LETTER

Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness

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Abstract

Recent research suggests that genetic diversity in plant populations can shape the diversity and abundance of consumer communities. We tested this hypothesis in a field experiment by manipulating patches of Evening Primrose (*Oenothera biennis*) to contain one, four or eight plant genotypes. We then surveyed 92 species of naturally colonizing arthropods. Genetically diverse plant patches had 18% more arthropod species, and a greater abundance of omnivorous and predacious arthropods, but not herbivores, compared with monocultures. The effects of genotypic diversity on arthropod communities were due to a combination of interactive and additive effects among genotypes within genetically diverse patches. Greater genetic diversity also led to a selective feedback, as mean genotype fitness was 27% higher in diverse patches than in monocultures. A comparison between our results and the literature reveals that genetic diversity and species diversity can have similar qualitative and quantitative effects on arthropod communities. Our findings also illustrate the benefit of preserving genetic variation to conserve species diversity and interactions within multitrophic communities.

Keywords

Biodiversity, coevolution, community genetics, diversity-ecosystem function, genetic variation, herbivory, insect ecology, *Oenothera biennis*, plant–insect interactions, tritrophic interactions.

Ecology Letters (2006) **9**: 24–34

INTRODUCTION

An emerging paradigm in biology is that biodiversity in natural ecosystems affects the stability and function of multitrophic communities (Hooper *et al.* 2005). Interest in such consequences of biodiversity stem, in part from a long-standing hypothesis that the composition and dynamics of animal communities are strongly shaped by plant species diversity (Elton 1958; Hutchinson 1959; Murdoch *et al.* 1972). Indeed, increases in the number of plant species in natural ecosystems consistently results in a higher diversity of herbivorous and predatory arthropod species (Siemann *et al.* 1998; Knops *et al.* 1999; Haddad *et al.* 2001). Plant diversity also affects the abundance of arthropods, but these effects are often less consistent, in part because the response in abundance varies by trophic level (Root 1973). Specifically, herbivores are predicted to decrease, while predators are predicted to increase, with the number of plant species in a community (Root 1973). Although the effects of plant species diversity have been the focus of numerous experiments in agricultural (Andow 1991) and natural ecosystems (Knops *et al.* 1999; Koricheva *et al.* 2000; Haddad *et al.* 2001; Parker *et al.* 2001; Otway *et al.* 2005), the effects of intraspecific genetic diversity on multitrophic interactions have received much less attention (Schmitt & Antonovics 1986; Power 1988).

Recent research in community genetics suggests that intraspecific genetic diversity may be an important factor shaping the diversity and structure of communities (Whitham *et al.* 2003). This hypothesis is largely based on the study of plant–arthropod interactions, where it is widely recognized that plant genotypes vary in their resistance and susceptibility to multiple species of herbivores (Maddox & Root 1987; Fritz & Price 1988) and predators (Fritz 1995; Stiling & Rossi 1996). More recently, it has been discovered that the effects of genetic variation scale-up to affect the composition and structure of diverse arthropod assemblages on individual plants (Dungey *et al.* 2000; Hochwender & Fritz 2004; Wimp *et al.* 2005). For example, in a previous study we reported that the total richness and abundance of

arthropods varied by as much as 2.4-fold and 3.9-fold between plant genotypes respectively (Johnson & Agrawal 2005). The variation in the arthropod community was correlated with several genetically variable plant traits, suggesting that different plant genotypes offer distinct niches for arthropods. This observation forms the basis of our hypothesis that plant populations with high genetic diversity offer a greater variety of niches than low diversity populations, leading to a greater diversity and altered abundance of arthropods.

Preliminary support for this hypothesis comes from several recent studies in terrestrial and marine ecosystems. In an observational study of hybridizing trees, genetic diversity across 11 natural tree stands positively correlated with arthropod diversity (Wimp *et al.* 2004). Experimental support stems from a marine system where two independent studies on *Zostera marina* found that high genotypic diversity led to a greater abundance, but not diversity, of invertebrates following stress imposed by geese grazing (Hughes & Stachowicz 2004) and extreme temperatures (Reusch *et al.* 2005).

Two general and non-exclusive mechanisms can account for an effect of plant genetic diversity on arthropod communities: interactive (i.e. non-additive) and additive effects of genotypes. Interactions among genotypes could result from: (i) plants directly affecting the phenotype of conspecific neighbouring plants (e.g. competition and facilitation) (Callaway & Walker 1997); (ii) plants indirectly interacting with neighbours via associational resistance and susceptibility (e.g. chemical masking of neighbours, spill-over of insects, etc.) (Tahvanainen & Root 1972); (iii) source–sink dynamics of arthropod populations between plants within patches (Underwood 2004); and (iv) arthropods perceiving mixtures of plants differently than monocultures when searching for microhabitats (Rodriguez-Saona & Thaler 2005). Conversely, additive effects would result from the independent influence of plant genotypes on the arthropod community, where diverse patches have a high richness and abundance of arthropods because of the increased probability of including genotypes with distinct communities (analogous to the so-called 'sampling effect', Loreau & Hector 2001).

The objective of our experiment was to examine the effects of plant genotypic diversity in populations of a native plant (*Oenothera biennis* L.), on the arthropod community associated with *O. biennis*. We first confirmed that genetic differences between individual plants (i.e. genotype identity) are an important factor affecting the richness (number of species) and abundance (number of individuals) of arthropods (Johnson & Agrawal 2005). We then tested the hypothesis that plant genotypic diversity (i.e. number of genotypes) affects both the richness and abundance of arthropods. If such an effect was detected, we then asked whether additive or interactive effects were responsible for the observed pattern. Finally, we examined the consequences of genotypic diversity for lifetime plant fitness to understand the potential evolutionary interactions between *O. biennis* and its arthropod fauna.

MATERIALS AND METHODS

Study site and species

This work was conducted at the Koffler Scientific Reserve at Jokers Hill (Jokers Hill; http://www.zoo.utoronto.ca/jokershill). Jokers Hill is a 350 ha field station owned by the University of Toronto, located 50 km North of Toronto, Ontario, Canada (44°03′ N, 79°29′ W, 300 m a.s.l.).

Common Evening Primrose (*O. biennis* L., Onagraceae) is an herbaceous, primarily self-pollinating species that forms discrete populations containing one to 14 genotypes (Steiner & Levin 1977). The genetic and mating system of *O. biennis* (complete translocative heterozygosity) is such that plants principally produce clonally related seeds (Cleland 1972), making it possible to obtain numerous replicates of genetically identical plants. The arthropod fauna associated with *O. biennis* is large and diverse, made-up of specialist and generalist herbivorous and carnivorous species (92 species in this study; see Appendix S1).

Experimental design

Seeds from 12 clonal families (hereafter genotypes) were collected between August 2001 and July 2002 from nine naturally occurring patches within 10 km of Jokers Hill, plus one patch in Toronto (50 km South of Jokers Hill) and another in Mississauga (50 km SW). Two of the genotypes from Jokers Hill came from a single patch. In this area, *O. biennis* typically forms small discrete patches of plants separated by hundreds of meters to kilometres and we operationally define these patches as populations. We germinated all seeds simultaneously on wet filter paper in July 2002 and randomized seedlings into 250 mL pots containing potting soil and 0.25 g of slow-release Nutricote pellets (13 : 13 : 13, N : P : K). After 11 weeks of growth in a common environment – a length of time that should effectively minimize maternal effects (Gross & Kromer 1986; Agrawal 2002) – we found evidence for genetic differences between all genotypes by comparing nine rosette traits using multivariate analysis of variance (M.T.J. Johnson, unpublished data). Plants were grown outside until late-October, when they were transplanted into an old field at Jokers Hill.

We created patches of plants that simulate the size and range of diversity found in small natural populations of *O. biennis*. Each patch was planted with eight plants of one,

four or eight genotypes, planted in equal relative abundance and arranged in a 40-cm diameter circle. Patches were arranged in a grid with 5 m spacing between the rows and columns of patches. Mixed patches were created by randomly sampling from our pool of 12 genotypes, with the constraint that no two patches could have identical composition. Thirteen plants (3%) died during winter, which resulted in nine patches with six to seven plants. We standardized abundance and richness in these patches to a density of eight plants by: (i) calculating the average abundance per plant, or average number of novel species contributed by each plant in the patch; and (ii) substituting this value for each dead plant in the patch. This correction reduces the bias imposed by unequal abundance because of mortality and does not change our conclusions if we do not make the correction. In total, there were 14 monocultures (one to two replicates/genotype), 20 four-genotype and 16 eight-genotype patches.

We non-destructively surveyed naturally colonizing arthropods on all plants five times between late-May and mid-August. On each plant, we identified and counted herbivorous (35 species), omnivorous (12) and predatory (45) arthropods by looking over the entire plant (Appendix S1). We did not record parasitoids, pollinators or pollen consumers (e.g. thrips) because we were unable to make accurate counts of these arthropods due to their small size or fleeting visits.

Statistical analyses

To examine the effects of genotype identity on total arthropod species richness and total abundance, we treated individual plants as replicates. Because monoculture and four-genotype patches contained more than one replicate plant per genotype, it was necessary to use only one replicate plant per genotype per patch so as to avoid pseudoreplication. We accomplished this by randomly sampling one plant from each monoculture, and one plant of each genotype from four-genotype patches; we used all plants from eight-genotype patches. In total, we used 215 of the original 400 plants. The data were analysed using mixed-model repeated measures analyses in Proc Mixed of SAS (SAS Institute, Cary, NC, USA) with the statistical model:

$$\text{variable} = \text{mean}_{\text{overall}} + \text{date} + \text{patch} + \text{genotype} + \text{genotype} \times \text{date} + \text{error}$$

individual plants were the repeated subjects. To determine the best covariance structure for the model we compared the performance of three commonly used structures (compound-symmetric, autoregressive order 1 and unstructured) using the Akaike information criterion (Littell *et al.* 1996). The best covariance structure was then used for all further

hypothesis tests of a given variable. Sampling date was treated as a fixed effect and the degrees of freedom were adjusted using the Kenward–Roger method (Kenward & Roger 1997). All other factors in the model were random effects and their statistical significance was assessed with log-likelihood ratio tests as the difference between $-2 \times$ log-likelihood in models with and without the random factor of interest. This statistic has a chi-squared distribution with 1 d.f. (Littell *et al.* 1996, p. 44), and the test is one-tailed because variance components cannot be negative. Our data were log transformed when needed to improve homogeneity of variance and normality.

We examined the effect of genotypic diversity on cumulative arthropod species richness (total richness and the richness for each trophic group) across the entire season using Proc Glm of SAS where the unit of replication was a patch of eight plants. To examine whether the effects of genotypic diversity varied across the season, we used a repeated measures design. The basic model was:

$$\text{variable} = \text{mean}_{\text{overall}} + \text{date} + \text{diversity} + \text{diversity} \times \text{date} + \text{error},$$

where patches were the repeated subject. In these analyses, we used mixed model analyses in Proc Mixed when raw or transformed data were normal and had equal variance among treatments; the best covariance structure was determined as before. Generalized linear models were used when data did not meet the assumptions of normality and homogeneity of variance. Omnivore abundance was normally distributed, but only the last two sampling dates were used because this trophic group was virtually absent prior to these dates. Data on the richness of individual trophic groups, as well as the abundance of predators, were analysed using generalized linear models for count data. Specifically, we fit these variables to Poisson or negative-binomial distributions with a log-link function using generalized estimating equations in Proc Genmod of SAS (Allison 1999). We chose the distribution type that minimized the deviance statistic: herbivore, omnivore, and predator richness were each fit to a Poisson distribution, and predator abundance was fit to a negative-binomial distribution.

As we were only interested in quantitative trends between plant diversity treatments, we employed orthogonal polynomial contrasts to determine whether variables changed linearly or nonlinearly as a function of genotypic diversity (Kuehl 2000). With three levels of genotypic diversity we were able to test for linear and quadratic effects. The contrast coefficients for detecting linear trends were: $-3.333, -0.334, +3.667$; one-genotype, four-genotypes, eight-genotypes and $+4.541, -7.946, +3.405$ for quadratic trends. We also examined linear and quadratic effects of sampling date because we previously found that community

variables frequently exhibit linear and hump-shaped patterns through the season (M.T.J. Johnson and A.A. Agrawal, unpublished results). The linear and quadratic coefficients for date were: -2, -1, 0, $+1$, $+2$; date 1, date 2, date 3, date 4, date 5 and $+2$, -1, -2, -2, $+2$ respectively. To test for genotypic diversity-by-date interactions we multiplied the contrast coefficients from each effect together. The significance of contrasts was determined using an *F*-statistic for normally distributed data, and chi-squared statistic (1 d.f.) calculated from a generalized score-function for data analysed using generalized linear models. For ease of interpretation, we present least-squared mean values (± 1 SE) of the raw data in all figures.

We assessed the effect of plant genetic diversity on plant fitness by comparing the mean fitness of genotypes between genetically mixed patches and monocultures. Fitness was measured as the number of unconsumed fruits on plants, which is a component of total lifetime fitness because plants are monocarpic and self-pollinating. We calculated the mean fitness of each genotype in mixed patches (four- and eight-genotype patches combined) and monocultures, and compared the mean fitness of genotypes in a single one-tailed paired *t*-test ($n = 12$ monoculture–mixture pairs, one for each genotype). A one-tailed test was used because the *a priori* prediction was that plant fitness increases with plant genetic diversity; a prediction based on results from both natural and managed systems (Kelley *et al.* 1988; Zhu *et al.* 2000; Mundt 2002). As this test was performed at the genotype-level, it also provides a direct test of whether the effects of plant genotypic diversity on fitness are additive or non-additive because a significant difference in the paired *t*-test can only result from an interactive effects among the genotypes in a genetic mixture.

Testing for additive vs. interactive effects on the arthropod community

We tested for interactions between genotypes in genetically mixed patches by comparing the data we observed in the field to the data we expected to find should there be no interactions between genotypes (i.e. an additive effect). Unlike univariate variables, such as plant productivity (Loreau & Hector 2001; Mulder *et al.* 2001), the expected species richness of diverse patches cannot be easily determined because richness is a composite measure of the number of unique arthropod species occurring in each patch. We thus coded resampling methods in Microsoft Visual C++ (Microsoft, Redmond, WA, USA) to generate expected datasets of genetically diverse patches using the data from monoculture patches. The data used in this analysis were based on the total cumulative species richness observed over the entire season on all plants from the monocultures ($n = 109$ plants) (recall there were one to two

replicate monoculture patches for each of twelve genotypes). Specifically, we filled the patch compositions of our mixed-genotype patches by randomly sampling plants from monocultures of the corresponding genotype. Take for instance a four-genotype patch of eight individual plants with genotypes A, B, C and D. To generate our expected data set, we recreated this patch by randomly substituting the data using randomly drawn individuals of genotypes A, B, C and D from monocultures (individual plants were not resampled). These monoculture plants had arthropod community data that were unbiased by the potential interactive effects between genotypes in mixed patches. We repeated this sampling for each non-monoculture patch in our experiment ($n = 36$ patches), and then calculated the expected mean species richness of all these four- and eight-genotype patches (as in the observed data set). This sampling and calculation of mean richness was repeated 10 000 times; a number typical of resampling generations (Efron & Tibshirani 1993) and sufficient to capture most of the resampling permutations of our monoculture data in mixed-patches. With these data, we calculated 95% confidence intervals using the bias-corrected percentile method (Efron & Tibshirani 1993). If the observed mean richness fell outside these confidence intervals, then observed and expected results were significantly different at the $P < 0.05$, indicating that genotypes interacted to affect arthropod diversity.

To identify interactive vs. additive mechanisms responsible for the effects on abundance of each trophic group (herbivores, omnivores and predators), we modified the conventional statistical approach used to calculate the net biodiversity effect (Loreau & Hector 2001; Mulder *et al.* 2001). Specifically, we tested for an interactive effect on the date in which abundance showed the greatest response to genotypic diversity. We first calculated the mean abundance of arthropods on each genotype in monoculture, and then created the expected data set for mixture patches based on their genotypic composition. No randomization was required for arthropod abundance. Instead, we created the expected data set for abundance by substituting the observed abundance on each plant in mixture with the appropriate genotype mean value from monoculture. We combined the observed data from four- and eight-genotype patches because there was no statistical difference between these diversity levels in any of the tests, and analysing them separately did not change the result. We then compared the observed abundance to the expected abundance in each of the 36 mixed patches using ANOVA blocked by patch, where the statistical model was:

$$\text{variable} = \text{mean}_{\text{overall}} + \text{patch} + \text{data set (observed vs. expected)} + \text{error.}$$

This test using ANOVA is equivalent to a paired t-test comparing the observed and expected values. A significant effect of 'dataset' reveals that the abundance of arthropods deviated from the additive expectation in genetically mixed patches. Because of unequal variance between the observed and expected mean values, we repeated the analyses on omnivore and predator abundance using an equivalent nonparametric method (Friedman test, Sokal & Rohlf 1995). The results from the nonparametric analyses did not change our conclusions and are not reported in the text.

RESULTS AND DISCUSSION

Consistent with our previous findings (Johnson & Agrawal 2005), genotype identity of *O. biennis* was an important determinant of the total richness and abundance of arthropods on individual plants (Table 1 and Fig. S1). Given that the individual genotypes varied in their resistance to diverse members of the arthropod community, we proceeded to test for community-level effects of genotypic diversity in plant patches.

Cumulative arthropod richness increased linearly with genotypic diversity, where patches with eight genotypes had 18% more arthropod species than monocultures (div$_{linear}$: $F_{1,47} = 8.53$, $P = 0.005$; Fig. 1a). This pattern was consistent throughout the season (Fig. 1a; $P < 0.10$ for all div × date interactions, Table 2) and was primarily caused by an increased number of predator species in diverse plant patches (cumulative predator richness div$_{linear}$: $F_{1,47} = 5.59$, $P = 0.02$; Fig. 1b). There was no effect of genotypic
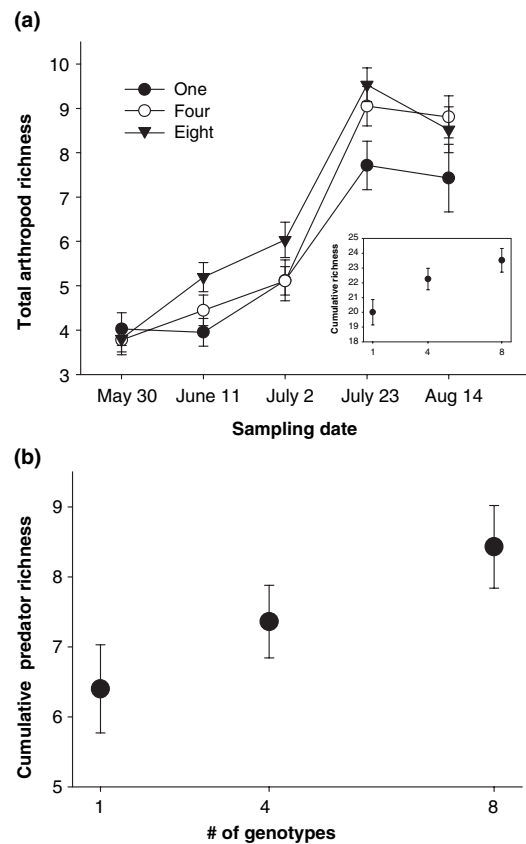
diversity on the cumulative richness of either herbivores or omnivores (div$_{linear}$: $F_{1,47} = 0.99$, $P = 0.32$; div$_{linear}$: $F_{1,47} = 0.51$, $P = 0.48$ respectively), but species richness of these trophic groups did appear to be affected at specific times during the spring or summer (Table 2). Herbivore richness in mixtures decreased by 20% relative to monocultures in late-May, and increased by 25% relative to monocultures in late-summer. Omnivore richness in mixtures also increased in late-summer by 71% relative to monocultures. These positive effects of genotypic diversity on herbivore and omnivore species richness corresponded to when plants were actively flowering.

Genotypic diversity had no measurable effect on the total abundance of arthropods, largely because of a lack of response by herbivores, the most abundant trophic group (Fig. 2a; Table 2). Nevertheless, plant genotypic diversity caused an increase in the abundance of both omnivorous

Figure 1 The influence of genotypic diversity in patches of *Oenothera biennis* on arthropod species richness. Genotypic diversity in *O. biennis* affected: (a) total arthropod richness throughout the season (figure of total cumulative richness inset); (b) predator richness. Points show the least-squared mean values ± 1 SE for patches containing one, four or eight genotypes, and lines connect the diversity levels between sampling dates.

Table 1 Effects of *Oenothera biennis* genotype on the total species richness and total abundance of arthropods

Effect	d.f.	F/χ^2	P
Richness			
Date	4,26	57.16	**< 0.001**
Patch	1	1.9	0.08
Genotype	1	7.9	**0.002**
Genotype × date	1	0.4	0.26
Abundance			
Date	4,10	49.18	**< 0.001**
Patch	1	5.8	**0.008**
Genotype	1	20.9	**< 0.001**
Genotype × date	1	4.6	**0.016**

Individual plants were the unit of replication. Date was treated as a fixed effect and its significance was determined using an F-statistic; denominator d.f. vary because they are adjusted using the Kenward–Roger method. The effects of patch, genotype and genotype × date were random effects and their significance was assessed using the chi-squared statistic with 1 d.f. from log-likelihood ratio tests. Effects in bold are significant at $P < 0.05$.

Table 2 The effects of genotypic diversity and date on arthropod richness and abundance

Effect	Estimate	F/χ^2	P
Total richness*			
Diversity$_{linear}$	3.36	7.78	**0.008**
Diversity$_{quad}$	− 1.39	0.41	0.52
Date$_{linear}$	13.00	267.00	**< 0.001**
Date$_{quad}$	0.10	0.01	0.92
Herbivore richness†			
Diversity$_{linear}$	0.14	0.34	0.56
Diversity$_{quad}$	− 0.07	0.03	0.87
Date$_{linear}$	2.55	319.9	**< 0.001**
Date$_{quad}$	0.05	0.07	0.79
Diversity$_{linear}$ × date$_{linear}$	2.03	2.99	0.084
Diversity$_{quad}$ × date$_{linear}$	− 7.13	8.13	**0.004**
Omnivore richness†			
Diversity$_{linear}$	1.11	3.15	0.076
Diversity$_{quad}$	− 0.62	0.32	0.57
Date		0.83	0.36
Diversity$_{quad}$ × date	4.28	4.14	**0.042**
Predator richness†			
Diversity$_{linear}$	0.93	4.42	**0.036**
Diversity$_{quad}$	− 0.1	0.02	0.90
Date$_{linear}$	− 0.23	0.45	0.50
Date$_{quad}$	− 1.11	7.24	**0.007**
Diversity$_{linear}$ × date$_{quad}$	− 6.57	3.16	0.076
Diversity$_{quad}$ × date$_{quad}$	− 11.08	2.73	0.099
Total abundance*			
Diversity$_{linear}$	− 0.017	0	0.99
Diversity$_{quad}$	− 2.98	1.96	0.17
Date$_{linear}$	9.49	59.91	**< 0.001**
Date$_{quad}$	8.51	166.67	**< 0.001**
Herbivore abundance*			
Diversity$_{linear}$	− 0.64	0.28	0.60
Diversity$_{quad}$	− 2.86	1.69	0.20
Date$_{linear}$	8.75	48.44	**< 0.001**
Date$_{quad}$	8.94	160.53	**< 0.001**
Omnivore abundance*			
Diversity$_{linear}$	3.07	6.71	**0.013**
Diversity$_{quad}$	− 1.81	0.71	0.40
Date	− 0.29	5.81	0.13
Predator abundance‡			
Diversity$_{linear}$	0.92	4.09	**0.043**
Diversity$_{quad}$	− 0.57	0.40	0.53
Date$_{linear}$	− 0.40	1.16	0.28
Date$_{quad}$	− 1.16	6.41	**0.011**
Diversity$_{linear}$ × date$_{quad}$	− 8.15	3.64	0.056

Orthogonal polynomial contrasts were used to estimate the linear and quadratic (quad) effects of genotypic diversity, sampling date, and their interaction. Interactions between all linear and nonlinear effects of diversity and date were examined, but we report only those interactions where $P < 0.1$.
*Data was normal and an F-statistic with d.f. = 1 and 47 was used to determine the significance of contrasts.
†Data fit to a Poisson distribution. A chi-squared statistic with 1 d.f. was used to determine the significance of contrasts.
‡Data fit to a negative binomial distribution. A chi-squared statistic with 1 d.f. was used to determine the significance of contrasts.

and predaceous arthropods (Fig. 2b,c; Table 2). Omnivores were 80% more abundant in mixed genotype patches than monocultures in late-summer (Fig. 2b; Table 2). Likewise, predator abundance linearly increased with genotypic diversity by 37% from monocultures to patches with eight genotypes (Fig. 2c; Table 2). A marginally significant interaction between diversity and date resulted from predators responding most strongly from late-spring to mid-summer (Table 2). The effects of genotypic diversity on the abundance and richness of predators cannot be separated as they are tightly correlated ($r = 0.93$, $P < 0.001$, $n = 50$). This strong positive relationship occurred because no predator species was common, so that the attraction of additional individual predators to a patch was necessarily accompanied by the attraction of additional predator species. Thus, predators as a whole exhibited a very general affinity for diverse plant patches.

The strong and consistent effects of plant genotypic diversity on predator richness and the abundance of both predators and omnivores may have been indirectly mediated by trophic interactions (Downing & Leibold 2002). To examine this potential mechanism, we included the richness and abundance of each trophic group as covariates in separate analyses. The inclusion of other trophic groups never ameliorated the effect of plant genotypic diversity on the richness and abundance of predators, or the abundance of omnivores (all P-values remained < 0.05). We therefore conclude that the plants themselves, and not indirect trophic interactions, directly attracted a greater abundance and diversity of omnivorous and predatory arthropods.

Our observed effects of genotypic diversity on arthropod community structure parallels similar research on plant species diversity in terrestrial ecosystems. Studies that have manipulated the number of plant species in natural communities have found that plant species richness explains 15–23% of the variation in total arthropod richness, and that the number of arthropod species increases at a rate of 0.35–1.54 per additional plant species (Siemann et al. 1998; Knops et al. 1999; Haddad et al. 2001). Genotypic diversity within patches of O. biennis explained a comparable amount of variation (16%) and led to a similar rate of increase (0.46 species/additional genotype) in cumulative arthropod richness. Our study represents the first experimental evidence that intraspecific genotypic diversity promotes diversity at higher trophic levels and supports recent correlative evidence showing a similar pattern in hybrid trees (Wimp et al. 2005). The effects of plant species diversity on arthropod abundance show all possible outcomes in the few studies that have distinguished trophic groups (Knops et al. 1999; Koricheva et al. 2000; Haddad et al. 2001; Otway et al. 2005). We found that genotypic diversity explained 7–8% of the variation in both omnivore and predator
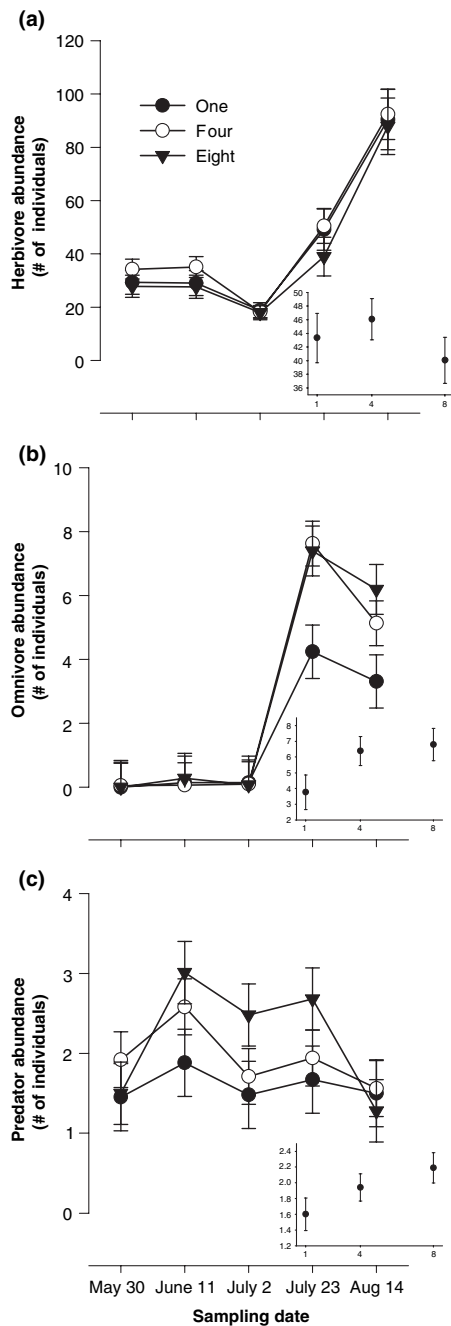
Figure 2 The response of arthropod abundance to genotypic diversity in patches of *Oenothera biennis*. (a) Herbivores were unaffected by genotypic diversity, while (b) omnivore and (c) predator abundance increased with increasing genotypic diversity. For all trophic groups, a figure depicting the mean abundance vs. the number of genotypes is inset. Points indicate the least-squared mean values ± 1 SE for patches with one, four or eight genotypes, and lines connect the points between sampling dates.

abundance, with abundance increasing at rates as high as 0.40 and 0.15 individuals per additional plant genotype respectively.

The effects of plant genetic diversity reported in our study likely reflect a general and widespread pattern in nature. Not only were multiple trophic groups affected by genotypic diversity, but the effects of diversity were typically consistent throughout the season. This result adds to a series of recent papers that reports similar strong ecological consequences of genetic diversity in a variety of ecosystems, suggesting that the patterns we observed may reflect a general, yet understudied factor affecting multitrophic interactions (Hughes & Stachowicz 2004; Wimp *et al.* 2004; Reusch *et al.* 2005). Furthermore, the qualitative and quantitative similarity between our results and those from species-level diversity studies suggest that our findings are of biological significance.

Mechanisms of the diversity effect

Interactions among *O. biennis* genotypes (i.e. non-additive effects) and additive effects were both responsible for the influence of genotypic diversity on the arthropod community (Fig. 3). The additive effects of individual plant genotypes in mixed patches almost perfectly explained the increase in arthropod richness (Fig. 3a), as both observed and expected mean values were similar in patches with four ($P = 0.17$) and eight genotypes ($P = 0.22$). Predator abundance was 22% higher than expected, but the increase was not significant ($P = 0.10$; Fig. 3c), indicating that the independent influence of each genotype explained the majority of effects of genotypic diversity on arthropod species richness and predator abundance. We did not distinguish the influence of these mechanisms on herbivore abundance, as they did not respond to genotypic diversity. In contrast, the observed abundance of omnivores in mixed genotype patches was 73% higher than expected under the additivity model (ANOVA: $F_{1,35} = 18.12$, $P < 0.001$; Fig. 3b). Here, interactions among plant genotypes in mixed patches resulted in an emergent property that attracted the omnivores.

Our method for distinguishing additive and interactive effects of genotypic diversity on the arthropod community has limitations. Because each genotype was only replicated in one or two monoculture patches, the expected values for mixture patches were less influenced by spatial variation than the corresponding observed values. This reduces the variance around the expected mean values compared with the observed mean values and increases the probability of falsely rejecting additive effects as the sole mechanism of the effect of genetic diversity. Although this is a potential problem in our study, it should not influence our conclusions concerning species richness and predator abundance, as additive effects were not rejected. However, this limitation does apply to our analysis of omnivore abundance where interactions between genotypes were detected. Although the statistical significance of this
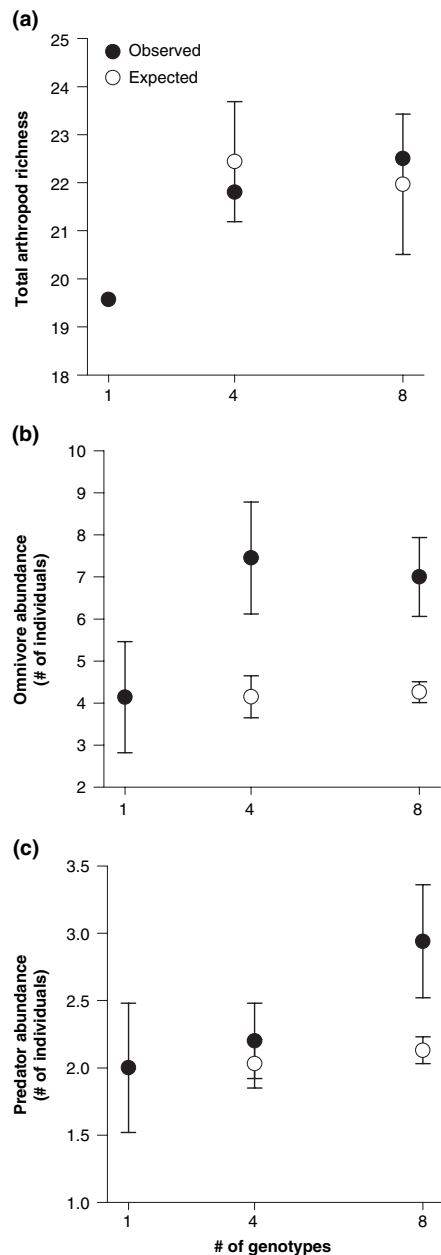
Figure 3 Interactive vs. additive effects of *Oenothera biennis* genotypes on arthropod communities. We compared the observed data (solid circles) from genetically mixed patches to the expected data (open circles) if there were no interactions among genotypes (i.e. an additive effect). We provide raw mean values for (a) species richness and show the 95% confidence intervals around expected mean values. If the confidence intervals overlap with the observed means then additive effects exclusively affected species richness. For (b) omnivore and (c) predator abundance we give the least-squared mean values ±1 SE. Tests for additive effects on abundance were performed on the sampling date that showed the strongest response to genotypic diversity.

interactive effect should be considered an upper limit, the magnitude of effect (73% difference) and the strength of the analysis ($P < 0.001$) strongly suggest that the result is real. This same issue is commonly encountered in biodiversity studies that attempt to identify both the ecological patterns and mechanisms associated with increasing biodiversity (Hector *et al.* 1999; Mulder *et al.* 2001; Tilman *et al.* 2001). Future studies should strive for greater replication of monocultures, combined with small-scale studies specifically designed to identify whether and how plant genotypes or species interact to affect ecosystem processes (Fargione *et al.* 2004) and higher trophic levels (Tahvanainen & Root 1972).

We believe that the additive and interactive effects are the result of individual plant genotypes offering distinct spatial and temporal niches for arthropods, where high genotypic diversity offers a wider range of niche space than monocultures. Several phenotypic traits genetically vary in *O. biennis* to affect the richness and abundance of arthropods (Appendices C and D in Johnson & Agrawal 2005), and we propose that these same traits may be responsible for the additive and interactive effects of genotypic diversity. For example, we previously found that early flowering was positively associated with omnivore and predator abundance on individual plants, but not herbivore abundance. Because we know that *O. biennis* is genetically variable for the date of first flower, diverse plant patches would have tended to flower earlier and longer than monocultures, simply because of the higher probability of containing early and late flowering genotypes. If omnivores or predators were attracted to these early flowering plants, as we had previously found (Johnson & Agrawal 2005), then it is likely that they would have recruited to and remained in diverse patches more readily. An interactive effect would result from these arthropods spilling over to nearby plants in the same patch. Similar niche-based mechanisms have been implicated in driving the ecological effects of species-level biodiversity studies in both terrestrial (Fargione *et al.* 2004) and marine communities (Stachowicz *et al.* 2002), indicating that common mechanisms may be responsible for the ecological effects of biodiversity at intra- and interspecific levels.

Our results have important implications for the conservation of species' populations and their communities. Much emphasis has been placed on conserving genetic variation within single species to maintain the long-term viability of populations (Falk & Holsinger 1991). Our results indicate that genetic impoverishment within plant populations may also have community-level consequences, supporting similar conclusions from recent studies (Hughes & Stachowicz 2004; Wimp *et al.* 2004; Reusch *et al.* 2005). Whether the mechanisms underlying the effects of diversity are additive or non-additive influences how we should approach the
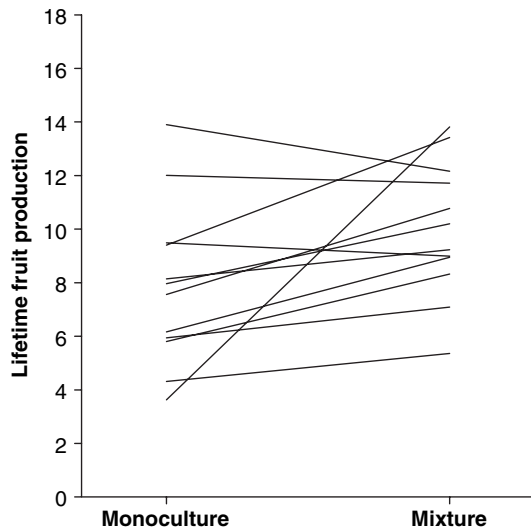
Figure 4 Mean genotype fitness of *Oenothera biennis* in monoculture and genetically mixed patches. Each line depicts the mean fitness of a single genotype, where fitness was measured as the mean number of unconsumed fruits. Patches containing four and eight genotypes were combined and are labelled as 'mixture' patches.

conservation of genetic variation. On the one hand, if the effects of diversity are additive, careful consideration should be paid to which genotypes are preserved and not necessarily the number of genotypes conserved. On the other hand, if genotypes interact and have non-additive effects on the community, it is important to consider the number and type of genotypes conserved. The development and implementation of conservation efforts should consider these community-wide consequences of maintaining genetic diversity for ecologically important phenotypic traits within species' populations (Bangert *et al.* 2005).

Plant fitness and evolutionary interactions

Compared to research on plant species diversity, the study of plant genotypic diversity has the unique potential for identifying evolutionary feedbacks between plants and their arthropod consumers (Rausher 2001). In our study, *O. biennis* genotypes had on average 27% higher lifetime fitness (i.e. fruit production) in genetically mixed patches compared with the fitness of the same genotypes in monocultures (mean$_{mix}$ = 10.0, SE = 0.73; mean$_{mono}$ = 7.86, SE = 0.87; one-tailed paired t_{11} = 2.45, P = 0.02; Fig. 4), which translates to a difference of approximately 300 seeds (M.T.J. Johnson, unpublished data). This indicates that a component of plant fitness was strongly influenced by the genetic diversity of plant neighbourhood. The specific agent of selection responsible for the increased fitness in diverse patches is unclear, but may include changes in

the abiotic environment because of facilitation or complimentarity between genotypes, altered levels of fungal pathogens or mutualistic interactions with plants, and/or changes in abundance and diversity of herbivores or predators. Both herbivory (Johnson & Agrawal 2005) and fungal pathogens (M.T.J. Johnson, unpublished data) are associated with reductions in plant fitness of *O. biennis*, but we have no direct evidence to suggest that these potential selective agents caused the increased fitness observed in genetically diverse plant patches. Nevertheless, our result suggests that a selective feedback between *O. biennis* and some aspect of the community may jointly influence the evolutionary and community dynamics between plants and arthropods if selection alters the genetic composition of plant populations (Johnson & Agrawal 2003).

CONCLUSIONS

Understanding the community- and ecosystem-level consequences of biodiversity is a central focus in ecology (Hooper *et al.* 2005). Although there has been considerable research on the effects of plant genetic variation on insects and pathogens in agricultural systems (Power 1988; Mundt 2002), the ecological consequences of genotype identity and genetic diversity within natural plant populations have received much less attention (Neuhauser *et al.* 2003; Whitham *et al.* 2003). We have demonstrated strong community and evolutionary consequences of plant genetic diversity for plant–arthropod interactions. Furthermore, our results suggest that conservation efforts should consider the community-level impacts of preserving genetic variation (Bangert *et al.* 2005).

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SUPPLEMENTARY MATERIAL

The following supplementary material is available online for this article from http://www.Blackwell-Synergy.com:

Appendix S1 Arthropods found on experimental plants.
Figure S1 The effects of plant genotype on arthropod richness and abundance across sampling dates.

SUPPLEMENTARY MATERIALS FOR ONLINE ARCHIVES

2
3   **Appendix S1** The assemblage of arthropods found on *Oenothera biennis* plants during the study.
4   The taxonomy and diet are indicated for the 92 taxa of herbivores (H), omnivores (O), and
5   predators (P) found on experimental *O. biennis* plants during the study.
6

	Order	Family	Species	Diet
Insects				
	Hemiptera (Homoptera)			
		Aphididae	*Aphis oestlundi*	H
		Cercopidae	*Aphrophora sp.*	H
			Philaenus spumarius	H
			species 1	H
			species 2	H
			species 3	H
		Cicadellidae	cf. *Endria* sp.	H
		Cicadellidae	*Cuerna sp.*	H
			species 1	H
			species 2	H
			species 3	H
			species 4	H
			species 5	H
		Flatidae	*Metcalfa pruinosa*	H
	Hemiptera (Heteroptera)			
		Alydidae	*Alydus eurinus*	H
		Lygaeidae	*Neortholomus scolopax*	H
			cf. *Ligyrocoris* sp.	O
		Miridae	*Adelphocoris lineolatus*	O
			Leptopterna dolobrata	H
			Lygus lineolaris	O
			Neurocolpus nubilus	O
			Plagiognatus brunneus	O
			Plagiognatus politus	O
			species 1	O
		Nabidae	*Nabicula subcoleoptrata*	P
			Nabis rufusculus	O
		Pentatomidae	*Coenus delius*	H
			Cosmopepla bimaculata	O
			Euschistus servus	H
			Podisus maculiventris	P
			species 1	O
			species 2	O
		Phymatidae	*Phymata americana*	P
		Unknown	species 1	O
			species 2	H
	Neuroptera	Chrysopidae	*Chrysopa* sp.	P
	Coleoptera			

	Lampyridae	cf. *Ellychnia* sp.	P
	Coccinellidae	*Coccinella trifasciata*	P
		Cocinella septumpunctata	P
		Hippodamia convergens	P
		Hippodamia glacialis	P
		Propylea quatuarodecipunctates	P
		species 1	P
		species 2	P
	Curculionidae	*Acanthoscelidius acephalus*	H
		Dietzella zimmermanni	H
		Phyllobius oblongus	H
		Tyloderma nigrum	H
		Tyloderma foveolata	H
	Chrysomelidae	*Altica* sp.	H
		Graphops pubescens	H
	unknown	species 1	H
Lepidoptera	Momphidae	*Mompha brevivittella*	H
		Mompha stellella	H
		Mompha sp. nov.	H
	Noctuidae	*Schinia florida*	H
	Pyralidae	*Desmia funeralis*	H
	Tortricidae	*Sparganothis reticulatana*	H
Diptera	Syrphidae	species 1	P
Arachnids			
Opiliones	Phalangiidae	species 1	P
Acari	Trombidiiae	species 2	P
Araneae	Araneidae	*Argiope trifasciata*	P
		Mangora gibberosa	P
	Clubionidae	*Clubiona johnsoni*	P
	Dictynidae	*Dictyna foliacea*	P
	Gnaphosidae	*Micaria/Zelotes* sp.	P
	Linyphiidae	*Hypselistes florens*	P
		Neriene montana	P
	Lycosidae	*Trochosa terricola*	P
	Philodromidae	*Philodromus cespitum*	P
		Thanatus formicinus	P
		Tibellus oblongus	P
	Salticidae	*Pelegrina flavipedes*	P
		Pelegrina sp. 2	P
		Phidippus clarus/princeps	P
		Salticus scenicus	P
		Tutelina similis	P
		species 1	P
	Tetragnathidae	*Leucauge venusta*	P
	Theridiidae	*Enoplognatha ovata*	P
		Neottiura bimaculata	P
		Theridula emertoni	P
		Thymoites unimaculatus	P

Thomisidae	*Misumenops* sp.	P
	Xysticus sp.	P
Unknown	species 1	P
	species 2	P
	species 3	P
	species 4	P
	species 5	P
	species 6	P
	species 7	P

7

8

8   **Figure S1** The effects of genotype identity on (a) total arthropod species richness and (b) total

9   arthropod abundance across sampling dates. Lines depict individual genotypes and connect best

10   linear unbiased predictors (similar to ls-means, Littell *et al.* 1996) between sampling dates. Total

11   arthropod richness and total arthropod abundance varied among genotypes.

12