuse selection, genetic diversity and herbivory, *Oenothera biennis*, phenotypic plasticity, plant–insect interactions.

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INTRODUCTION

The top-down effects of consumers are integral to the structure and functioning of ecosystems (Duffy 2002). Only recently, however, have biodiversity–ecosystem functioning studies been expanded to include multitrophic interactions. Theory and limited empirical evidence indicate that the sign, magnitude and mechanism of diversity effects at lower trophic levels (i.e. ‘horizontal diversity’) can be altered by the presence of higher trophic levels (i.e. ‘vertical diversity’, Thebault & Loreau 2003; Gamfeldt *et al.* 2005; Duffy *et al.* 2007). Herbivores, for example, can alter diversity–function relationships by changing the composition of producer communities (Mulder *et al.* 1999; Gamfeldt *et al.* 2005). Few studies to date, however, have simultaneously manipulated producer diversity and trophic complexity, limiting our ability to understand how diversity at multiple trophic levels influences ecological processes.

Recent studies suggest that an often-overlooked component of biological diversity, intraspecific genetic diversity, can have wide-ranging impacts on the structure, functioning and persistence of ecological communities (Hughes *et al.*

2008). Genetically diverse plant communities, for example, are more productive (Crutsinger *et al.* 2006), support more diverse animal communities (Wimp *et al.* 2004; Crutsinger *et al.* 2006; Johnson *et al.* 2006), and are more resistant to disturbance than communities with lower genetic diversity (Hughes & Stachowicz 2004; Reusch *et al.* 2005; Crutsinger *et al.* 2008). The top–down effects of herbivores have been commonly implicated in these effects (e.g. Hughes & Stachowicz 2004; Wimp *et al.* 2004; Johnson *et al.* 2006), but explicit manipulations of genotypic diversity and herbivory are lacking.

Genotypic diversity is the raw material for evolution by natural selection, and thus manipulations of intraspecific diversity may have both ecological and evolutionary implications (Antonovics 1992; Whitham *et al.* 2006). For example, the ecological effects of biological diversity at any level depend on phenotypic variation among members of diverse communities (Hughes *et al.* 2008), and natural selection occurs when phenotypes are heritable and result in fitness differences among genotypes. Among plants, two chief forces of natural selection on plant traits are competition with neighbouring plants and consumption by

natural enemies (Gurevitch *et al.* 2000). Competition and enemies, however, generally select for opposing suites of traits due to physiological and ecological trade-offs among traits related to growth vs. defence (Agrawal *et al.* 2006). These effects may also be interactive, including when the identity of plants and herbivores in a given neighbourhood directly and indirectly alters the likelihood and strength of their interactions (e.g. 'associational effects', Gurevitch *et al.* 2000; Agrawal *et al.* 2006). This suggests that the ecological and evolutionary consequences of genotypic diversity will be context-dependent and altered by increasing community complexity.

We conducted a field experiment with 476 experimental diversity patches to examine the ecological and evolutionary effects of plant genotypic diversity and herbivory on the lifetime fitness (i.e. total reproductive output) of the native plant *Oenothera biennis*. Plant genotypic richness and herbivory were manipulated by planting either eight different or eight identical genotypes in patches exposed to or protected from herbivory by white-tailed deer, *Odocoileus virginianus*. We also monitored plant damage and mortality resulting from unmanipulated herbivory by meadow voles, *Microtus pennsylvanicus*. Genetic selection analyses examined whether increased genetic diversity and deer herbivory altered patterns of natural selection on nine growth and defence-related traits.

MATERIALS AND METHODS

Study organisms

Evening primrose (*O. biennis* L., Onagraceae) is an herbaceous annual or biennial old-field plant found in patches typically ranging from 1 to 14 genotypes (Johnson 2007). Because it is primarily self-pollinating and exhibits a permanent translocation heterozygote genetic system, maternal plants produce seeds which are genetically identical to the parent (Cleland 1972). This functional asexuality makes it an ideal system to investigate the impacts of intraspecific genetic diversity, and previous work with *O. biennis* indicates high genotypic variability for many traits related to growth and resistance to insect herbivores (Johnson & Agrawal 2005, 2007; Johnson *et al.* 2009). *Oenothera biennis* is also palatable to and consumed by vertebrate herbivores including white-tailed deer *Odocoileus virginianus* and meadow voles *M. pennsylvanicus*.

Experimental design

Seeds were collected from 20 distinct *O. biennis* genotypes (distinguished by microsatellites) from separate populations around Ithaca, NY (Larson *et al.* 2008; Johnson *et al.* 2009), grown for 60 days in a temperature- and light-controlled

glasshouse, and then hardened off in cold frames for 30 days prior to out-planting into a 2-ha old-field. We manipulated plant genotypic richness by planting 480, 40-cm diameter patches with either eight identical or eight different genotypes, resulting in 240 'monocultures' and 240 'polycultures', respectively, with 5 m separating each patch in a randomized grid design. For monocultures, each of the 20 genotypes was replicated 12 times. Polyculture patches were created by randomly selecting combinations without replacement from the pool of 20 genotypes. The natural vegetation in the field was not disturbed. To manipulate deer herbivory, we placed 1-m² cages constructed of 1.75-m tall DuPont[®], E. I. du Pont de Nemours and Company, Wilmington, DE, USA Deer Netting attached to four bamboo support poles around half the patches in each genetic diversity treatment. Our final experimental design included 476 patches containing 3808 plants. All plants were watered as needed for the initial three weeks of the experiment.

During the growing season, plants were monitored periodically for survival, date of bolting and evidence of deer browsing. Shortly after planting, we observed unexpected but intense vole herbivory occurring throughout the field that resulted in significant plant mortality (see Section 'Results'). To determine if vole attacks differed across our deer exclusion and genetic diversity treatments, *c.* 3 weeks after planting we scored dead and alive plants for the presence or absence of vole damage, evident as girdled stems or excavations leading to consumed roots.

We harvested the fruits from reproductive plants in late September after the first frost. In the laboratory, we quantified the total number of fruits per plant and the number of fruits eaten by the specialist seed predators *Mompha brevivitella* and *Schinia florida*, Lepidoptera. Consumption by *S. florida* was evident as large, irregular cavities and quantified as the percentage of each fruit missing. Consumption by *M. brevivitella* was evident as small bore holes and quantified as 20% loss per fruit for each individually penetrated fruit capsule (M.T.J. Johnson and A.A. Agrawal, unpublished data). The number of fruits per plant was then converted to the number of seeds per plant by gravimetrically determining the number of seeds per fruit for three to seven randomly selected fruits from each genotype in each treatment. The lifetime fitness for each plant was then estimated as the number of unconsumed seeds per plant.

Measurement of plant growth and resistance traits

For each genotype we measured nine plant traits commonly associated with either tolerance or resistance to herbivores, including plant growth rate, stem diameter, root:shoot ratio, bolting phenology, leaf toughness, foliar trichome density, specific leaf area and total phenolics of both root and shoot

tissues. All traits except for root:shoot ratio, growth rate and phenolics (which we measured in a separate experiment because they required destructive sampling) were collected in August from plants growing in polycultures in both uncaged and caged treatments (four to five replicate plants for each genotype in each treatment). We measured traits in both the caged and uncaged treatments because vertebrate browsing can induce changes in plant growth and resistance traits (Gómez & González 2007). Stem diameter was measured with callipers at the soil surface. Leaf toughness was measured as the grams of force required to pierce a fully expanded leaf below the inflorescence using a force gauge penetrometer (Type 516; Chatillon, Largo, FL, USA). For each leaf we used an average of two piercings, one from each side of the mid-rib. Foliar trichome density was calculated by counting the trichomes on the tops and bottoms of leaf discs (28 mm²) under a dissection microscope. Specific leaf area was calculated as the area of a leaf disc (28 mm²) divided by its dry mass. Bolting phenology (i.e. the number days until a rosette bolted in preparation for flowering) was measured during the course of the experiment in both diversity treatments and across both herbivore treatments.

Plant growth rate, root:shoot ratio and total phenolics of root and leaf tissues required destructive sampling of whole plants and were measured on four to six replicate plants of each genotype grown on an open-air rooftop patio on the Cornell campus. Plants were grown and harvested during the same time period that we collected traits from the experimental site. Plant growth rate was calculated as the dry biomass of washed roots plus shoot tissues divided by the number of days the plant had been alive. The ratio of below to aboveground tissue biomass was calculated using the dry mass of each tissue type. Total phenolics of root and shoot tissues were measured by HPLC with a diode array detector following the methods of Johnson *et al.* (2009).

Statistical analysis

Treatment effects on plant fitness

We assessed treatment effects at the patch and genotypic levels. At the patch level, we analysed whether the number of surviving plants in each patch over time was influenced by genotypic diversity, deer exclusion and their interaction, with a repeated measures factorial ANOVA in Proc Mixed of SAS (SAS Institute, Cary, NC, USA). Data best fit a lognormal distribution and thus were $\ln + 1$ transformed prior to analysis. We used a factorial two-way ANOVA with the fixed factors plant genotypic diversity, deer exclusion and their interaction, to determine the effects of diversity and deer on the number of vole-damaged plants per patch, the number of seeds per patch at the end of the experiment and the percentage seeds per patch lost to specialist insects.

Seed output data were log transformed to improve heteroscedasticity. We also used a one-way ANOVA to determine if the mean onset of deer browsing (uncaged treatments only) differed by genotypic environment.

To examine the mechanisms behind the effects of diversity on plant fitness, we partitioned the net biodiversity effects on the number of seeds per patch into components of complementarity and selection using standard techniques (Loreau & Hector 2001). One genotype failed to produce seeds in the monoculture/deer environment; we added a small amount ('1') to these zero values in order to make the calculations. Our conclusions were the same if we simply excluded patches with this genotype. We considered effects of biodiversity, complementarity and selection to be significant when 95% likelihood confidence intervals (CIs) did not cross zero. The equations of Loreau & Hector (2001) give the net biodiversity effect, that is, the difference between observed seed output of genotypes in polyculture and their mean output in monoculture. We were also interested in the relative effects of genotypic diversity (i.e. 'horizontal diversity') in the presence vs. absence of deer herbivory (i.e. 'vertical diversity'). Thus, we extended the equations of Loreau & Hector (2001) to calculate whether the percentage change in seed output due to plant genotypic diversity differed in the presence vs. absence of deer (analysed with a *t*-test).

At the genotypic level, we also compared the mean number of seeds per plant for genotypes grown in monoculture vs. polycultures using a paired *t*-test in each deer herbivory treatment (e.g. Johnson *et al.* 2006). Importantly, a significant paired *t*-test at the genotypic level indicates a non-additive effect of genotypic diversity because the analysis tests whether a genotype's fitness is higher in one environment (polyculture) compared with the other (monoculture). We ran these analyses first by including a zero for plants that perished and thus produced no seeds, and second by considering only plants that survived to the end of the experiment. To test whether deer herbivory altered the strength of the diversity effect, we used a paired *t*-test to examine whether the effect size of plant genotypic diversity [$\ln(\text{poly}/\text{mono})$] on mean genotype seed output differed in the presence vs. absence of deer.

Plant growth and resistance traits

To test genotypic variation in plant growth and resistance traits, we employed separate ANOVAs in each of the deer exclusion treatments with genotype as a random effect. There was no effect of patch genetic diversity on flowering phenology (e.g. the mean number of days after planting until a genotype bolted, $F_{1,254} = 0.614$, $P = 0.434$), thus for consistency with other traits, we used phenology data from only the polyculture patches. For each trait we then used paired *t*-tests at the genotypic level to determine whether

deer browsing altered mean trait expression (i.e. phenotypic plasticity). For rooftop grown plants, we used one-way ANOVAs with genotype as a random effect to test for genotypic variation in growth rate, root:shoot ratio, and total phenolics of root and leaf tissues.

The heritability of each trait was calculated by dividing total genotypic variance by total variance, which equates to the broad-sense heritability for clonally or asexually reproducing organisms (Lynch & Walsh 1998). All trait-level analyses were performed on untransformed data as recommended by Houle (1992). Genetic covariation among traits (i.e. physiological trade-offs) was calculated as Pearson product-moment correlations between all pairwise combinations of standardized trait values.

Linking genotype traits to plant fitness

We used three methods to link genotype traits to diversity effects. First, we investigated whether mean trait values for each genotype were correlated with vole-induced mortality and resistance to deer browsing, the two primary enemies in this study. We used survival data from early in the experiment when the effects of voles on plant mortality were strongest (see section 'Results'). Second, we examined whether we could link genotype traits to the effects of genotypic diversity on genotype performance. For these analyses we calculated a 'polyculture effect size' for each genotype in polyculture using the equation: $\ln(\text{Genotype}_{\text{Poly}}/\text{Genotype}_{\text{Mono}})$, where $\text{Genotype}_{\text{Poly}}$ and $\text{Genotype}_{\text{Mono}}$ were the mean reproductive output of genotypes in polyculture and monoculture, respectively, in each deer herbivory environment. We then regressed 'polyculture effect size' against our nine measured plant traits to determine which traits were correlated with the relative effects of polyculture.

Third, we assessed whether deer exclusion and/or genetic neighbourhood influenced direct and indirect natural selection on plant traits. We estimated the strength of total selection, including both direct and indirect selection caused by covariation among traits, by estimating the Pearson

product-moment correlations between each genotype's relative fitness and standardized normal trait values (i.e. selection differentials, Price 1970). To measure the strength of direct adaptive selection after correcting for trait covariation, we used stepwise forward regression testing whether each genotype's relative fitness was influenced by standardized normal trait values, with $P = 0.1$ as the criterion for each variable to enter and leave the model (i.e. selection gradients, Stinchcombe *et al.* 2002; Johnson *et al.* 2009). We used stepwise regression because the fully parameterized models had limited statistical power.

RESULTS

Treatment effects on plant fitness

Of the 3808 plants, 2699 (71%) died by the end of the experiment. Dead plants always showed signs of vole attack, including consumed roots and girdled stems. We never observed plants that wilted and died from physiological stress alone. Deer browsed bolting stalks but did not consume entire plants. Thus, we attribute all plant deaths to vole herbivory, although some plants may have perished due to the combined effects of voles and other stressors. Of the 1109 survivors, all but seven bolted and attempted to reproduce. The surviving population was thus >99% annual and survival and seed production were accurate estimates of lifetime fitness.

The proportion of plants surviving per patch varied over the course of the experiment (Fig. 1a, time effect, $F_{9,472} = 137.7$, $P < 0.001$, repeated measures ANOVA), but survival was consistently higher in polyculture than monoculture (genotypic diversity effect: $F_{1,472} = 4.38$, $P = 0.037$). Deer herbivory decreased plant survival, but this effect only became evident later in the growing season (Fig. 1a, deer herbivory \times time interaction, $F_{9,472} = 6.48$, $P < 0.001$). Deer herbivory also reduced the number of seeds produced per patch ($F_{1,472} = 86.2$, $P < 0.001$), but polyculture patches produced more seeds than monocultures in both deer

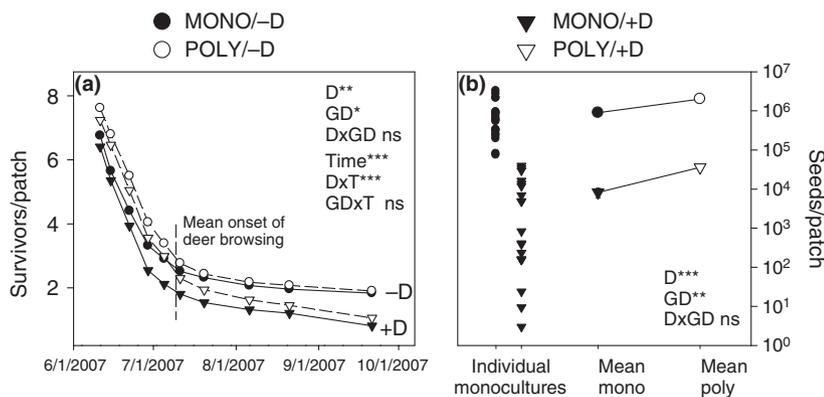
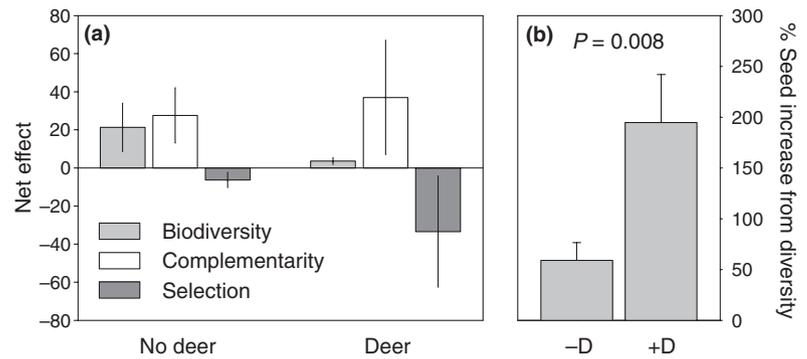


Figure 1 Influence of deer herbivory (D) and genotypic diversity (GD) on (a) mean number of surviving plants per patch over time (T), and (b) mean (± 1 SE) number of unconsumed seeds per patch at the end of the experiment. Error bars are omitted from individual dates in (a) and individual monocultures in (b) for clarity. * $P < 0.05$, *** $P < 0.010$, *** $P < 0.001$, ns: not significant.

Figure 2 (a) Mean ($\pm 95\%$ CI) effects of biodiversity, complementarity and selection on seed output per patch in the absence and presence of deer herbivory. Y-axis is seed output in 1000s. (b) Percentage increase (± 1 SE) in seed output per patch due to plant genotypic diversity in each deer herbivory environment.



herbivory environments (Fig. 1b, $F_{1,472} = 9.48$, $P = 0.002$). The positive effects of diversity on seed output were driven by positive complementarity effects that swamped negative selection effects in both the presence and absence of deer (Fig. 2a). Importantly, the relative impacts of genotypic diversity on seed output were nearly fourfold stronger in the presence vs. the absence of deer (Fig. 2b).

At the genotypic level, plants in diverse patches produced 59 and 200% more seeds in the absence and presence of deer, respectively, than did genotypes in monocultures (Fig. 3, $P \leq 0.008$, paired t -tests). Importantly, the presence of deer herbivory again strengthened the effects of genotypic diversity on fitness, in this case leading to a threefold increase in seed output per plant ($P = 0.022$, paired t -test, Fig. 3 inset). The patterns for reproductive output of only the surviving plants were similar. Genotypes from diverse patches produced 35 and 62% more seeds per

plant than plants from genetically homogenous patches in the absence and presence of deer, respectively ($P \leq 0.001$, paired t -tests, data not shown).

Voles attacked fewer plants in polycultures vs. monocultures: 4.5 (± 0.17) damaged plants in monoculture vs. 4.00 (± 0.17) damaged plants in polyculture ($F_{1,472} = 3.99$, $P = 0.046$). The presence of deer herbivory had no effect on the number of vole damaged plants per patch ($F_{1,472} = 0.63$, $P = 0.429$), nor was there an interaction between diversity and deer exclusion ($F_{1,472} = 1.54$, $P = 0.216$). Thus, vole attack was equivalent across deer herbivory treatments. Polyculture patches lost 76% fewer seeds to specialist insects than did monocultures ($0.41 \pm 0.11\%$ of seeds eaten per patch in monoculture vs. $0.10 \pm 0.10\%$ of seeds in polyculture, $F_{1,244} = 4.09$, $P = 0.044$), with no effect of deer browsing on percentage seed loss to insects (deer: $F_{1,244} = 0.207$, $P = 0.650$), and no interaction between diversity and deer exclusion ($F_{1,244} = 0.030$, $P = 0.863$). The mean onset of deer browsing did not differ in monoculture vs. polyculture (37.1 ± 1.8 days in monoculture vs. 40.3 ± 1.7 days in polyculture, $F_{1,170} = 1.68$, $P = 0.197$).

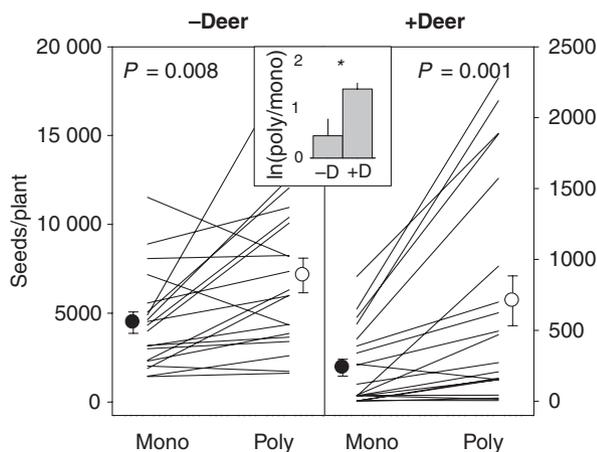


Figure 3 Genotypic diversity enhanced mean lifetime seed output of genotypes by 59 and 200% in the absence and presence of deer herbivory, respectively (paired t -tests). Each line depicts the mean number of unconsumed seeds for a single genotype in monoculture vs. polyculture. Symbols depict overall means (± 1 SE) in each treatment. Inset: the positive effects of genotypic diversity on seed output were over threefold larger in the presence vs. absence of deer ($P = 0.022$, paired t -test).

Plant traits and the mechanisms of diversity

We detected significant heritable variation among our 20 *O. biennis* genotypes for eight of nine measured plant traits (all traits except for leaf toughness, Appendix 1). Heritabilities were highest for plant secondary compounds (range: 0.70–0.70), intermediate for growth rate and bolting phenology (range: 0.43–0.54), and lowest for the four leaf and stem traits we measured (range: 0–0.27, Appendix 1). All five traits from the field experiment were plastic in response to deer herbivory. Browsed plants had significantly larger stem diameters, softer leaves, increased specific leaf area, fewer trichomes and bolted earlier than unbrowsed plants (Appendix 1).

Bolting phenology was the only trait significantly linked to vole damage and deer browsing. Early in the experiment, when vole-induced mortality was greatest (Fig. 1), early

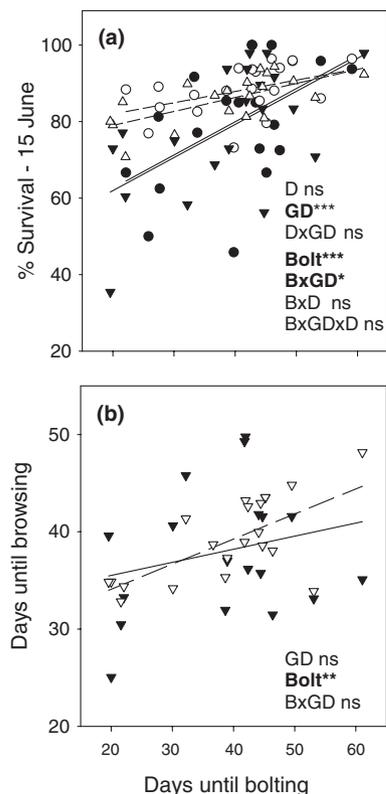


Figure 4 Early in the experiment when the effects of genetic diversity on plant survival were strongest (Fig. 1), early bolting genotypes had disproportionately lower rates of survival in monocultures (a). Deer also preferentially browsed early bolting genotypes, but being in a polyculture did not delay the onset of browsing (b). Filled symbols and solid lines are for monocultures; open symbols and dashed lines are for polycultures. Circles are for –deer treatments; triangles are for +deer treatments. * $P < 0.05$, *** $P < 0.010$, **** $P < 0.001$, ns: not significant.

bolting genotypes had lower rates of survival than later bolting genotypes (Fig. 4a). However, early bolting genotypes had disproportionately higher survival in polycultures, suggesting an associational effect of diversity on vole attack rate. Early bolting genotypes were also browsed earlier by deer, but polycultures did not delay the onset of browsing (Fig. 4b), suggesting no associational effect on deer browsing.

In contrast, two traits explained the enhanced relative performance of genotypes in polyculture vs. monocultures. The presence of deer herbivory, however, altered which traits improved genotype performance. In the absence of deer, genotypes that bolted later in the growing season performed relatively better in polycultures than early bolting genotypes (Fig. 5a). In the presence of deer, however, fast-growing genotypes performed relatively better than slow growing genotypes (Fig. 5b). Other traits were not substantial predictors of relative genotype performance in polyculture. Growth was not correlated with bolting phenology in either deer herbivory treatment ($r^2 \geq 0.049$, $P \geq 0.345$, linear regressions).

Natural selection acted on multiple plant traits, but the targets and strength of natural selection depended on the genetic neighbourhood and the presence of deer herbivory. In monoculture environments, the only detectable selection on plant traits was for reduced trichome density in both the presence and absence of deer (Table 1). In contrast, we detected selection for multiple plant traits in polycultures in the absence of deer (but presence of voles), including increased leaf toughness, larger stem diameters, later bolting date and smaller root:shoot ratios. None of these traits were under significant natural selection in polycultures in the presence of deer, although

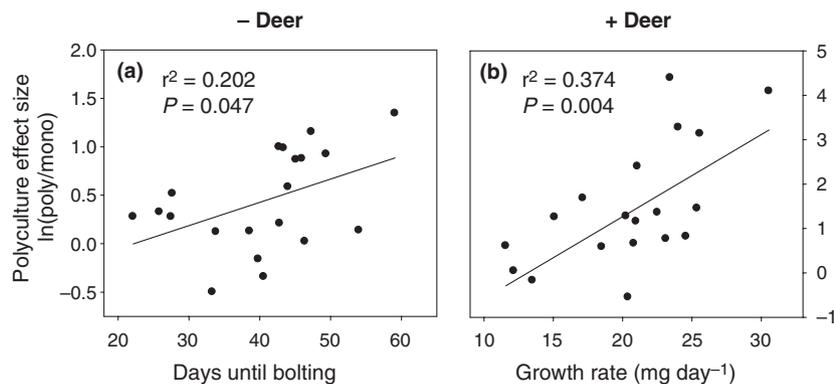


Figure 5 In the absence of deer herbivory (a), the positive effects of genotypic diversity on genotype seed output were explained by enhanced performance of later bolting genotypes. In the presence of deer herbivory (b), the positive effects of genotypic diversity on genotype seed output were explained by enhanced performance of fast-growing genotypes. Statistics are from linear regressions comparing relative performance of genotypes in polyculture vs. monoculture [$\ln(\text{poly}/\text{mono})$] against mean genotype traits. Genotypes are the unit of replication.

Table 1 Linear genotypic selection differentials (S) and gradients (β) on growth and defence traits in *Oenothera biennis* plants living in monocultures vs. polycultures in the presence and absence of deer herbivory (D)

Trait	Mono/-D		Mono/+D		Poly/-D		Poly/+D	
	S	β	S	β	S	β	S	β
Leaf toughness	0.055		0.110		0.205	0.439**	0.086	
SLA (mm ² mg ⁻¹)	-0.190		-0.301		-0.315		-0.163	
Trichomes cm ⁻²	-0.460*	-0.460*	-0.517*	-0.670**	-0.128		-0.415	
Stem diameter (mm)	0.157		-0.243		0.331	0.570***	-0.241	
Days to bolt	0.070		-0.110	-0.380	0.455*	0.818***	-0.111	
Growth (mg day ⁻¹)	-0.066		-0.146		-0.183		-0.041	
Root:Shoot	-0.316		-0.275		-0.225	-0.453**	-0.178	
Root phenolics	-0.221		-0.234		-0.185		-0.094	
Shoot phenolics	-0.313		-0.394		0.009		-0.453*	-0.453*

Selection differentials are the Pearson product moment correlations between the relative fitness of each of 20 *O. biennis* genotypes and their standardized normal trait values in each environment. Selection gradients were determined using stepwise multiple linear regression on the relative fitness of each genotype vs. their standardized normal trait values, with $P = 0.1$ as the criterion to enter and leave the model. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Bold values are statistically significant at $P < 0.05$.

we did detect selection for decreased phenolics in shoot tissues (Table 1).

DISCUSSION

The interaction between horizontal and vertical diversity has received little attention to date despite the pervasive impacts of consumers on community structure and function (Duffy 2002; Hooper *et al.* 2005). Although a plague of meadow voles (*M. pennsylvanicus*) killed over 70% of our nearly 4000 experimental evening primrose plants (*O. biennis*) and complicated our manipulation of vertical diversity, this unmanipulated herbivory provided novel insight into the interaction between trophic complexity and genotypic diversity. Plant genotypic diversity increased resistance to voles, reduced seed loss to specialist insects, increased plant survival and increased plant reproductive output. These results are consistent with previous findings showing that more genetically diverse plant communities are more productive (Crutsinger *et al.* 2006; Johnson *et al.* 2006) and more resistant to disturbance (Hughes & Stachowicz 2004; Reusch *et al.* 2005; Crutsinger *et al.* 2008) than genetically depauperate communities. Our results are also similar to those of Hughes & Stachowicz (2004), who found positive effects of seagrass genetic diversity on several ecosystem properties only after their experimental plots were grazed by migrating geese. The broad concordance of these 'accidental' experiments in marine seagrass meadows and terrestrial old fields indicates that intraspecific genetic diversity is an important component of biodiversity.

Moreover, because we explicitly manipulated herbivory by white-tailed deer (*Odocoileus virginianus*), we were able to show that the effects of genotypic diversity were enhanced

by added trophic-level complexity. In the presence of both deer and voles, plant genotypic diversity had a three to fourfold stronger effect on plant lifetime fitness relative to the effects of genotypic diversity in the presence of voles alone (Figs 2 and 3). Although the absolute increase in seed output per patch was higher in the absence of deer, the positive effects of diversity were relatively stronger after accounting for the strong suppressive effect that deer had on plant seed output. This suggests that increasing diversity can have a disproportionately advantageous effect on plant fitness when disturbance, in this case herbivory from multiple natural enemies, is most severe. Similarly, Duffy *et al.* (2005) found effects of herbivore diversity only in the presence of predators, and Gamfeldt *et al.* (2005) found the strongest effects of prey diversity at the highest levels of consumer diversity. In contrast, other studies have shown that adding consumers can eliminate, reverse, or have idiosyncratic effects on diversity-function relationships (Mulder *et al.* 1999; Naeem *et al.* 2000; Fox 2004; Wojdak 2005). Although the limited number of available tests and disparate outcomes makes broad generalizations premature, our results indicate that experiments manipulating just horizontal diversity may underestimate the positive effects of biodiversity, and increased trophic-level complexity may reinforce the positive effects of producer diversity. This result has significant implications for the conservation of higher trophic levels and the community consequences of maintaining intact trophic systems.

Plant diversity and herbivore impacts

The ability of prey diversity to dampen the impacts of consumers has been a matter of debate for decades and is

widely thought to limit the strength of trophic cascades in diverse ecosystems (Strong 1992). Three mechanisms are believed to limit consumer impact in diverse ecosystems: positive interactions among members of diverse communities, dominance of inedible prey in diverse multitrophic communities, and trade-offs between prey resistance and competitive ability (Hillebrand & Cardinale 2004; Duffy *et al.* 2007). We found clear support for the first two mechanisms and partial support for the third.

First, one of the most striking aspects of this study is the presence of positive and non-additive effects of genotypic diversity on plant lifetime fitness at both the patch (Figs 1b and 2a) and genotypic levels (Fig. 3). Given that we also found positive complementarity effects (see section 'Results'), we can attribute these effects to positive interactions in genetic polycultures, and in particular to associational resistance of vole herbivory. Early bolting genotypes, for example, were likely to perish in the initial wave of vole attacks, but mortality for these susceptible genotypes was reduced in genotypically diverse patches (Fig. 4a). This suggests that plant phenological diversity in our patches enhanced resistance to consumers, similar to studies showing that phenological complementarity is an important characteristic of diverse communities (e.g. Stachowicz *et al.* 2002).

Second, the 'variance in edibility hypothesis' argues that more diverse communities are more resistant because they are likely to contain less-preferred taxa (Leibold 1989). In agreement with this hypothesis, we found negative selection effects in both deer herbivory treatments (Fig. 2a). Negative selection effects are common in multitrophic studies and indicative of consumers shifting polycultures towards more resistant but less fecund taxa (Jiang *et al.* 2008). In our experiment, later-bolting genotypes were less preferred by voles and deer (Fig. 4a,b), and in the presence of vole herbivory alone, these less-preferred genotypes performed relatively better than preferred genotypes in polyculture (Fig. 5a). However, herbivory by voles and deer together shifted polyculture output towards dominance by relatively fast-growing genotypes (Fig. 5b), likely because fast-growing genotypes were better able to tolerate browsing and still produce fruits before the end of the growing season. Thus, negative selection effects arose via different ecological mechanisms in the presence of different enemies, suggesting that trophic complexity can qualitatively alter which taxa maximize the performance of diverse assemblages.

Third, we found partial support for evidence of trade-offs among *O. biennis* growth and resistance traits. Bolting phenology was negatively correlated with only one trait, plant stem diameter, and unrelated to plant growth rate and a suite of traits related to growth and defence (Appendix 2). Other studies with *O. biennis* have found trade-offs among growth/resistance traits (Johnson *et al.* 2009), and it is

possible that trade-offs in our study were obscured by phenotypic plasticity (Appendix 1). However, bivariate trade-offs between growth and resistance traits appear relatively uncommon, and it has been argued that the ecological costs of different defence strategies outweigh internal allocation costs (Koricheva 2002). Our findings are consistent with this hypothesis. For example, delayed bolting allowed plants to escape voles but exposed them to deer herbivory (Fig. 4a,b), and only the capacity for rapid regrowth (i.e. tolerance of herbivore damage) maximized fecundity in the presence of both herbivores (Fig. 5b). We interpret these results to mean that vole herbivory favoured a strategy of phenological escape, whereas vole and deer herbivory together favoured a strategy of herbivore tolerance, consistent with the hypothesis of ecological trade-offs among defence strategies.

The pattern that thus emerges from our study is that genotypic diversity promotes plant performance, but the interaction between plant diversity and trophic complexity alters the mechanisms and thus the genotypes that maximize performance. This interaction hinges on several key features, including the emergent properties of diverse assemblages, herbivore feeding selectivity and prey that are constrained by trade-offs between growth and resistance to variable consumer types. These complex interactions are fundamentally important because they indicate that increasing both vertical and horizontal diversity can promote and maintain ecosystem function (Lankau & Strauss 2008; Duffy 2009).

Plant diversity, herbivory and natural selection

Vertebrates can have large impacts on plant communities (Danell *et al.* 2006), yet surprisingly few studies have examined their ability to select for the evolution of plant defences. Both voles and deer selectively attacked different *O. biennis* genotypes in our study (Fig. 4), supporting a growing body of literature showing that generalist vertebrates can feed selectively among genotypes within a species, including voles (Pusenius *et al.* 2002), beavers (Bailey *et al.* 2004), hares (Laitinen *et al.* 2004), porcupine (Diner *et al.* 2009), deer (Duncan *et al.* 2001) and moose (Jia *et al.* 1997). More importantly, selective feeding in our study differentially influenced genotype fitness and resulted in natural selection for plant traits. For example, in polyculture/no-deer treatments, we found natural selection for genotypes with tougher leaves, larger stem diameters, delayed bolting, and decreased root:shoot ratios (Table 1). We attribute these impacts to voles because we effectively excluded deer but not voles from these plots, and selection for these traits is largely consistent with resistance to girdling of bolting stems, one of the primary sources of vole mortality in this experiment.

Understanding the agents of natural selection in polyculture/deer treatments, where we found selection for decreased phenolics in the shoot tissues (Table 1), is more complicated given that these plots were exposed to both voles and deer. We cautiously interpret these results, and selection for decreased trichome densities in monocultures (Table 1), to indicate that we found natural selection for some traits in some environments but not in others. This finding supports a growing literature showing that diffuse interactions in communities can alter the targets of natural selection (Stinchcombe & Rausher 2001; Lankau & Strauss 2008), and suggests that vertebrate herbivores can have stronger impacts on the evolution of plant defence than is currently appreciated.

CONCLUSION

Numerous studies have shown that plant diversity has positive effects on consumer abundance and diversity (Siemann *et al.* 1998; Wimp *et al.* 2004; Crutsinger *et al.* 2006; Johnson *et al.* 2006), but relatively few have examined effects of diversity on consumer impact, an important metric because of its potential relationship to secondary production. Our genotypically diverse plant patches reduced herbivore impact, suggesting that diverse prey communities may reduce secondary production when consumers promote dominance by less preferred prey. In contrast, other studies have seen enhanced secondary production when diverse prey communities contained equally edible species (Gamfeldt *et al.* 2005). Thus, the composition of diverse prey communities and the feeding specificity of consumers are likely to have pivotal effects on energy transfer in multitrophic communities. Moreover, although consumer feeding selectivity can decrease consumer impact (this study), the same mechanism increases consumer abundance and diversity (Siemann *et al.* 1998; Wimp *et al.* 2004; Crutsinger *et al.* 2006; Johnson *et al.* 2006), and consumer diversity itself can feedback to increase secondary production (Duffy *et al.* 2005). The complexity of these potential outcomes highlights the importance of examining multiple functional aspects of ecosystems in response to changing diversity (Jiang *et al.* 2008; Duffy 2009). Overall, our study demonstrates that incorporating consumers into biodiversity-ecosystem function studies could fundamentally change the notion of how producer diversity affects ecosystems, where, in this case, the positive effects of plant genotypic diversity on plant fitness were enhanced by trophic complexity.

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SUPPORTING INFORMATION

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

Appendix S1 Mean, range of variation and heritability for nine growth and defence-related traits of evening primrose, *Oenothera biennis*.

Appendix S2 Matrix of Pearson product–moment genetic correlations among plant traits (standardized means) in polycultures in the absence of deer herbivory (above the diagonal), and in polycultures in the presence of deer herbivory (below the diagonal).

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1 Appendix 1. Mean, range of variation, and heritability for nine growth and defense-related traits of
 2 evening primrose, *Oenothera biennis*. The first five traits were measured on plants grown in
 3 polycultures exposed to or protected from deer herbivory in the field experiment; the latter four traits
 4 were measured on a separate set of plants. Bold text and asterisks in ‘Plant trait’ column are from paired
 5 t-tests and test phenotypic plasticity of the traits in response to deer herbivory in polycultures. Bold text
 6 and asterisks in heritability columns test for the presence of heritable, genotypic variation for each trait
 7 within each herbivore treatment. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Bold values are statistically
 8 significant at $P < 0.05$. n/a = not applicable.
 9

Plant trait	- Deer		+ Deer	
	Mean (Range of variation)	Heritability	Mean (Range of variation)	Heritability
Toughness***	80.1 (79-81)	0.006	53.3 (49-59)	0.0
SLA (mm²/mg)***	18.7 (18-19)	0.036	24 (20-34)	0.269***
Trichomes/cm²***	886 (718-1150)	0.229**	768 (754-789)	0.016
Stem dia (mm)***	7.6 (6.3-8.9)	0.211**	8.3 (7.3-9.5)	0.164*
Days to bolt*	40.5 (22-59)	0.429***	38.7(20-61)	0.543***
Growth (mg/day)	20.5 (12-31)	0.479***	n/a	
Root:Shoot	0.31 (0.23-0.47)	0.646***	n/a	
Root phenolics	99 (59-146)	0.696***	n/a	
Shoot phenolics	107 (82-142)	0.702***	n/a	

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13 Appendix 2. Matrix of Pearson product-moment genetic correlations among plant traits (standardized
 14 means) in polycultures in the absence of deer herbivory (above the diagonal), and in polycultures in the
 15 presence of deer herbivory (below the diagonal). Growth, root:shoot ratio (R:S), root phenolics, and
 16 shoot phenolics were measured on plants grown separately in one environment (correlations are thus
 17 identical above and below the diagonal). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Bold values are
 18 statistically significant at $P \leq 0.05$. Relatively few traits were statistically correlated with each other.
 19 Of the 45 pairwise tests among traits measured in the absence of deer, only three traits covaried ($P =$
 20 0.206 , binomial expansion test). There were seven significant correlations among the 39 unique
 21 pairwise combinations in the presence of deer ($P = 0.002$, binomial expansion test).
 22
 23

Trait	% Water	Leaf toughness	SLA	Trichomes	Growth	Stem dia	Days to bolt	R:S	Root Phenolics	Shoot Phenolics
% Water		-0.518	-0.118	-0.361	-0.089	0.144	0.304	0.216	0.013	-0.049
Leaf toughness	-0.079		-0.422	0.368	0.332	0.349	-0.398	0.238	0.068	0.140
SLA (mm ² /mg)	0.558	0.130		-0.025	-0.220	-0.105	0.136	0.241	-0.097	-0.045
Trichomes/cm ²	-0.418	-0.419	0.069		0.474*	0.135	-0.392	0.285	0.362	0.182
Growth (mg/day)	-0.084	-0.650**	0.073	0.547**		0.042	-0.222	0.254	0.742***	0.148
Stem dia (mm)	-0.188	-0.388	-0.078	0.503*	0.433		-0.345	0.242	-0.187	-0.120
Days to bolt	0.220	0.444*	0.232	-0.404	-0.223	-0.504*		-0.017	-0.007	0.239
R:S	0.333	-0.094	0.544**	0.304	0.254	0.420	0.017		0.186	0.105
Root phenolics	-0.189	-0.481*	0.071	0.442	0.742***	0.242	0.038	0.186		0.569**
Shoot phenolics	-0.193	-0.082	-0.041	0.165	0.148	0.230	0.344	0.105	0.569**	

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