PLANT GENOTYPIC DIVERSITY AND ITS INFLUENCE ON ARTHROPOD COMMUNITIES

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Scott Harold McArt

May 2012

PLANT GENOTYPIC DIVERSITY AND ITS INFLUENCE ON ARTHROPOD COMMUNITIES

Scott Harold McArt, Ph. D.

Cornell University 2012

Genotypic diversity varies markedly among populations of organisms, however the ecological consequences of intraspecific diversity are poorly understood. Here I directly compare the effects of plant species and genotypic diversity on arthropod communities and ecosystem functioning. Through behavioral observations, field experiments, and laboratory assays, I show contrasting mechanisms by which arthropod species richness and evenness are altered by each type of plant diversity. I then show how genotypic diversity of the common evening primrose (*Oenothera biennis*) reduces herbivory by changing herbivore behavior and physiology, ultimately decreasing consumption efficiency. Finally, I show how *O. biennis* genotypic diversity attenuates induced plant resistance to the Japanese beetle (*Popillia japonica*), indirectly increasing plant susceptibility to three native seed predators. As a result, this highly invasive beetle actually increases the fitness of *O. biennis* by consuming it. Overall, I show that plant genotypic diversity contributes substantially to the structure and functioning of arthropod communities through both direct and indirect mechanisms.

BIOGRAPHICAL SKETCH

Scott grew up in upstate New York and has been interested in nature for as long as he can remember. His lifelong passion for running and cross-country skiing has always kept him outdoors with a healthy dose of respect for the beauty and complexity of ecological communities. Week long summer canoe trips to the Temagami region of Ontario, Canada were especially influential in cultivating his love of the outdoors during his middle school and high school years. During his undergraduate years at Dartmouth College he became interested in the formal study of ecology while taking classes and participating in research with numerous excellent professors and graduate students.

Upon completion of his undergraduate degree he moved to Alaska to train full time for competitive cross-country skiing. At the same time he started his first job in the field of ecology as a laboratory technician at the University of Alaska Anchorage (UAA). He was hooked from day number one and transitioned into a Masters student at UAA studying large herbivore nutritional ecology after two years of technician work. During his Masters he became interested in community ecology and chemical ecology. Cornell had recently hired numerous excellent community and chemical ecologists and Scott was very happy that one of them was recruiting Ph. D. students when he finished his Masters!

During his Ph. D. Scott has been trained as a field-oriented community ecologist who uses chemical ecology to understand the mechanistic basis of plant-animal interactions. He tests theory via a combination of field natural history, manipulative field and laboratory experiments, detailed chemical and nutritional analyses, and multivariate statistical techniques. He thoroughly enjoys research, teaching, and mentoring students, and plans to continue these activities as a professor at a university.

To my daughter Sigrid

Follow your dreams, live the life you have imagined

ACKNOWLEDGMENTS

First and foremost, I would like to acknowledge the incredible support of my wife Jess. You have made my life and this dissertation better in countless ways. My parents, Ebba and Craig, have nurtured my curiosity and ingrained me with my work ethic.

This dissertation would never have happened without the advice, support, innovation, and energy of my advisor, Dr. Jennifer Thaler. In addition, my committee members – Drs. Anurag Agrawal and Andre Kessler – have contributed substantially to how I currently view ecological communities, species interactions, and the world as a whole. It is rare to find true mentors in life, and I have had the great pleasure to work with and learn from these three mentors for the past five years.

In addition to my committee I have greatly benefited from an amazing community of scientists at Cornell. My dissertation started via an excellent collaboration with a fellow graduate student in Ecology and Evolutionary Biology – Susan Cook-Patton. I can only hope that my future professional collaborations will be as fruitful and enjoyable. Rick Hoebeke generously loaned his expertise on the local arthropod fauna on numerous occasions. I am indebted to Paul Cooper, who not only took care of my plants for five years but also taught me how to ski moguls. Joe Simonis taught me the use (and potential abuse) of nonmetric multidimensional scaling. Amy Hastings helped me start to understand the most exciting field pattern I have found thus far in my career by genotyping plants I brought to her at the drop of a hat. I have also thoroughly enjoyed our friendship and commiseration about being new parents outside of our professional endeavors. Bernd Blossey generously loaned the large field cages that were critical for my Japanese beetle induction experiments. Rayko Halitschke has helped me learn more practical knowledge about plant chemical analyses than anyone I have ever interacted with. Juha-Pekka Salminen has allowed me to continue my pursuit of the chemical ecology of phenolics at the highest level available. Finally, I have greatly benefited from and enjoyed interacting with Katja Poveda and Rob Raguso – both of whom I have considered "informal advisors" during my Ph. D.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function

Introduction

Rapid human alterations of the environment are leading to substantial reductions in biodiversity (Pimm et al. 1995, Chapin et al. 2000). These changes may have profound consequences, as diverse systems can be more productive (Tilman et al. 1996, Cardinale et al. 2007), stable (Reusch et al. 2005, Tilman et al. 2006) and resistant to invasions (Levine 2000) than less diverse systems. While most biodiversity research has focused on species diversity, recent work has found that genotypic diversity within species can also have pronounced ecological consequences (Wimp et al. 2004, Hughes et al. 2008, Parker et al. 2010). However, to date, there has been no direct comparison of either the relative importance of genotypic and species diversity, or the mechanisms by which genotypic and species diversity alter community structure and ecosystem functioning.

Greater productivity in diverse mixtures may be due to the increased probability of including a highly productive species (*i.e.,* the sampling effect), dominance of highly productive species in polycultures (*i.e.,* a positive selection effect), or reduced competition in polycultures due to niche partitioning or facilitation among the interacting species (*i.e.,* positive complementarity) (Loreau and Hector 2001, Hooper et al. 2005). Niche partitioning, in particular, should be affected by trait variation and relatedness among interacting organisms (Petchey et al. 2004, Villeger et al. 2008, Cadotte et al. 2009, Hillebrand and Matthiessen 2009). Plant assemblages with greater trait variation are predicted to exhibit less niche overlap, more efficiently utilize resources, and achieve higher productivity than less variable assemblages

(Cadotte et al. 2009, Hillebrand and Matthiessen 2009). Because trait variation within a single species is expected to be lower than trait variation among multiple species, one would predict that biomass increases in response to plant genotypic diversity would be less pronounced than that of species diversity. Despite these expectations, a few recent studies have suggested that plant genotypic diversity may have similar impacts to species diversity on biomass, fitness, and other ecosystem functions (Schweitzer et al. 2005, Crutsinger et al. 2006, Johnson et al. 2006). However, these studies did not manipulate plant genotypic and species diversity simultaneously.

Two alternative hypotheses predict how general patterns of arthropod community diversity will respond to plant diversity (for hypotheses addressing responses of specific trophic levels, see Root 1973, Barbosa et al. 2009). The *resource specialization hypothesis* posits that because many arthropods specialize on distinct host plant species, increasing the number of plant species in a patch will attract a more diverse fauna (Hutchinson 1959, Strong et al. 1984). Alternatively, the *more individuals hypothesis* suggests that as available energy (*e.g.,* plant biomass) increases, there will be a greater number of arthropod individuals present, and thus a higher probability of observing more arthropod species (Srivastava and Lawton 1998). Because plant biomass is expected to increase with plant diversity, arthropod diversity is expected to also increase through abundance-driven accumulation of species. When considered in the context of plant trait variation, both of these hypotheses predict that the response of arthropods to plant species diversity will be greater than to plant genotypic diversity. In contrast, two recent studies have suggested that plant genotypic and species diversity may similarly impact the structure of higher trophic level communities (Crutsinger et al. 2006, Johnson et al. 2006).

In this study, we present the first direct comparison of the effects of plant genotypic and species diversity on arthropod species diversity and plant productivity (an ecosystem function) by simultaneously manipulating these two levels of diversity within a single field experiment.

Materials and Methods

Study species and plant propagation

We manipulated plant genotypic diversity with *Oenothera biennis* L (Common Evening Primrose, Onagraceae), a native herbaceous plant that is common to old-fields and disturbed areas in eastern North America. *O. biennis* reproduces via a permanent translocation heterozygosity mating system, which results in clonally-related seeds (Cleland 1972) (*i.e.,* all seeds produced by an individual plant are genetically identical to each other and the parent). *O. biennis* genotypes vary from an annual to perennial life-history strategy that is known to plastically respond to environment (Johnson 2007).

We collected *O. biennis* seeds from individual plants in 24 distinct populations around Ithaca, NY. Each genotype used in this experiment was determined to be unique using nine polymorphic microsatellite loci specifically developed for *O. biennis* (Larson et al. 2008). To reduce maternal effects, we first grew the seeds in a common garden in 2007, which was sprayed with insecticide at regular intervals throughout the growing season, and we used seeds collected from these plants (24 genotypes) for our experiment.

We focus on comparing the effects of plant genotypic versus species diversity exclusively (and not functional group diversity) because genotypic variation within a species presumably offers no functional group diversity. Thus, for the species treatments we did not have nitrogen-fixers in the species pool, because the presence of this functional group can overwhelm effects of

richness (Hooper et al. 2005, Cadotte et al. 2009). We used 24 species that are common in old-fields, co-occur with *O. biennis*, germinate easily, and do not possess particularly notable functional attributes: *Carex* sp.1, *Carex* sp.2*, Cichorium intybus, Daucus carota, Dianthus armeria, Dipsacus sativus, Elymus repens, Epilobium parviflorum, Galium mollugo, Leucanthemum vulgare, Pastinaca sativa, Penstemon digitalis, Phleum pratense, Plantago lanceolata, Rudbeckia hirta, Rumex crispus, Saponaria officinalis, Silene vulgaris, Solidago altissima, Symphyotrichum simplex, Symphyotrichum lateriflorum, Verbascum blattaria, Verbascum thapsus,* and *Verbena hastata*. Seeds were collected from multiple individuals at three separate fields around Ithaca, NY in 2007 and pooled to generate genetically-diverse seed sources for each species. This species pool includes three annuals, six biennials, and fifteen perennials (Table S2).

We cold stratified (4°C, four days) all seeds in April 2007, sowed them into 96-well trays filled with soil (Pro-mix "BX" with biofungicide, Premier), and thinned germinated seedlings to a single individual per well. Plants were watered *ad libitum* and fertilized weekly (21-5-20 NPK, 150 ppm) while in the greenhouse (14:10 hour light:dark cycle, 5 weeks) and then field-hardened in an outdoor mesh cage (one week) prior to planting in the field.

Field establishment

In late May 2008, we established the experiment in an abandoned agricultural field near Ithaca, NY where the soil was plowed, but otherwise untreated. Using a substitutive design and our pools of 24 *O. biennis* genotypes and 24 old-field species, we constructed four treatments: genotypic monocultures ("GM", one *O. biennis* genotype), genotypic polycultures ("GP", eight *O. biennis* genotypes), species monocultures ("SM", multiple genotypes of a single species that did not include *O. biennis*), and species polycultures ("SP", eight species that did not include *O.*

biennis). All plots contained eight equally spaced individuals arrayed in a ring 0.5 m in diameter. This density of plants is common in old-field plant communities and *O. biennis* populations (McArt and Cook, *pers. obs.*). The original design included 264 plots, but due to the loss of individuals within plots, we restricted our analyses to the 230 plots that experienced no mortality (GM: $n = 46$; GP: $n = 69$; SM: $n = 66$; and SP: $n = 49$). Every genotype or species appeared ~20 times in polyculture and 2-3 times in monoculture (except for two *O. biennis* genotypes that only had one monoculture each due to mortality, and *Verbascum thapsus* which had no monocultures due to mortality).

In addition to the ring of plants, we grew a single *O. biennis* focal plant in the middle of every plot to test how the diversity treatments impacted natural selection on *O. biennis*. We ensured that the focal plant was always a different genotype than the *O. biennis* ring plants. Thus, our treatments are balanced such that species "monocultures" always contained two species (eight plants of the same species in a ring and one *O. biennis* focal plant) and genotype "monocultures" always contained two genotypes (eight plants of the same *O. biennis* genotype in a ring and one *O. biennis* focal plant of a different genotype), while polycultures always contained nine genotypes or nine species. The natural selection data will be presented elsewhere, but here we include the focal plant in analyses for completeness and accuracy (see *Plant analyses*).

Plots were separated by 1.5 m and we clipped encroaching weeds by hand every 2-3 weeks to ensure treatments remained consistent throughout the summer. During the experiment 18 of the 24 species bolted and flowered, and all of the *O. biennis* genotypes bolted and flowered. For *O. biennis* genotypes and plant species that bolted, nearly every individual plant bolted and bolting did not vary by diversity treatments (*O. biennis* genotypes: Pearson $\chi^2 = 0.06$,

$P = 0.80$; plant species: Pearson $\chi^2 = 0.39$, $P = 0.53$). Thus, diversity did not affect life-history expression of the plants.

Plant analyses

During the 2nd and 3rd week of October, we harvested the aboveground biomass of every plant, which was then dried (65°C) and weighed to the nearest 0.1g. We analyzed plant productivity via a two-way analysis of variance with main effects of diversity level (monocultures or polycultures) and level of plant relatedness (genotypic or species), plus their interaction (JMP, Version 7. SAS Institute Inc., Cary, NC, 2007). An alternative approach is to view this experiment as four distinct treatments and conduct analyses via a one-way ANOVA, which we have also done to verify that all two-way ANOVA results were similar to one-way ANOVA results. To account for spatial heterogeneity in the field, we divided the experiment into six blocks, where each block contained equal proportions of the four treatments, and included block as a random effect in all analyses. We analyzed both the full plot data (the sum of eight ring plants plus the focal plant) as well as the ring data alone (sum of the eight ring plants) for all of our analyses. Excluding the focal plant from our analyses (*i.e.,* analyzing only the ring plants) did not alter the direction or significance of any of our results. We present the full plot data because it includes all the interactions that occurred in the plot.

Loreau and Hector (2001) devised a method to partition diversity effects into complementarity and selection effects. We modified this technique slightly to account for the absence of true monocultures (due to the focal plant in the middle of the ring). Whether a genotype occurred in the center or the ring had a substantial effect; for example, a single, representative genotype produced on average 110 g biomass in the ring versus 69 g as a focal plant. Thus, to determine the expected biomass of a ring plant in polyculture, we used the

average value of an individual genotype or species from the monoculture ring. To determine the expected biomass of a focal plant, we took the average value of the 2 or 3 times that this genotype occurred in the middle of a genotypic monoculture (if calculating expected values for a genotypic polyculture) or a species monoculture (if calculating expected values for a species polyculture). Our modifications to Loreau and Hector's methods (2001) are indicated in bold, while the remainder of the text is replicated from the original paper.

Define for any polyculture:

M_i = **average yield of an individual from species or genotype *i* in the low diversity treatment; for species this is the average of all individuals in a ring, for genotypes this was either the average of all individuals in a ring or of all individuals in the center of a genotypic or species monoculture**

Y_{Oi} = observed yield of species *i* **or genotype *i*** in the polyculture

$Y_O = \sum_i Y_{Oi}$ Y_O = total observed yield of the polyculture

$RY_{Ei} = 1$ = expected relative yield of species *i* or genotype *i* in a polyculture (**which is 1 because the yield is expected to be identical to that in the monoculture)**

$RY_{Oi} = Y_{Oi} / M_i$ = observed relative yield of species *i* **or genotype *i*** in the polyculture

$Y_{Ei} = RY_{Ei} M_i = M_i =$ **expected yield of an individual from species or genotype *i* in the polyculture**

$Y_E = \sum_i Y_{Ei}$ = total expected yield of the polyculture

$\Delta Y = Y_O - Y_E$ = deviation from total expected yield in the polyculture

$\Delta RY = RY_{Oi} - RY_{Ei}$ = deviation from expected relative yield of species i **or genotype *i*** in the polyculture

N = number of species in the polyculture

Complementarity is calculated as $N\overline{\Delta RY}\,\overline{M_i}$ and selection as $N\mathrm{cov}(\Delta RY, M)$. If we exclude the focal plant, the modification produces mathematically equivalent results to the original method and our results do not qualitatively change (see Fig. S1 in the *Ecological Archives*). Note that one species, *Verbascum thapsus*, did not survive in monoculture, so the three monocultures and ten species polycultures with this species were excluded from the complementarity and selection analyses.

To examine how competition intensity changed from monoculture to polyculture we calculated the corrected index of relative competition intensity (CRCI) (Oksanen et al. 2006). This index reduces bias inherent to other indices by extending the range of arguments where the function behaves linearly. To minimize errors due to the aberrant behavior of individuals, we first calculated mean values of individual genotype or species performance in each treatment. We then calculated competition intensity as CRCI = arc sin$((X_r - X_c)/(\max X_r, X_c))$ (Oksanen et al. 2006) where X_r is the mean performance of a particular genotype or species in monoculture and X_c is the mean value in polyculture. Note that CRCI is unitless, and values further from 0 indicate greater differences in competition intensity between treatments.

Arthropod analyses

In mid-July and again in mid-August, we censused arthropods by visually surveying every plant in the experiment ($N = 2070$ plants). We identified familiar arthropods in the field or collected specimens of unknown arthropods for later identification. To identify arthropods, we consulted relevant literature and the expertise of E. R. Hoebeke (Dept. of Entomology, Cornell University). Arthropods were identified to the lowest taxonomic level possible, generally species or genus and occasionally family. We also assigned arthropods to a feeding guild (herbivore, predator, omnivore or detritivore) based on relevant literature and the expertise of E. R.

Hoebeke. We lumped together parasitoids that were less than 3mm in length (n = 10) because of logistical difficulties associated with their field identification. We did not attempt to count or identify arthropods that were less than 1 mm in length (*e.g.,* thrips, collembola).

Similar to the plant analyses, we used a two-way ANOVA with block as a random effect to test for the effects of plant diversity on cumulative arthropod abundance and richness. Repeated-measures analyses yielded qualitatively identical results to the cumulative dataset, so we chose the latter to facilitate more sophisticated follow-up analyses. We used a log+1 transformation on the abundance data to improve normality.

To test for the effect of plant biomass on arthropod abundance we divided arthropod abundance by the biomass of each plant and log-transformed the resulting data to improve normality. Division assumes a linear relationship between these two variables and indeed a linear function provided the best fit for the data (R^2_{linear} = 0.40, $R^2_{logarithmic}$ =0.34). Next, because of the well known non-linear relationship between arthropod abundance and richness, we used individual-based rarefaction (Ecosim 7.0, (Gotelli and Entsminger 2006)) to test the effect of cumulative arthropod abundance on cumulative richness. We conducted rarefaction at each level of plant relatedness independently in order to compare arthropod communities drawn from the same distribution (Gotelli and Graves 1996). To test for differences in rarefied arthropod richness we used ANOVA with post-hoc independent contrasts.

We visualized the similarity among arthropod assemblages on genotypes and species with nonmetric multidimensional scaling (NMDS, Vegan 1.15-1, R version 2.8.1). The semimetric Bray-Curtis dissimilarity coefficient was used to compare arthropod assemblages on monocultures of *O. biennis* genotypes and plant species using a presence/absence dataset. We then conducted 500 simulations on a random dataset with identical parameters (McCune and

Grace 2002) to verify that random stress (mean = 0.28) was significantly higher than model stress (mean = 0.23).

Results

We found an overall positive effect of diversity on plot-level plant productivity (diversity: $F_{1,221.4} = 15.62$, $P = 0.0001$). Genotypic and species polycultures showed nearly equivalent increases in productivity (diversity × relatedness level: $F_{1,221.4} = 1.84$, $P = 0.18$): total biomass was 16.8% and 16.9% greater in genotypic and species polycultures than in monocultures, respectively (Fig. 1.1a). Analysis via one-way ANOVA produced similar results ($F_{3,221.3} = 122.6$, $P < 0.0001$): *post-hoc* independent contrasts on plant biomass indicated that genotypic polycultures were more productive than genotypic monocultures ($F_{1,221.4} = 14.0$, $P = 0.0002$) and that species polycultures were marginally more productive than species monocultures ($F_{1,221.1} = 3.4$, $P = 0.065$). While selection effects were weak to negative (Fig. 1.1d), we found that complementarity among individuals contributed to the increases in plant productivity and did not differ between each level of relatedness ($F_{6,102}=1.06$, $P = 0.39$, Fig. 1.1d). Another metric more commonly employed in the plant competition literature – the *corrected index of relative competition intensity* (CRCI) (Oksanen et al. 2006) – showed similar results: there were similar decreases in competition intensity with increasing plant diversity (-0.79 for genotypic diversity and -0.56 for species diversity, $F_{1,45} = 0.07$, $P = 0.79$). Thus, our comparable changes in complementarity and competition intensity may explain the remarkably similar increases in plot-level productivity that we observed in both genotypic and species polycultures of plants.

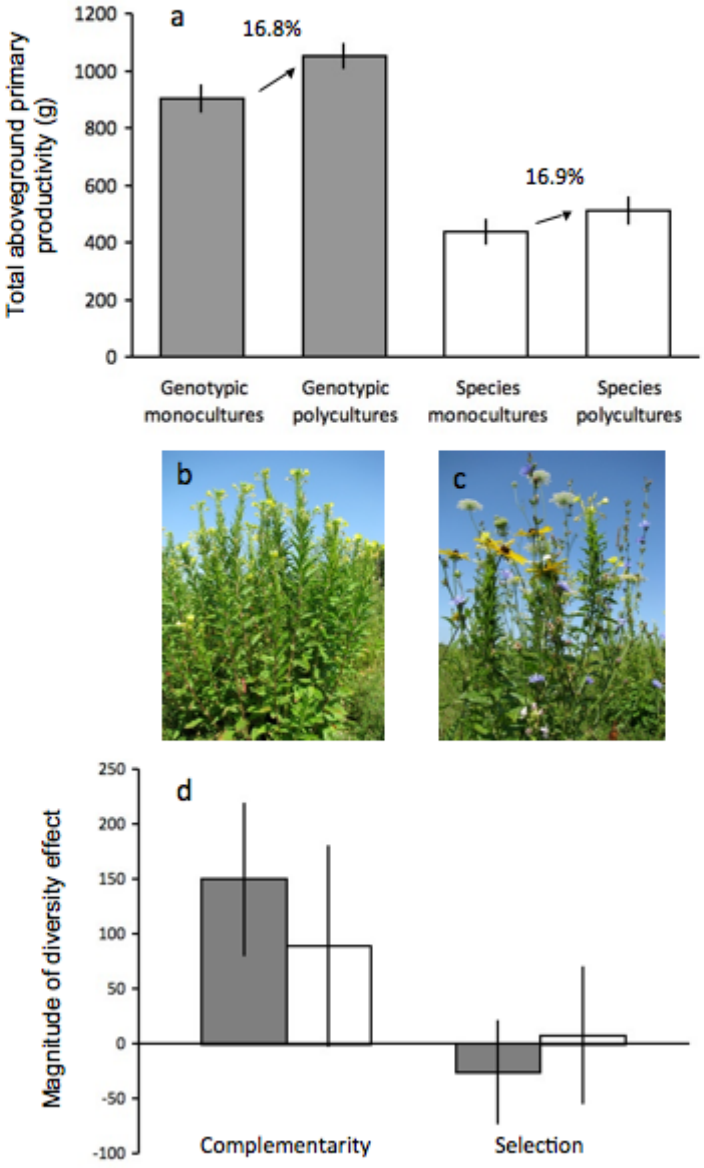

Figure 1.1: Plant diversity effects on productivity. (a) Genotypic and species polycultures had ~17% more biomass than their respective monocultures (LS means ± s.e.); (b) Genotypic polyculture; (c) Species polyculture. (d) The overall diversity effect can be partitioned into *complementarity* or *selection effects* (Loreau and Hector 2001) for genotype polycultures (dark columns) and species polycultures (light columns), means ± 95% confidence intervals.

To determine the effects of plant biodiversity on higher trophic-level communities, we non-destructively surveyed arthropods that naturally recruited to each plant twice during peak growing season. In total, we made 76,753 observations of ~252 arthropod species. We found that arthropod richness increased with both types of plant diversity, but changed more dramatically in plant species polycultures (diversity × relatedness level: $F_{1,221.5} = 10.96$, $P = 0.001$; Fig. 1.2a).

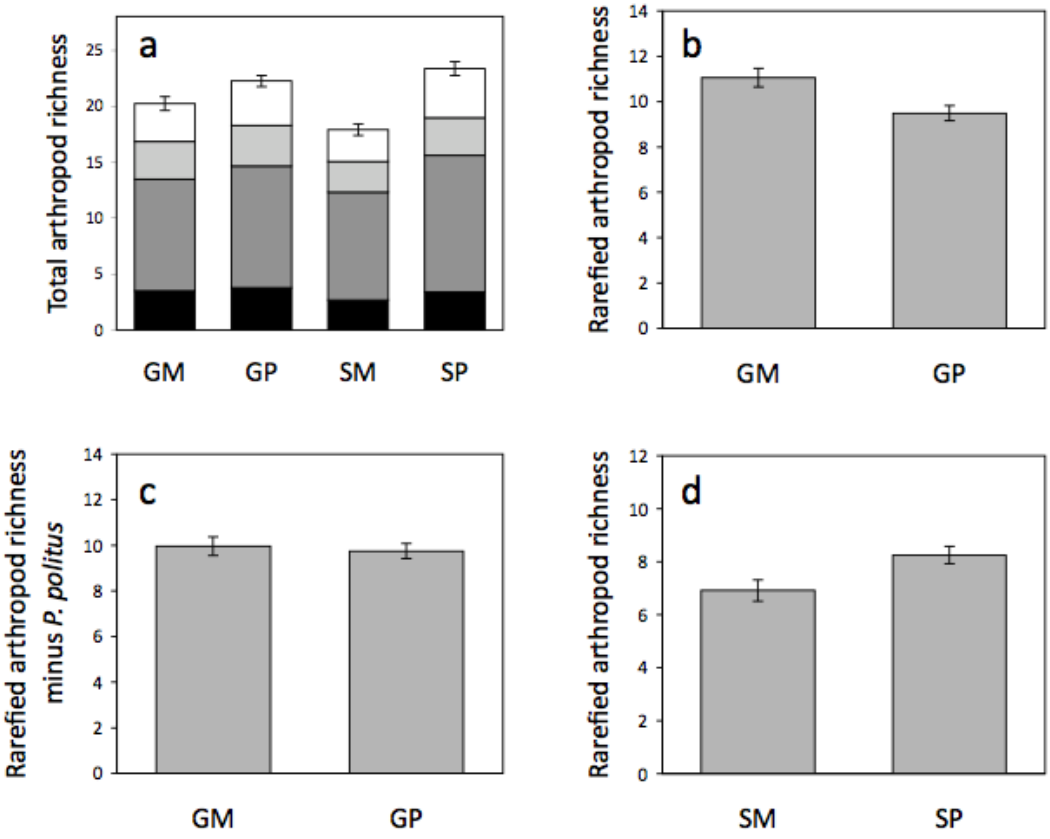

Figure 1.2: Relationship between plant diversity and arthropod richness. (a) Predators are represented in white, omnivores in light gray, herbivores in dark gray, and detritivores in black (Overall arthropod richness LS means ± s.e.); (b) Rarefied arthropod richness decreased with plant genotypic diversity (LS means ± s.e.); (c) After removing the dominant insect, *Plagiognathus politus*, from the dataset (*see* Results), rarefied arthropod richness showed no change with plant genotypic diversity (LS means ± s.e.); (d) Rarefied arthropod richness increased with plant species diversity (LS means ± s.e.); GM = genotypic monocultures, GP = genotypic polycultures, SM = species monocultures, SP = species polycultures.

Predators showed the most pronounced response to plant diversity, increasing in abundance 80% in species polycultures and 30% in genotypic polycultures (diversity: $F_{1,221}$ = 18.62, $P < 0.0001$; diversity × relatedness level: $F_{1,221.6} = 4.42$, $P = 0.037$), while increasing in richness 54% and 17% respectively (diversity: $F_{1,221.3} = 17.92$, $P < 0.0001$; diversity × relatedness level: $F_{1,221.8} = 3.87$, $P = 0.051$; Fig. 1.2a). Herbivores increased in abundance 44% and 30% in plant species and genotypic polycultures (diversity: $F_{1,221} = 8.54$, $P = 0.004$; diversity × level of relatedness level: $F_{1,221.2} = 0.007$, $P = 0.93$), while increasing in richness 30% and 10%, respectively (diversity: $F_{1,221.4} = 28.76$, $P < 0.0001$; diversity × relatedness level: $F_{1,220.9}$ = 6.80, $P = 0.010$; Fig. 1.2a). Omnivores and detritivores showed similar patterns of increases in abundance and richness at both levels of relatedness (Fig. 1.2a), although responses were not as pronounced. A one-way ANOVA approach to these analyses produced qualitatively identical results (not shown).

To further understand how plant diversity at each level of relatedness affected arthropod community structure, we first evaluated the influence of plant productivity on the number of arthropod individuals. After dividing arthropod abundance by plant biomass, the previously significant effect of plant diversity on arthropod abundance disappeared ($F_{1,221.6} = 0.19$, P = 0.66). Thus, arthropod abundance at both levels of relatedness was largely controlled by plant productivity and not by plant diversity *per se*.

We next used rarefaction to determine whether increases in arthropod species richness would be best explained by arthropod abundance (*more individuals hypothesis*) or by arthropod specialization on distinct host plants (*resource specialization hypothesis*). Contrary to expectations, rarefied richness decreased with plant genotypic diversity (post-hoc contrast: $F_{1,212}$

= 9.04, $P = 0.003$; Fig. 1.2b). This decrease in genotypic polycultures derives from a non-additive increase in the abundance of a single dominant species, *Plagiognathus politus* (Miridae), resulting in a lower richness than expected for that insect abundance. Removing *P. politus* from the dataset resulted in no difference in rarefied richness between treatments (Fig. 1.2c). Both of these results are consistent with greater arthropod abundances causing higher arthropod species richness in genotypic polycultures, supporting the *more individuals hypothesis.* Conversely, rarefied richness increased with plant species diversity (post-hoc contrast: $F_{1,212} = 6.27$, $P = 0.01$; Fig. 1.2d), indicating that the diversity of host-specific resources was important for the increase in arthropod richness. This result, in addition to the fact that the arthropod communities found on each plant species were far more divergent than the arthropod communities on each plant genotype (npMANOVA $F_{1,46} = 6.78$, $P < 0.0001$, Fig. 1.3), highlights the importance of *resource specialization* for the arthropod community response to plant species polycultures.

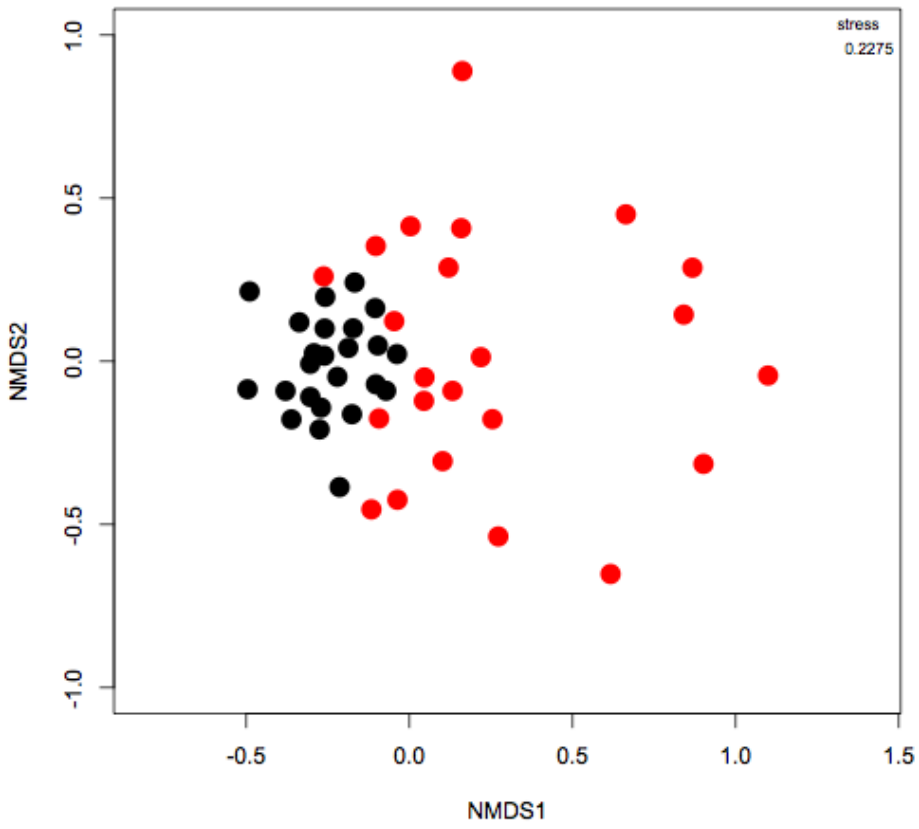

Figure 1.3: Nonmetric multidimensional scaling (NMDS) ordination of arthropod communities on each *O. biennis* genotype (black circles) and each old-field species (red circles) obtained using two dimensions and 100 permutations. Each point represents the summed community of three monoculture plots of either an individual genotype or an individual species. Analysis of Bray-Curtis dissimilarity coefficients indicate that arthropod community assemblages are more dissimilar among species than among genotypes (npMANOVA: $R^2 = 0.13$, $F_{1,46} = 6.78$, $P < 0.0001$). 500 simulations on a random dataset with identical parameters were used to verify that random stress (mean = 0.28) was significantly higher than model stress (mean = 0.23).

Discussion

We found that increasing either plant genotypic or species diversity led to quantitatively similar increases in primary production, and that the plausible mechanisms responsible for these effects – niche complementarity or decreased intensity of competition – were also similar for each type of diversity. A recent meta-analysis of the effects of biodiversity on primary

productivity found that the most diverse species assemblages had on average 1.7 times more biomass than monocultures (Cardinale et al. 2007). However, effect sizes ranged dramatically, and nearly 21% of studies showed negative to no effect of increasing diversity (Cardinale et al. 2007). The limited genotypic diversity literature also reports a wide range of increases in productivity across a diverse set of species: ~0 % in *Poa pratensis* (Vellend et al. 2010), ~14% in *Cakile edentula* ((Dudley and File 2007), ~17% (Kotowska et al. 2010) and ~69% (Crawford and Whitney 2010) in *Arabidopsis thaliana*, ~36% in *Solidago altissima* (Crutsinger et al. 2006), ~39 % in *Lupinus angustifolius* (Milla et al. 2009), and ~58% in *Zostera marina* (Reusch et al. 2005); mean = 33%). Thus, the 17% increases in productivity that we observed at both levels of plant diversity were lower than average, but not atypical for genotypic or species diversity experiments. This variation among experiments, in addition to the comparison of vastly different experimental designs, highlights the importance of comparing the effects of genotypic and species diversity within a single field experiment, under similar conditions, and for the same duration of time.

Several factors may have contributed to the similar increases in plant productivity we observed with each type of diversity in this study. First, because the effect of species diversity on plant productivity generally increases with time (Cardinale et al. 2007), the similar effects of genotypic and species diversity that we observed may be a short-term phenomenon. Because plants comprising genotypic monocultures acquire resources very similarly, genotypic monocultures may become resource-limited more quickly than genotypic and/or species polycultures (where plants may differ in their patterns of resource utilization, and thus may utilize a larger pool of resources). Resource limitation is believed to be a key mechanism of increased plant productivity in response to diversity (Hooper et al. 2005), and temporal

variability in post-disturbance resource limitation along a continuum of plant genotypic to species diversity may be critical in predicting the effect size of increases in productivity. For example, a recent study investigating the effects of *Solidago altissima* genotypic diversity found that the standardized effect size of genotypic diversity on plant productivity over one growing season was similar to the effect size of species diversity from a multi-year experiment (Crutsinger et al. 2006). Understanding how trait variation and plant diversity interact temporally to affect ecosystem functioning represents an important gap in the literature, and we suggest that further studies are needed in this area of research.

A second factor that may have impacted our plant productivity results are the specific species selected for this experiment. Genotypic diversity-productivity relationships have only been investigated in a handful of species (Reusch et al. 2005, Crutsinger et al. 2006, Dudley and File 2007, Milla et al. 2009, Bischoff et al. 2010, Crawford and Whitney 2010, Kotowska et al. 2010). Some of these species are particularly abundant in their communities (*i.e.,* dominant species) – for example, goldenrods (*Solidago altissima* (Crutsinger et al. 2006)) in old-field communities and eelgrass (*Zostera marina* (Reusch et al. 2005)) in coastal estuaries. Due to the myriad biotic and abiotic conditions experienced by dominant species, they may accumulate relatively large amounts of intraspecific trait variation, thus increasing the likelihood that the species will show a genotypic diversity-productivity effect. While *O. biennis* is not particularly dominant in old-field communities, it did respond positively to the growing conditions at our field site, producing the greatest amount of above-ground biomass of all species in our study (Fig. 1, Table S2). It is possible that larger plants are more likely to manifest a diversity effect since they may more fully fill the available niche space, thus accentuating the importance of niche partitioning. An ideal future experiment, though logistically large, might simultaneously

manipulate genotypic diversity in multiple different species with species diversity from a broad range of functional groups or phylogenetic distances.

A third possible mechanism for the similar increases in plant productivity we observed in this study may be that higher trophic levels are dampening the response of species polycultures and/or amplifying the response of genotypic polycultures. For example, in a separate experiment with *O. biennis*, levels of arthropod herbivory were 26% higher in genotypic monocultures compared to polycultures (McArt, *unpuplished data*). If greater differences in herbivory occur between genotypic diversity treatments compared to those that occur between species diversity treatments, interactions with higher trophic levels may amplify the biomass increases observed with genotypic diversity. The contribution of herbivory to overyielding in plant diversity experiments has received some recent attention (*e.g.,* (Haddad et al. 2009, Parker et al. 2010), but has yet to be compared among different types of plant diversity.

Lastly, non-linear declines in competition intensity with increasing genetic distance may explain the similar increases in plant productivity we observed in the genotypic and species diversity treatments. In other words, small changes in genetic distance among plants in genotypic monocultures versus genotypic polycultures may reduce competition to the same degree as much larger changes in genetic distance among plants in species monocultures versus species polycultures. Our data cannot distinguish among these multiple possibilities, yet each hypothesis is testable.

The second part of our study links arthropod community responses to each type of plant diversity. As expected, arthropod species richness responded less to plant genotypic diversity than species diversity (Fig. 1.2a). Interestingly, divergent mechanisms led to the increases in arthropod richness with each type of plant diversity (Figs. 1.2b-d, 1.3). Our data support the

hypothesis that resource specialization influenced the arthropod response to plant species diversity while abundance-driven accumulation of species (*more individuals hypothesis*) influenced the arthropod response to plant genotypic diversity. These patterns fit the notion that insects are more likely to specialize on host plant species than host plant genotypes. However, resource specialization may be an important driver of arthropod responses to plant species hybrids and their backcrossed progeny (Dungey et al. 2000, Wimp et al. 2004, Evans et al. 2008) suggesting that comparing the similarity of arthropod communities (*e.g.*, Fig. 1.3) across wider and more quantitative ranges of plant relatedness could greatly inform how plant genetics influences patterns of specialization, and ultimately shapes arthropod community structure.

Overall, our results emphasize that diversity is inherently hierarchical and that within-species diversity can play a more important role in competitive interactions and community structure than previously realized. It is currently unclear whether the same factors causing declines in species diversity similarly impact genotypic diversity, or whether these two levels of biodiversity are causally connected (Vellend 2005, Lankau 2009). Nonetheless, variation within species is inevitably lost before species themselves go extinct (Vitousek et al. 1997). Considering our results in relation to the longstanding focus on plant species diversity and ecosystem functioning (Chapin et al. 2000, Reich et al. 2001, Hooper et al. 2005, Tilman et al. 2006), we suggest that more emphasis be placed on conserving variation within species, elucidating the ecological consequences of genotypic diversity, and discerning how diversity among traits, relatedness, and trophic levels interact.

Acknowledgements

We thank J. Simonis for assistance with R code for the ordination plot and analyses, and E.R. Hoebeke for assistance with insect identifications. We thank M. Hunter, N. Gotelli, L. Oksanen, and R. Bingham for advice, M. Stastny, A. Hastings, A. Erwin, S. Campbell, S. Rasmann, B. Blossey, M. Geber, M. Weber, M. Johnson, M. Vellend, and several anonymous reviewers for manuscript critique, and G. Petchenka, M. Kersch-Becker, M. Stastny, C. Becker, S. Campbell, A. Erwin, P. Llambias and numerous Cornell undergraduates for field assistance. The NSF (DEB- 0950231 to A.A.A. and IGERT small grant in Biogeochemistry and Environmental Biocomplexity to S.H.M., S.C.C., and A.L.P.) and USDA-NRI (2006-35302-17431 to JST) supported this work.

REFERENCES

Barbosa, P., J. Hines, I. Kaplan, H. Martinson, A. Szczepaniec, and Z. Szendrei. 2009. Associational resistance and associational susceptibility: having right or wrong neighbors. Annual Review of Ecology, Evolution and Systematics **40**:1-20.

Bischoff, A., T. Steinger, and Müller-Schärer. 2010. The importance of plant provenance and genotypic diversity of seed material used for ecological restoration. Restoration Ecology **18**:338-348.

Cadotte, M. W., J. Cavender-Bares, D. Tilman, and T. H. Oakley. 2009. Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. PLoS ONE **4**:e5695.

Cardinale, B. J., J. P. Wright, M. W. Cadotte, I. T. Carroll, A. Hector, D. S. Srivastava, M. Loreau, and J. J. Weis. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. PNAS **104**:18123-18128.

Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. Nature **405**:234-242.

Cleland, R. E. 1972. Oenothera: cytogenetics and evolution. Academic P, New York.

Crawford, K. M. and K. D. Whitney. 2010. Population genetic diversity influences colonization success. Molecular Ecology **19**:1253-1263.

Crutsinger, G. M., M. D. Collins, J. A. Fordyce, Z. Gompert, C. C. Nice, and N. J. Sanders. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. Science **313**:966-968.

Dudley, S. A. and A. L. File. 2007. Kin recognition in an annual plant. Biology Letters **3**:435-438.

Dungey, H. S., B. M. Potts, T. G. Whitham, and H. F. Li. 2000. Plant genetics affects arthropod community richness and composition: evidence from a synthetic eucalypt hybrid population. Evolution **54**:1938-1946.

Evans, L. M., G. J. Allan, S. M. Shuster, S. A. Woolbright, and T. G. Whitham. 2008. Tree hybridization and genotypic variation drive cryptic speciation of a specialist mite herbivore. Evolution **62**:3027-3040.

Gotelli, N. J. and G. L. Entsminger. 2006. Ecosim: Null models software for Ecology. Acquired Intelligence Inc. & Kesey-Bear, Jericho, VT.

Gotelli, N. J. and G. R. Graves, editors. 1996. Null Models in Ecology. Smithsonian Institution, Washington, D.C.

Haddad, N. M., G. M. Crutsinger, K. Gross, J. Haarstad, J. M. H. Knops, and D. Tilman. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. Ecology Letters **12**:1029-1039.

Hillebrand, H. and B. Matthiessen. 2009. Biodiversity in a complex world: consolidation and progress in functional biodiversity research. Ecology Letters **12**:1405-1419.

Hooper, D. U., F. S. Chapin III, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setala, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A conensus of current knowledge. Ecological Monographs **75**:3-35.

Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood, and M. Vellend. 2008. Ecological consequences of genetic diversity. Ecology Letters **11**:609-623.

Hutchinson, G. E. 1959. Homage to Santa-Rosalia or Why are there so many kinds of Animals. American Naturalist **93**:145-159.

Johnson, M. T. J. 2007. Genotype-by-environment interactions lead to variable selection on life-history strategy in Common Evening Primrose (*Oenothera biennis*). Journal of Evolutionary Biology **20**:190-200.

Johnson, M. T. J., M. J. Lajeunesse, and A. A. Agrawal. 2006. Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. Ecology Letters **9**:24-34.

Kotowska, A. M., J. F. Cahill Jr., and B. A. Keddie. 2010. Plant genetic diversity yields increased plant productivity and herbivore performance. Journal of Ecology **98**:237-245.

Lankau, R. A. 2009. Genetic variation promotes long-term coexistence of *Brassica nigra* and its competitors. American Naturalist **174**:E40-E53.

Larson, E. L., S. M. Bogdanowicz, A. A. Agrawal, M. T. J. Johnson, and R. G. Harrison. 2008. Isolation and characterization of polymorphic microsatellite loci in common evening primrose (Oenothera biennis). Molecular Ecology Resources **8**:434-436.

Levine, J. M. 2000. Species diversity and biological invasions: Relating local process to community pattern. Science **288**:852-854.

Loreau, M. and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature **412**:72-76.

McCune, B. and J. B. Grace, editors. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, OR.

Milla, R., D. M. Forero, A. Escudero, and J. M. Iriondo. 2009. Growing with siblings: a common ground for cooperation or for fiercer competition among plants? Proceedings of the Royal Society B-Biological Sciences **276**:2531-2540.

Oksanen, L., M. Sammul, and M. Magi. 2006. On the indices of plant-plant competition and their pitfalls. Oikos **112**:149-155.

Parker, J. D., J.-P. Salminen, and A. A. Agrawal. 2010. Herbivory enhances positive effects of plant genotypic diversity. Ecology Letters **13**:553-563.

Petchey, O. L., A. Hector, and K. J. Gaston. 2004. How do different measures of functional diversity perform? Ecology **85**:847-857.

Pimm, S. L., G. J. Russell, J. L. Gittleman, and T. M. Brooks. 1995. The future of biodiversity. Science (Washington) **269**:347-350.

Reich, P. B., J. Knops, D. Tilman, J. Craine, D. Ellsworth, M. Tjoelker, T. Lee, D. Wedin, S. Naeem, D. Bahauddin, G. Hendrey, S. Jose, K. Wrage, J. Goth, and W. Bengston. 2001. Plant diversity enhances ecosystem responses to elevated CO2 and nitrogen deposition. Nature **410**:809-812.

Reusch, T. B. H., A. Ehlers, A. Haemmerli, and B. Worm. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. PNAS **102**.

Root, R. B. 1973. Organization of a plant-arthropod association in simple and diverse habitats - fauna of collards (*Brassica oleracea*). Ecological Monographs **43**:95-120.

Schweitzer, J. A., J. K. Bailey, S. C. Hart, and T. G. Whitham. 2005. Nonadditive effects of mixing cottonwood genotypes on litter decomposition and nutrient dynamics. Ecology **86**:2834-2840.

Srivastava, D. S. and J. H. Lawton. 1998. Why more productive sites have more species: A experimental test of the theory using tree-hole communities. American Naturalist **152**:510-529.

Strong, D. R., J. H. Lawton, and R. Southwood. 1984. Insect on Plants: Community Patterns and Mechanisms. Harvard UP, Cambridge, MA.

Tilman, D., P. B. Reich, and J. M. Knops. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature **441**:629-632.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature **379**:718-720.

Vellend, M. 2005. Species diversity and genetic diversity: Parallel processes and correlated patterns. American Naturalist **166**:199-215.

Vellend, M., E. B. M. Drummond, and H. Tomimatsu. 2010. Effects of genotype identity and diversity on the invasiveness and invasibility of plant populations. Oecologia **162**:371-381.

Villeger, S., N. W. H. Mason, and D. Mouillot. 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. Ecology **89**:2290-2301.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. Science **277**:494-499.

Wimp, G. M., W. P. Young, S. A. Woolbright, G. D. Martinsen, P. Kelm, and T. G. Whitham. 2004. Conserving plant genetic diversity for dependent animal communities. Ecology Letters **7**:776-780.

CHAPTER 2

Relationships between arthropod richness, evenness, and diversity are altered by complementarity among plant genotypes

Introduction

Biodiversity is known to affect the stability (Elton 1958, Tilman et al. 2006, Haddad et al. 2010), productivity (Tilman et al. 1996, Crutsinger et al. 2006, Cardinale et al. 2007), and trophic interactions (Duffy et al. 2007, Parker et al. 2010) of communities. Although richness (*e.g.,* the number of species) has recently dominated as the primary description of biodiversity in the literature (Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006), biodiversity can be quantified via richness, evenness (the relative abundance distribution of species in a community (Smith and Wilson 1996)), or the combination of these two metrics (*e.g.,* Shannon proportional diversity (Margalef 1958, Stirling and Wilsey 2001)).

Since diversity-function relationships have largely relied upon richness as a representative measure of biodiversity (Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006), but evenness also affects community processes and ecosystem functions (Hillebrand et al. 2008, Dickson and Wilsey 2009, Wittebolle et al. 2009, Crowder et al. 2010), it is important to know when (and how) these aspects of biodiversity are related, or whether they should be considered separately. Some theory, based on mathematical models, predicts strong and positive relationships between richness, evenness, and proportional diversity (De Benedictis 1973, May 1975). Other theory suggests that evenness and richness are independent measures of biodiversity (Whittaker 1965, Hurlbert 1971, Magurran 1988), and that their association must be tested empirically (Bell 2000). Accordingly, studies that have examined relationships between

richness, evenness, and proportional diversity have found mixed results: while some positive relationships exist (Sugihara 1980, Stirling & Wilsey 2001, Willig et al. 2003), numerous null (Willig et al. 2003, Ma 2005, Bock et al. 2007) or negative (Cook & Graham 1996, Weiher and Keddy 1999, Wilsey et al. 2005) relationships also occur.

Empirical studies addressing relationships between richness, evenness, and proportional diversity have attempted to understand the mechanisms leading to variable relationships. For example, Stirling & Wilsey (2001) hypothesize that richness and evenness are altered by different ecological processes (dispersal and biotic interactions, respectively) that may vary independently. While mechanisms such as these may exist, a striking pattern emerging from these studies is that they almost exclusively restrict their analyses to within-taxa and within-trophic level relationships of richness, evenness, and proportional diversity (Stirling & Wilsey 2001, Willig et al. 2003, Bock et al. 2007, *but see* Root 1973).

Studies that do investigate among-trophic level relationships (*e.g.*, plant vs. animal richness) typically focus on the same aspect of biodiversity at each trophic level. For example, across-trophic level studies have shown that arthropod richness can be altered by plant richness (Siemann et al. 1998, Crutsinger et al. 2006, Johnson et al. 2006, Cook-Patton et al. 2011), arthropod evenness can be altered by plant evenness (Murdoch et al. 1972), and arthropod proportional diversity can be altered by plant proportional diversity (Murdoch et al. 1972, Parker et al. 2001, Wimp et al. 2004). However, whether biodiversity-mediated interactions *among* trophic levels alter the relationships between different aspects of biodiversity *within* a trophic level has received little attention. Interactions between different aspects of diversity across trophic levels could have important consequences for community processes and ecosystem functions (Duffy et al. 2007).

Here, we directly test how relationships between arthropod richness, evenness, and proportional diversity are altered by genotypic richness of the Common Evening Primrose (*Oenothera biennis*). The two questions we address in this paper are: (1) How are relationships between arthropod richness, evenness, and proportional diversity modified by plant genotypic richness, and (2) What mechanisms drive these patterns?

Materials and Methods

Study species and plant propagation

We manipulated genotypic richness of *Oenothera biennis* L (Common Evening Primrose, Onagraceae), a native herbaceous plant that is common to old-fields and disturbed areas in eastern North America. *O. biennis* reproduces via a permanent translocation heterozygosity mating system, which results in seeds that are genetically identical to each other and the parent (Cleland 1972, Johnson 2010).

We collected *O. biennis* seeds from individual plants in 24 distinct populations around Ithaca, NY. Each genotype used in this experiment was determined to be unique using nine polymorphic microsatellite loci developed for *O. biennis* (Larson et al. 2008). To reduce maternal effects, we first grew the seeds in a common garden in 2007, which was sprayed with insecticide at regular intervals throughout the growing season, and we used seeds collected from these plants (24 genotypes) for our experiment. We cold stratified (4°C, four days) all seeds for the first field experiment in April 2008, sowed them into 96-well trays filled with soil (Pro-mix "BX" with biofungicide, Premier), and thinned germinated seedlings to a single individual per well. Plants were watered *ad libitum* and fertilized weekly (21-5-20 NPK, 150 ppm) while in the greenhouse (14:10 hour light:dark cycle, 5 weeks) and then field-hardened in an outdoor mesh

cage (one week) prior to planting in the field. These conditions were replicated for the follow-up field experiment in 2009, which employed 21 genotypes – a subset of the original 24 genotypes.

Field establishment

In late May 2008, we established the first field experiment in an abandoned agricultural field near Ithaca, NY where the soil was plowed, but otherwise untreated. Using our pool of 24 *O. biennis* genotypes, we constructed two treatments: genotypic monocultures (one *O. biennis* genotype) and genotypic polycultures (eight *O. biennis* genotypes). All plots contained eight equally spaced individual plants arrayed in a ring 0.5 m in diameter, and plots were separated by 1.5 m. We clipped encroaching weeds by hand every 2-3 weeks to ensure treatments remained consistent throughout the summer. The original *O. biennis* design included 120 plots, but due to the loss of individuals within plots, we restricted our analyses to the 115 plots that experienced no mortality (monocultures: $n = 46$; polycultures: $n = 69$). Every genotype appeared ~20 times in polyculture and two times in monoculture (except for two *O. biennis* genotypes that had one monoculture each due to mortality). Due to its large size, we divided our experiment into six spatial blocks where each block contained the same proportion of monocultures and polycultures. Additional details of the experimental design of this field experiment can be found in Cook-Patton et al. (2011).

In addition to the ring of plants, we grew a single *O. biennis* focal plant in the middle of every plot to test how plant richness impacted natural selection on *O. biennis* (the natural selection data will be presented elsewhere). Including or excluding the focal plant did not affect the direction or significance of any of our analyses. Thus, for simplicity, we have restricted our analyses to the ring plants.

In order to elucidate mechanisms for the patterns we observed in 2008, we replicated the above field establishment protocols for a follow-up experiment in 2009 with a few minor alterations. To reduce logistical effort, a subset of 21 of the original 24 genotypes were utilized, seven (instead of eight) plants were planted in each plot, and no focal plant was planted in 2009. Equal numbers of monocultures and polycultures were planted (n = 84 total), however there were 20 plots where one or more plants died or remained as rosettes. Thus, our analysis was restricted to intact monocultures (n = 36) and polycultures (n = 28). Each genotype occurred ~10 times in polyculture and at least one monoculture was complete for every genotype used in the experiment.

Arthropod and plant analyses

In the 2008 field experiment, we censused arthropods in mid-July and mid-August by visually surveying every plant in the experiment (n = 1080 plants). We identified familiar arthropods in the field or collected specimens of unknown arthropods for later identification. To identify collected specimens, we consulted relevant literature and the expertise of E. R. Hoebeke (Dept. of Entomology, Cornell University). Arthropods were identified to the lowest taxonomic level possible, generally species or genus and occasionally family. We lumped together parasitoids that were less than 3mm in length (n ~ 10 species) because of logistical difficulties associated with their field identification. We did not attempt to count or identify arthropods that were less than 1 mm in length (*e.g.,* thrips, collembola). In total, we made 36,006 observations of ~167 arthropod species. On average, we sampled 274 individuals and 20 species in each plot.

To address how *O. biennis* genotypic richness altered relationships among arthropod richness, evenness, and proportional diversity, we used ANCOVA with block as a random effect, *O. biennis* treatment as a nominal variable, factor (*i.e.,* richness, evenness, or proportional

diversity) as a covariate, and tested for the interaction of treatment × factor in each full model (JMP Version 8.0.1). A significant interaction indicates that the relationship between two arthropod diversity metrics (*e.g.*, richness and evenness) was altered by *O. biennis* richness. Due to significance in each full model (*see* Table 2.1), relationships among arthropod richness, evenness, and proportional diversity were also analyzed separately for each *O. biennis* treatment. Arthropod richness was ln-transformed so it occurred on the same scale as evenness and diversity indices (Alatalo 1981, Stirling & Wilsey 2001). Arthropod diversity was calculated via the Shannon index (Shannon 1948, Margalef 1958) using the equation $H' = -\Sigma\left(p_i \ln p_i\right)$, where p_i = the proportion of individuals of a given species to the total number of individuals in the community. Evenness (E_{var}) was calculated as variance in species' abundance using the equation $E_{\text{var}} = 1 - \frac{2}{\pi \arctan}\left[\Sigma\left(\ln\left(x_s\right) - \Sigma\ln\left(x_t\right)/S\right)^2 \frac{1}{S}\right]$ where x_s and x_t are abundances of the sth species (Smith & Wilson 1996). E_{var} was chosen as an evenness index because it performs the best of all evenness indices over the widest range of circumstances (Smith & Wilson 1996) and is not correlated with *S* for purely mathematical reasons (Alatalo 1981).

To elucidate mechanisms for the altered arthropod relationships we observed, we first used ANOVA with block as a random effect to test for differences in arthropod richness, evenness, proportional diversity, and *P. politus* abundance among *O. biennis* genotypic richness treatments. Due to the importance of *P. politus* in altering arthropod evenness and proportional diversity, we tested whether the response of *P. politus* to *O. biennis* genotypic richness was additive or interactive following the methods of Johnson et al. (2006). Briefly, we first calculated the mean abundance of *P. politus* on each genotype in monoculture. Then, we created an expected data set for polyculture patches based on their genotypic composition. We used ANOVA with block as a random effect to test whether observed versus expected values differed.

A difference in observed versus expected values using this method is indicative of a non-additive response (*i.e.*, an interactive effect of plant genotypic diversity on *P. politus* abundance).

In the follow-up field experiment in 2009, we further investigated the response of *P. politus*. We censused all *P. politus* individuals, the number of *O. biennis* flowers, and the number of buds on every plant in the experiment three times during the peak of flowering in mid-late August. Sampling occurred on Aug. 15, Aug. 21, and Sept. 1. During two of these surveys (Aug. 21 and Sept. 1) we also noted where each *P. politus* individual occurred on the plant (flower, bud, or stem/leaf). In total, we made 17,586 observations of *P. politus* during these surveys. In early October, just prior to plant senescence, we counted fruits, and harvested and weighed all above-ground biomass. Above-ground biomass was dried in an oven (40° C) for 72 hours, then weighed to the nearest 0.1 g. Biomass and fruit data were collected because these two traits commonly respond to plant richness (Cardinale et al. 2006, Hughes et al. 2008) and are known to be a mechanism affecting positive richness-richness relationships among plant and animal trophic levels (Haddad et al. 2009, Genung et al. 2010, Cook-Patton et al. 2011).

We used ANOVA to test for the effect of *O. biennis* genotypic richness on cumulative flower + bud abundance, fruit abundance, and above-ground dry mass. No blocking factor was used on this data set since block was never significant. While biomass and fruit data were normally distributed, flower + bud abundance was log transformed to improve normality. To test whether increased production of flowers + buds and fruits were due to complementarity or selection we followed the methods of Loreau and Hector (2001). Positive complementarity implies that non-additive increases in polyculture yield are due to resource partitioning or facilitation among plant genotypes, whereas negative selection implies that smaller genotypes grow proportionally better in polycultures than monocultures compared to larger genotypes.

Finally, we used linear regression to test for the effect of plot-level flower + bud abundance and above-ground biomass on *P. politus* abundance. Flower + bud abundance and *P. politus* abundance were both log transformed to improve normality.

Plagiognathas politus bioassay

To test whether potential differences in floral quality between monocultures and polycultures could influence *P. politus,* in mid-August 2009 we performed a choice test with *P. politus* using floral tissues from monoculture and polyculture plants. *P. politus* is an opportunistic florivore and agricultural pest (Wheeler 2001), and has occasionally been observed to consume an omnivorous diet (*e.g.,* Hunt-Joshi et al. 2005). On *O. biennis*, most *P. politus* interact with flowers and buds (*see* Table 2.1), where they consume pollen and nectar (McArt, *personal observation*). Three flowers and three buds were removed from each of 19 genotypes in each treatment and immediately placed in a Petri dish (9 cm diameter) containing a moist filter paper disk. Floral tissues were arranged such that flowers and buds from a genotype in monoculture were placed on one side of the Petri dish while flowers and buds from the same genotype in polyculture were placed on the other side of the dish. Three choice tests were set up for each genotype that was flowering in the experiment (n = 57 choice tests). Petri dishes were immediately transported back to the lab where five field-collected *P. politus* adults were introduced and allowed to choose among floral tissues from each treatment over a period of 14 hrs. At the end of 14 hrs *P. politus* individuals were counted on the tissues from each genotype and treatment. We used ANOVA with treatment as the factor, *P. politus* abundance as the response, and Petri dish as a blocking factor to test whether *P. politus* showed a preference for floral tissues acquired from plant genotypes growing in monocultures vs. polycultures.

Results

Relationships between arthropod richness and evenness, and richness and proportional diversity were altered by *O. biennis* genotypic richness (significant treatment × factor interactions for each response, Table 2.1).

Table 2.1: Pair-wise relationships between three aspects of arthropod biodiversity (richness, evenness, and proportional diversity) as affected by each factor, *Oenothera biennis* genotypic richness (monocultures vs. polycultures), and their interaction.

Full Analysis

Factor	Response	Factor			*O. biennis* richness		Factor × *O. biennis* richness	
		F	*P*	*slope*	*F*	*P*	*F*	*P*
Richness	Evenness	0.24	0.62	0.02	11.82	**<0.001**	8.65	**0.004**
Diversity	Richness	0.41	0.53	0.14	6.79	**0.011**	4.57	**0.035**
Evenness	Diversity	102.4	**<0.001**	3.14	0.03	0.87	1.86	0.18

Monocultures

		F	*P*	*slope*
Richness	Evenness	1.12	0.30	-0.11
Diversity	Richness	0.53	0.47	-0.33
Evenness	Diversity	85.04	**<0.001**	3.59

Polycultures

		F	*P*	*slope*
Richness	Evenness	13.65	**<0.001**	0.18
Diversity	Richness	7.04	**0.010**	0.66
Evenness	Diversity	28.34	**<0.001**	2.69

Arthropod species richness (*S*) was natural logarithm transformed to be on the same scale as evenness and diversity indices (Alatalo 1981), evenness calculated as the variance in species' abundance (E_{var}, Smith and Wilson 1996), diversity calculated as the Shannon index (*H'*, Margalef 1958). Slope indicates direction of relationship (positive, negative, or null if $P > 0.05$).

When analyzed separately, we found positive richness-evenness and richness-proportional diversity relationships in *O. biennis* polycultures ($F_{1,62} = 13.65$, $P < 0.001$, and $F_{1,62} = 7.04$, $P = 0.010$, respectively, Table 2.1) while there was no relationship between either of these aspects of arthropod biodiversity in monocultures ($P > 0.29$). These altered relationships

were not an artifact of different numbers of monocultures ($n = 46$) and polycultures ($n = 69$), as a power analysis indicated that only 23 and 40 polycultures, respectively, were necessary to achieve significance for richness-evenness and richness-proportional diversity relationships at $\alpha = 0.05$. The relationship between arthropod evenness and proportional diversity was positive and did not differ between *O. biennis* treatments (factor: $F_{1,106} = 102.4$, $P < 0.001$, treatment × factor interaction: $F_{1,106} = 1.86$, $P = 0.176$, Table 2.1).

To understand the mechanisms for these responses we first analyzed how each aspect of arthropod biodiversity responded to *O. biennis* genotypic richness independently. We found that plant genotypic richness decreased arthropod evenness by 19% ($F_{1,108} = 11.80$, $P < 0.001$, Fig 2.1a) and proportional diversity by 15% ($F_{1,108} = 6.57$, $P = 0.012$, Fig. 2.1b), while arthropod richness increased by 17% ($P = 0.011$, Cook-Patton et al. 2011).

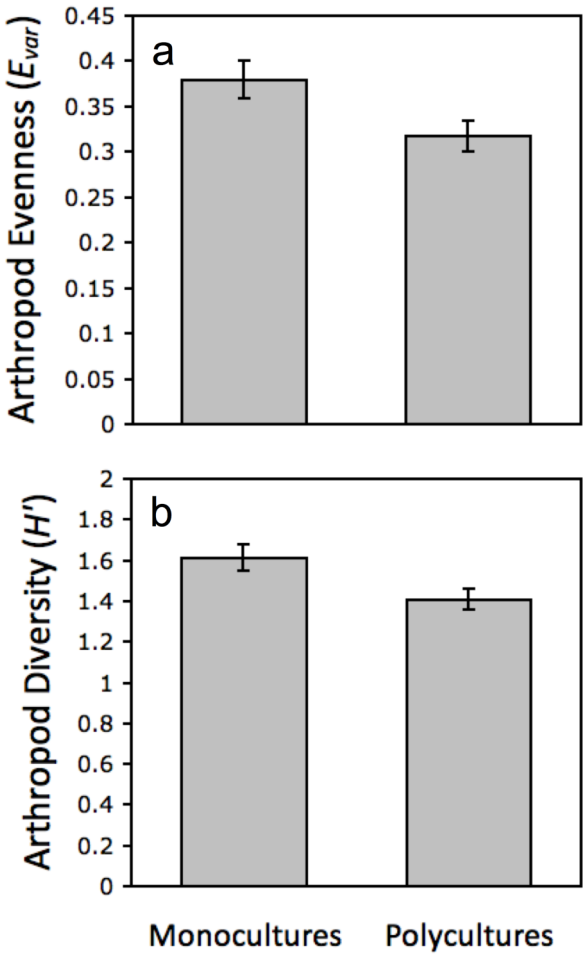

Figure 2.1: Relationship between plant genotypic richness and arthropod species evenness and diversity. Arthropod evenness calculated as variance in species' abundance, E_{var} (**a**), proportional diversity calculated via the Shannon index, *H'* (**b**). LS means ± SE shown.

Reduced evenness in communities can result from a greater proportion of rare species, a greater proportion of individuals comprising dominant species, or a combination of these two mechanisms. While the proportion of rare arthropod species was nearly identical among treatments (species where 5 or fewer individuals were observed: monocultures = 63%, polycultures = 62%), we observed a striking non-additive increase in abundance of the numerically dominant arthropod, *Plagiognathas politus*, in response to plant genotypic richness ($F_{1,108} = 27.78$, $P < 0.001$, Fig. 2.2). Nearly twice as many *P. politus* were found in *O. biennis*

polycultures compared to monocultures, and *P. politus* abundance was 47% greater than expected in polycultures ($F_{1,131} = 6.80$, $P = 0.010$, Fig. 2.2). When we removed *P. politus* from our evenness analysis, we found that evenness did not differ among treatments ($F_{1,108} = 0.41$, $P = 0.52$), suggesting that *P. politus* alone altered arthropod evenness in response to *O. biennis* genotypic richness.

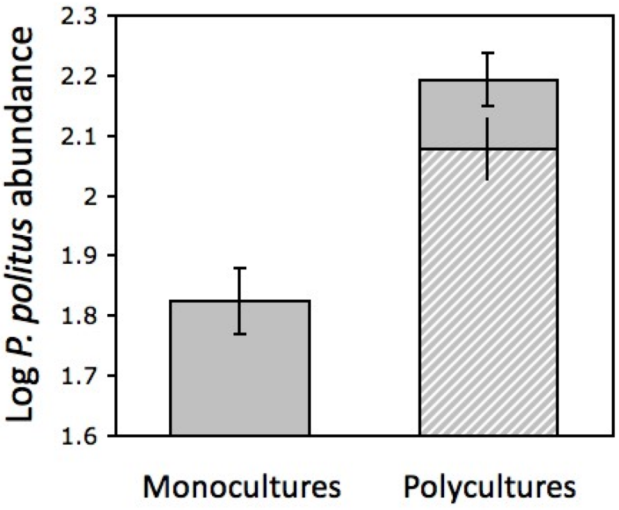

Figure 2.2: Relationship between plant genotypic richness and abundance of *Plagiognathas politus*. Gray bars show values from monocultures and polycultures (LS means ± SE). Hatched gray bar shows additive prediction from monoculture values (see *Methods*). Additive prediction shown with 95% confidence intervals surrounding LS mean.

Similar to evenness, low proportional diversity communities can result from differences in species' abundance distributions. However, proportional diversity also responds to the number of species present (*S*), which we found increased 17% in response to *O. biennis* genotypic richness. Since proportional diversity indices such as *H'* increase with greater *S* (see equation in methods), the decrease in proportional diversity that we observed shows that the increased number of arthropod species in polycultures were not able to counteract the influence

of reduced arthropod evenness. Again, when we removed *P. politus* from our analysis, we found that proportional diversity did not differ among treatments ($F_{1,108} = 0.12$, $P = 0.72$), suggesting that the increased numbers of *P. politus* in response to *O. biennis* genotypic richness were primarily responsible for decreased arthropod proportional diversity.

Due to the large influence of *P. politus* on arthropod evenness and proportional diversity that we observed in 2008, we conducted a series of follow-up experiments to understand the mechanism for its striking increase in abundance from monocultures to polycultures. In the 2009 follow-up field experiment, we observed that 77% of *P. politus* individuals were associated with flowers and buds compared to stems and leaves (Table 2.2). Therefore, we tested two plausible mechanisms for the response of *P. politus* to *O. biennis* floral tissues. First, we tested whether floral *quality* differed among *O. biennis* treatments. Via a choice bioassay, we found that *P. politus* did not preferentially choose floral tissues from either *O. biennis* treatment ($F_{1,56} = 0.00$, $P = 1.00$).

Table 2.2: Association of *Plagiognathas politus* (Miridae) with *Oenothera biennis* tissues during the 2009 field experiment.

Survey date	Proportion *P. politus* on flowers	Proportion *P. politus* on buds	Proportion *P. politus* on stems and leaves
Aug. 21	0.50	0.30	0.20
Sept. 1	0.42	0.32	0.26
Average	0.46	0.31	0.23

Next, we tested whether floral *quantity* influenced *P. politus*. After surveying the number of flowers + buds on every plant in the experiment, we found that cumulative flower + bud abundance increased in response to genotypic richness ($t_{1,62} = 2.24$, $P = 0.028$, Fig 2.3a), and that

this increase was due primarily to positive complementarity among *O. biennis* genotypes (95% confidence: 43.6 - 249.1, Fig 2.3b). Positive complementarity implies that non-additive increases in polyculture yield are due to resource partitioning or facilitation among plant genotypes (Loreau and Hector 2001).

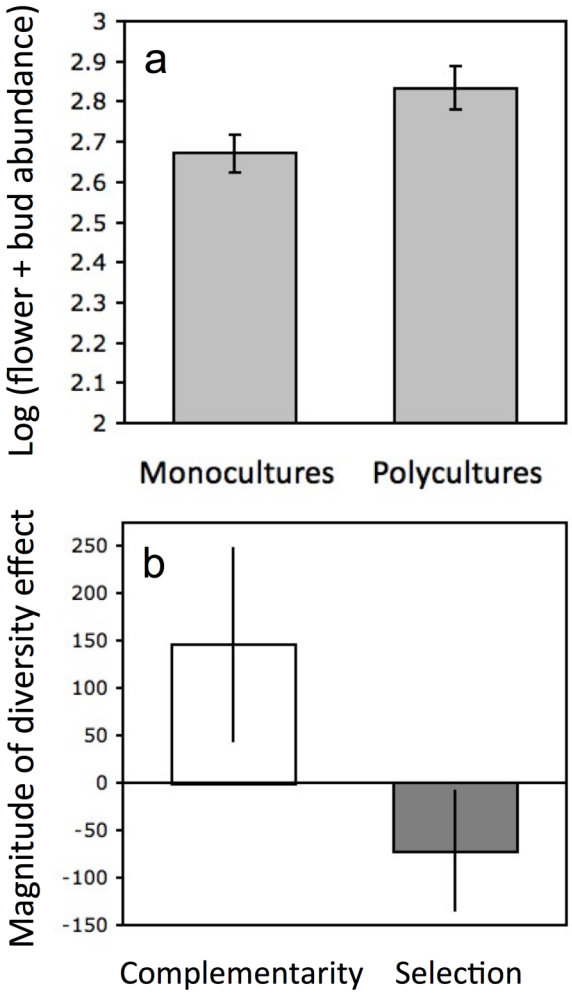

Figure 2.3: Relationship between plant genotypic richness and flower + bud abundance (**a**). LS mean ± SE shown. The overall diversity effect can be partitioned into complementarity or selection effects (Loreau and Hector 2001) (**b**). Positive complementarity (white bar) indicates that, on average, genotypes are more productive in polyculture than would be predicted from their monoculture values. Negative selection (dark gray bar) indicates that smaller genotypes are showing a disproportionally large increase in flowers and buds in polyculture compared to larger genotypes. Mean diversity effect ± 95% CI shown.

Increased flower + bud abundance in polycultures resulted in increased fruit production ($t_{1,62} = 2.27$, $P = 0.026$), which was also due primarily to positive complementarity (95% confidence: 85.3 – 284.5). Although a trend existed for greater above-ground biomass in polyculture, biomass did not differ significantly among treatments in the 2009 field experiment ($t_{1,62} = 1.53$, $P = 0.13$). Supporting the importance of floral tissue quantity in driving the abundance of *P. politus*, we found a strong positive correlation between plot-level flower + bud abundance and *P. politus* abundance ($R^2 = 0.65$, $P < 0.0001$, Fig. 2.4).

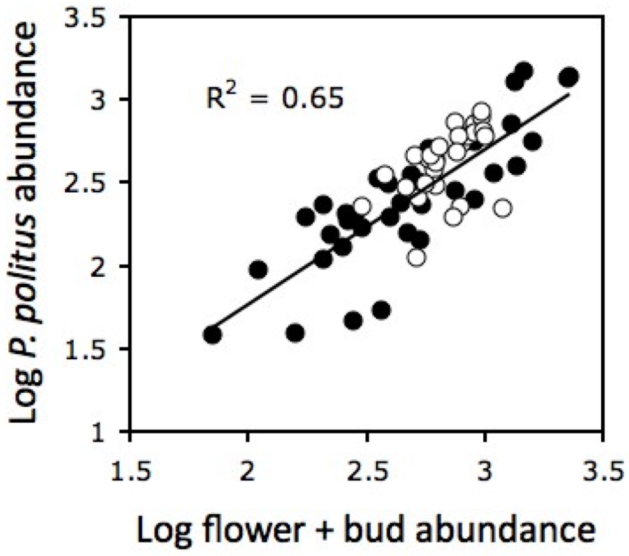

Figure 2.4: Relationship between flower + bud abundance and *P. politus* abundance in monocultures and polycultures. Each point represents the summed abundance in a monoculture plot (black circles) or polyculture plot (white circles).

Above-ground plant biomass was also correlated with *P. politus* abundance ($R^2 = 0.36$, $P < 0.0001$), however a model including both of these factors showed that flower + bud abundance drove *P. politus* abundance (flower + bud abundance: $P < 0.001$, above-ground biomass: $P =$

0.68). Finally, further supporting the importance of flower quantity in driving patterns of *P. politus* abundance, the positive correlation between flower + bud abundance and *P. politus* abundance did not differ among *O. biennis* monocultures and polycultures ($F_{1,59} = 0.37$, $P = 0.54$, Fig. 2.4).

Discussion

Our finding of variable relationships between arthropod richness, evenness, and proportional diversity (Table 2.1) reflects a growing literature that has found positive (Sugihara 1980, Stirling & Wilsey 2001, Willig et al. 2003), null (Stirling & Wilsey 2001, Willig et al. 2003, Ma 2005, Bock et al. 2007), and negative (Cook & Graham 1996, Weiher and Keddy 1999, Stirling & Wilsey 2001, Willig et al. 2003, Wilsey et al. 2005, Bock et al. 2007, Wilsey & Stirling 2007) relationships between these aspects of biodiversity in communities. Here, we show a novel mechanism that may contribute to these inconsistent results: plant genotypic richness modified relationships among different aspects of arthropod biodiversity (Table 2.1). Thus, interactions with plants altered relationships between different aspects of animal biodiversity.

Interactions between richness, evenness, and proportional diversity across trophic levels could have important consequences for communities and ecosystems. Within-trophic level richness (Hooper et al. 2005, Balvanera et al. 2006) and evenness (Hillebrand et al. 2008, Wittebolle et al. 2009, Crowder et al. 2010) are each separately known to impact functions such as resource utilization, population/community stability, and pest control. In addition, trophic complexity is known to modify community and ecosystem functions (Duffy et al. 2007). However, few diversity-function studies simultaneously measure or manipulate richness *and*

evenness across trophic levels. Theory and experiments addressing how biodiversity at one trophic level alters functions at other trophic levels have provided mixed results (Duffy et al. 2007), and we suggest that one reason for these inconsistencies may be a failure to consider how multiple aspects of biodiversity interact across trophic levels.

The second part of our study elucidates mechanisms for the arthropod patterns we observed. In *O. biennis* polycultures, where arthropod richness was high and evenness and proportional diversity were low, we observed positive relationships between these aspects of biodiversity. Conversely, in *O. biennis* monocultures, where arthropod richness was low and evenness and proportional diversity were high, richness-evenness and richness-proportional diversity relationships did not occur. Thus, determining the mechanisms altering each aspect of arthropod biodiversity provides insight into the mechanisms altering relationships among them. In a previous paper (Cook-Patton et al. 2011), we show how arthropod richness increased with *O. biennis* genotypic richness. Via rarefaction analyses, we found that arthropod richness increased due to increases in arthropod abundance, and arthropod abundance responded to increased plant biomass. Increased productivity is common in experiments manipulating plant richness (Cardinale et al. 2007, Hughes et al. 2008), and by increasing the amount of bottom-up energy, greater numbers of animals can be supported in a community, which results in an abundance-driven accumulation of species (Gotelli and Colwell 2001). This effect has been called the "more individuals" hypothesis, where animal richness is dependent on available plant energy (Srivastava and Lawton 1998, Crutsinger et al. 2006, Haddad et al. 2009).

Here, we show an analogous effect of biodiversity-driven productivity on arthropod evenness and proportional diversity. Flowers and buds – resources utilized by the numerically dominant insect on *O. biennis*, *P. politus* – increased in response to plant genotypic richness

(Fig. 2.3). In turn, this greater abundance of floral tissues attracted more *P. politus* individuals to polycultures (Figs. 2.2, 2.4), which resulted in decreased arthropod evenness and proportional diversity. When above-ground plant biomass and floral tissue abundance were compared, we found that plant biomass was not a significant predictor of *P. politus* abundance. Thus, knowing the specific plant resource utilized by *P. politus* allowed us to delve further into the relationship between plant productivity and animal evenness and proportional diversity.

A great deal of controversy surrounds the meaning or usefulness of proportional diversity indices such as *H'* (*e.g.,* Hairston et al. 1968, Hurlbert 1971). Some authors suggest that richness and evenness represent two components of biodiversity (*e.g.,* Stirling & Wilsey 2001) while other authors suggest that they represent inherently different aspects (*i.e.,* range vs. variance, respectively) of biodiversity, and therefore attempts to combine them into a single index are not justified (*e.g.,* Bell 2000). In this study we show that arthropod proportional diversity decreased despite an increase in arthropod richness in response to *O. biennis* genotypic richness, and that arthropod evenness and proportional diversity showed similar relationships with arthropod richness. From a mathematical standpoint, this points to the fact that arthropod evenness drove patterns of proportional diversity in this community much more than arthropod richness. Although our study was not designed to explore the consequences of this pattern, the relative contribution of evenness vs. richness in altering patterns of diversity may be important in predicting, for example, community responses to environmental change (Wilsey & Stirling 2007).

In summary, our results add to our knowledge of the relationship between plant and animal biodiversity by showing how relationships between arthropod richness, evenness, and proportional diversity are modified by interactions with plant genotypic richness. Since both

richness (Hooper et al. 2005, Balvanera et al. 2006) and evenness (Hillebrand et al. 2008, Crowder et al. 2010) are known to impact community and ecosystem processes, yet no consistent relationship between these aspects of biodiversity has been observed, we suggest that further mechanistic studies explicitly evaluating trophic interactions may greatly benefit our understanding of biodiversity and ecosystem functioning.

Acknowledgements

We thank Monica Kersch-Becker, Gui Becker, Alexis Erwin, Sergio Rasmann, Mike Stastny, Stuart Campbell, and Ordom Huot for assistance with field work. Katja Poveda, Anurag Agrawal, Andre Kessler, and Brian Wilsey provided constructive feedback that greatly improved this manuscript. This work was funded by a Small Grant in Biogeochemistry and Environmental Biocomplexity (NSF IGERT) to SHM and SCC, and a National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, Grant Number # 2006-35302-17431 to JST.

REFERENCES

Alatalo, R. V. 1981. Problems in the measurement of evenness in ecology. Oikos 37:199-204.

Balvanera, P., A. B. Pfisterer, N. Buchmann, J. S. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters 9:1146–1156.

Bock, C. E., Z. F. Jones, and J. H. Bock. 2007. Relationships between species richness, evenness, and abundance in a southwestern Savanna. Ecology 88:1322–1327.

Bell, G. 2000. The distribution of abundance in neutral communities. American Naturalist 155:606-617.

Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature 443:989–992.

Cardinale, B. J., J. P. Wright, M. W. Cadotte, I. T. Carroll, A. Hector, D. S. Srivastava, M. Loreau, and J. J. Weis. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Sciences USA 104:18123–18128.

Cleland, R. E. 1972. Oenothera: cytogenetics and evolution. Academic Press, New York, New York, USA.

Cook, L. M. and C. S. Graham. 1996. Evenness and species number in some moth populations. Biological Journal of the Linnean Society 58:75-84.

Cook-Patton, S. C., McArt, S. H., Parachnowitsch, A. L., Thaler, J. S., and Agrawal, A. A. 2011. A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function. Ecology 92:915-923.

Crowder, D. W., Northfield, T. D., Strand, M. R., and Snyder, W. E. 2010. Organic agriculture promotes evenness and natural pest control. Nature 466:109-112.

Crutsinger, G. M., M. D. Collins, J. A. Fordyce, Z. Gompert, C. C. Nice, and N. J. Sanders. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. Science 313:966–968.

De Benedictis, P. A. 1973. On the correlations between certain diversity indices. American Naturalist 107:295-302.

Dickson, T.L. and Wilsey, B.J. 2009. Biodiversity and tallgrass prairie decomposition: the relative importance of species identity, evenness, richness, and micro-topography. Plant Ecology 201:639-649.

Duffy, J. E., Cardinale, B. J., France, K. E., McIntyre, P. B., Thebault, E., and Loreau, M. 2007. The functional role of biodiversity in ecosystems: incorporating trophic complexity. Ecology Letters 10:522-538.

Elton, C.S. 1958. The Ecology of Invasions by Animals and Plants. Methuen & Co. Ltd, London.

Genung, M. A., Lessard, J-P., Brown, C. B., Bunn, W. A., Cregger, M. A., Reynolds, W. N., Felker-Quinn, E., Stevenson, M. L., Hartley, A. S., Crutsinger, G. M., Schweitzer, J. A., and Bailey, J. K. Non-additive effects of genotypic diversity increase floral abundance and abundance of floral visitors. PlosOne 5:e8711.

Gotelli, N. J., and Colwell, R. K. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters 4:379-391.

Hairston, N. G., J. D. Allan, R. K. Colwell, D. J. Futuyma, J. Howell, M. D. Lubin, J. Mathias, and J. H. Vandermeer. 1968. The relationship between species diversity and stability: an experimental approach with protozoa and bacteria. Ecology 49:1091.

Haddad, N.M., Crutsinger, G.M., Gross, K., Haarstad, J., Knops, J.M.H. & Tilman, D. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. Ecology Letters 12:1029-1039.

Haddad, N. M., Crutsinger, G. M., Gross, K., Haarstad, J., and Tilman, D. 2010. Plant diversity and the stability of food webs. Ecology Letters 14:42-46.

Hillebrand, H., Danuta M. B., and M. W. Cadotte. 2008. Consequences of dominance: A review of evenness effects on local and regional ecosystem processes. Ecology 89:1510–1520.

Hooper, D. U. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75:3–35.

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., and Vellend, M. 2008. Ecological consequences of genetic diversity. Ecology Letters 11:609-623.

Hunt-Joshi, T. R., Root, R. B., and Blossey, B. 2005. Disruption of weed biological control by an opportunistic mirid predator. Ecological Applications 15:861-870.

Hurlbert, S. H. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology 52:577–586.

Johnson, M. T. J. 2010. The contribution of evening primrose (*Oenothera biennis*) to a modern synthesis of evolutionary ecology. Population Ecology 53:9-12.

Johnson, M. T. J., M. J. Lajeunesse, and A. A. Agrawal. 2006. Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. Ecology Letters 9:24–34.

Larson, E. L., S. M. Bogdanowicz, A. A. Agrawal, M. T. J. Johnson, and R. G. Harrison. 2008. Isolation and characterization of polymorphic microsatellite loci in common evening primrose (*Oenothera biennis*). Molecular Ecology Resources 8:434–436.

Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412:72–76.

Ma, M. 2005. Species richness vs evenness: independent relationship and different responses to edaphic factors. Oikos 111:192–198.

Magurran, A. E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, N.J. May 1975

Margalef, D.R. 1958. Information theory in ecology. General Systems 3:36-71.

May, R. M. 1975. Patterns of species abundance and diversity. Pages 81-120 in M. L. Cody and J. L. Diamond, eds. Ecology and evolution of communities. Harvard University Press, Cambridge, Mass.

Murdoch, W. W., Peterson, C. H., and Evans, F. C. 1972. Diversity and pattern in plants and insects. Ecology 53:819-827.

Parker, J. D., J. E. Duffy, and R. J. Orth. 2001. Plant species diversity and composition: experimental effects on marine epifaunal assemblages. Marine Ecology-Progress Series 224:55-67.

Parker, J. D., J.-P. Salminen, and A. A. Agrawal. 2010. Herbivory enhances positive effects of plant genotypic diversity. Ecology Letters 13:553–563.

Root, R. B. 1973. Organization of a plant–arthropod association in simple and diverse habitats: fauna of collards (*Brassica oleracea*). Ecological Monographs 43:95–120.

Shannon, C. E. 1948. A mathematical theory of communication. Bell System Technical Journal 27:379-423.

Siemann, E., Tilman, D., Haarstad, J. & Ritchie, M. 1998. Experimental tests of the dependence of arthropod diversity on plant diversity. American Naturalist 152:738–750.

Smith, N. and Wilson, J. B. 1996. A consumer's guide to evenness indices. Oikos 76:70-82.

Srivastava, D. S. and Lawton, J. H. 1998. Why more productive sites have more species: An experimental test of theory using tree-hole communities. American Naturalist 152:510-529.

Stirling, G., and B. Wilsey. 2001. Empirical relationships between species richness, evenness and proportional diversity. American Naturalist 158:286–299.

Sugihara, G. 1980. Minimal community structure: An explanation of species abundance patterns. American Naturalist 133:458-463.

Tilman, D., D. Wedin, and J. M. H. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379:718–720.

Tilman, D., P. B. Reich, and J. M. H. Knops. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441:629-632.

Wheeler, A.G. 2001. Biology of the plant bugs (Hemiptera: Miridae): pests, predators, opportunists. Comstock Pub. Associates, Ithaca, NY.

Whittaker, R. H. 1965. Dominance and diversity in land plant communities – numerical relations of species express importance of competition in community function and evolution. Science 147:250-253.

Wieher, E. and Keddy, P. A. 1999. Relative abundance and evenness patterns along diversity and biomass gradients. Oikos 87:355-361.

Willig, M. R., Kaufman, D. M. and Stevens, R. D. 2003. Latitudinal gradients of biodiversity: Pattern, process, scale, and synthesis. Annual Review of Ecology Evolution and Systematics 34:273-309.

Wilsey, B. J., and G. Stirling. 2007. Species richness and evenness respond in a different manner to propagule density in developing prairie microcosm communities. Plant Ecology 2:259-273.

Wilsey, B. J., D. R. Chalcraft, C. M. Bowles, and M. R. Willig. 2005. Relationships among indices suggest that richness is an incomplete surrogate for grassland biodiversity. Ecology 86:1178–1184.

Wimp, G. M., Young, W. P., Woolbright, S. A., Martinsen, G. D., Keim, P., and Whitham, T. G. 2004. Conserving plant genetic diversity for dependent animal communities. Ecology Letters 7: 776-780.

Wittbolle, L., Marzorati, M., Clement, L., Balloi, A., Daffonchio, D., Heylen, K., De Vos, P., Verstraete, W., and Boon, N. 2009. Initial community evenness favours functionality under selective stress. Nature 458:623-626.

CHAPTER 3

Invasive herbivore increases plant fitness via induced resistance to seed predators

Introduction

Plants have evolved myriad ways to defend themselves against herbivores (Fritz and Simms 1992, Rosenthal and Berenbaum 1992, Karban and Baldwin 1997). Although costly (Strauss et al. 2002), constitutive (*i.e.,* always present) defenses are often associated with reduced herbivore damage (Mauricio 1998, Wittstock and Gershenzon 2002). Plants can also induce resistance following initial attack (Karban and Baldwin 1997), and the adaptive benefit of this induced defensive strategy has been demonstrated twice previously when subsequent vegetative feeding herbivores impacted plant fitness (Agrawal 1998, Baldwin 1998). However, it is increasingly clear that leaf herbivory also induces defenses in plant reproductive tissues (Baldwin and Karb 1995, Euler and Baldwin 1996, Ohnmeiss and Baldwin 2000, Strauss et al. 2004, Adler et al. 2006, Halpern et al. 2010), which may broaden the community-wide consequences of induction via altered interactions with reproductive tissue mutualists (Kessler et al. 2011, Whitehead and Poveda 2011) as well as antagonists (McCall and Karban 2006).

Toxins in flowers and fruits are known to deter pollinators (Adler and Irwin 2005, Gegear et al. 2007) and seed dispersers (Herrera 1982, Cipollini and Levey 1997), and deterrence of these reproductive tissue mutualists can negatively impact plant fitness (Howe and Smallwood 1982, Burd 1994). Therefore, leaf-to-reproductive tissue induction may be maladaptive in plants. Alternatively, defenses in reproductive tissues can deter antagonists such as florivores (McCall and Irwin 2006) and seed predators (Cipollini and Levey 1997, Tewksbury and Nabhan 2001). Due to their direct interaction with reproductive tissues, these antagonists can have large

negative impacts on plant fitness (Crawley 1992, Louda and Potvin 1995, McCall and Irwin 2006). Thus, if leaf herbivory is a reliable predictor of risk of florivory or seed predation (Karban et al. 1999), and the negative impacts of these reproductive tissue antagonists outweigh the positive effects of mutualists, leaf-to-reproductive tissue induction can be adaptive in plants.

Despite this potential conflict, it is unknown whether leaf-to-reproductive tissue induction is maladaptive or adaptive. More basically, while an increasing number of studies show that leaf herbivory can alter interactions with plant reproductive tissue mutualists such as pollinators (Kessler and Halitschke 2009, Kessler et al. 2011) and seed dispersers (Whitehead and Poveda 2011), or antagonists such as seed predators (McCall and Karban 2006), a direct link between leaf-to-reproductive tissue induction of defenses and altered animal behavior has yet to be documented.

Here we use a combination of natural population surveys, field manipulations, behavioral assays, and plant chemical analyses to investigate the fitness consequences of leaf-to-reproductive tissue induction in the common evening primrose (*Oenothera biennis*). An herbaceous plant native to eastern North America, *O. biennis* is a preferred host plant of the highly invasive Japanese beetle (*Popillia japonica*) in its introduced range (Potter and Held 2002). Japanese beetles are the dominant folivore on *O. biennis* in Tompkins Co., NY, consuming leaves prior to and during the emergence of its three dominant native herbivores – *Mompha stellella, Mompha brevivittella,* and *Schinia florida*. All three native herbivores are specialist Lepidoptera that prey on *O. biennis* reproductive tissues (flower buds, seeds, and both, respectively). While *O. biennis* lacks any seed dispersing mutualists, numerous pollinators interact with *O. biennis* flowers, including hummingbirds, hawkmoths, and other Lepidoptera.

However, reproduction and seed set in *O. biennis* occurs regardless of pollination via its permanent translocation heterozygote genetic system (Johnson 2011a).

Materials and methods

Study system

The common evening primrose (*Oenothera biennis*, Onagraceae) is a native herbaceous plant that is common to old-fields and disturbed areas in eastern North America (Cleland 1972, Johnson 2011b). The herbivore fauna on *O. biennis* in Tompkins County, NY, is dominated by the leaf-chewing Japanese beetle (*Popillia japonica,* Scarabaeidae) and three seed predators: the primrose moth (*Schinia florida,* Noctuidae), and two microlepidopterans (*Mompha stellella* and *Mompha brevivittella,* Momphidae). *Popillia japonica* is an invasive dietary generalist that was first discovered in New Jersey in 1916 (Fleming 1976). The first documentation of *P. japonica* herbivory on *O. biennis* in Tompkins County was in 1976 (Kinsman 1982), so *P. japonica* has utilized *O. biennis* as a host plant in the Ithaca area for between 35 and 95 years. *Schinia florida*, *M. stellella*, and *M. brevivittella* are native specialists that have coevolved with plants in the genus *Oenothera* (Hardwick 1970a, Powell 1980) and locally feed exclusively on *O. biennis* (Hardwick 1970b, Kinsman 1982).

In Tompkins County, *P. japonica* is univoltine; adults emerge and feed on *O. biennis* in mid-June, peak in abundance in mid-late July, and are absent by early September. Adult *S. florida* oviposit on *O. biennis* flower buds from July-August. Larvae usually remain on their initial host plants and preferentially consume flower buds (Kinsman 1982). Near the end of their development they will also consume maturing fruits if buds are not available, occasionally destroying every seed on a plant, before dropping to the ground to pupate in the soil. Adult *M.*

stellella oviposit on or near developing flower buds from July-August. Each larva feeds alone within one bud, destroying the stamens, style, stigma, and occasionally the petals, which terminates development of the flower/fruit. The larva then emerges and drops to the ground to pupate in the soil (Kinsman 1982). Adult *M. brevivittella* oviposit in maturing *O. biennis* fruits in late July-early September. A single larva stays within one of the four locules, consuming 80% of developing seeds on average (Agrawal et al. 2012). Up to four larvae inhabit each seed capsule and pupate in a cocoon spun within the capsule. Adults emerge from September-October through a conspicuous exit hole cut by the larva in the wall of the seed capsule (Kinsman 1982)

Population surveys

In mid-July 2009, we surveyed four local populations of *O. biennis* for leaf damage by *P. japonica* and abundance of the two seed predators that are present in mid-July, *S. florida* and *M. stellella* (populations separated by ~ 10 km). We counted the number of leaves damaged by *P. japonica* the number of eggs, larvae, and adults of each seed predator species on each plant. Because this count data conformed to a Poisson distribution, we tested for a relationship between *P. japonica* leaf herbivory and seed predator abundance via Poisson regression (R version 2.9.2).

In mid-September 2009, once all herbivores had finished damaging the plants but leaves had not dropped, we revisited these four populations to record end of season damage patterns. Two populations that were adjacent agricultural fields were destroyed due to mowing. Therefore, we surveyed the remaining two populations plus two additional populations ~5 km from the original sites. We estimated percent leaf damage from *P. japonica* by scoring the amount of leaf area removed on each leaf (0, 25, 50, 75, or 100%), adding these values from all leaves on a plant, dividing this number by the total number of leaves, and multiplying by 100.

We estimated percent fruit damage from *M. stellella*, *S. florida*, and *M. brevivittella* by counting the number of flower buds or fruits consumed (taking into account partial consumption), dividing this number by the total number of fruits, and multiplying by 100. Data from each herbivore conformed to a normal distribution, so we tested for relationships between *P. japonica* leaf damage and seed predation via linear regression.

Phytohormone analysis

We measured the concentration of jasmonic acid (JA) in leaves, flower buds, and fruits from tissues collected during mid-July, 2009 in one of the four populations surveyed. Samples were not collected in the remaining three populations to minimize costs. Tissue was collected from randomly sampled plants that either had *P. japonica* leaf damage or did not have leaf damage. Tissue was immediately frozen on dry ice and stored in a -80 freezer. Prior to analysis, 0.1-0.3 grams of frozen tissue from each sample was weighed to the nearest 0.1 mg, 80 ng of D_5-jasmonic acid was added to each tube as an internal standard, and samples were extracted in 1 mL of an isopropanol:H_2O:HCl buffer (2:1:0.005 vol/vol) using a Fastprep 24 homogenizer (MP Biomedicals LLC, Solon, OH). JA concentrations were then determined via the protocol outlined in Thaler et al. (Thaler et al. 2010) using HPLC-MS.

Choice experiment

We set up eight large mesh cages (12' × 12' × 6', Lumite Inc., Baldwin, GA) in an abandoned and untreated agricultural field in 2010. Each cage enclosed 28-32 bolting *O. biennis* plants that naturally occurred in the field. In mid-July, we randomly assigned plants to two treatments: beetle-induced or control. We placed a mesh bag (Agrifabrics Pro-17 material, American Agrifabrics, Alphretta GA) over the foliage of each plant and tied the bag off in two places: at the base of the stem and below the floral/fruit tissues. Beetle-treated plants received

five *P. japonica* per bag while control plants received no beetles (empty bag). After five days of leaf herbivory, we performed a choice experiment with *S. florida* by adding 4-5 adult moths to each cage. Moths were obtained from wild populations, immediately added to the cages, allowed to oviposit on the exposed flower buds of each plant for 3-4 days, then removed from the cages and released. The number of eggs oviposited by *S. florida* was counted on each plant, all mesh bags were removed, and the amount of leaf damage from *P. japonica* was quantified. In early August, after larvae had completed development and dropped to the soil to pupate, the number of flower buds and fruits consumed by *S. florida* were recorded for each plant. We tested for differences in oviposition, flower bud, and fruit consumption between treatments via ANOVA with cage as random effect.

Phenolics quantification

We measured the concentration of phenolics (ellagitannins and flavonoids) in flower buds collected from control and beetle-induced plants immediately prior to the introduction of *S. florida* in the choice experiment. Tissue was collected, immediately frozen on dry ice, and stored in a -80 freezer until analysis. We lyophilized the frozen tissue for 5 days, then extracted dried tissue in acetone:H_2O (70:30 vol/vol) via Fastprep homogenization. We then followed the methods outlined in Johnson et al. (Johnson et al. 2009a) to quantify eleven ellagitannins and five flavonoids using HPLC-DAD via analyses on two separate dates.

As a multivariate test of overall differences in individual flower bud phenolics we used the semimetric Bray-Curtis dissimilarity coefficient to compare assemblages of individual ellagitannins or flavonoids from control vs. beetle-induced plants using phytochemical concentrations as abundance data (Vegan 1.15-1, R version 2.9.2 (McCune and Grace 2002)). Flower bud ellagitannins were dissimilar between control vs. beetle-induced plants ($F_{1,64} = 2.0$, P

= 0.039) while flavonoids were not dissimilar between treatments ($F_{1,64}$ = 0.7, P = 0.52). Therefore, we proceeded with univariate comparisons of individual ellagitannins via ANOVA with cage and analysis date as random effects (Table 3.1). Four individual ellagitannin peaks were induced ($P < 0.05$). These four peaks corresponded to two compounds only, since both exist naturally as isomeric α- and β-glucose mixtures (see *Ellagitannin characterization*). We therefore combined data from each appropriate compound and isomer to obtain total concentrations of the two compounds (Table 3.1).

Ellagitannin characterization

All the ellagitannins were first classified by their UV spectra. Characterization of oenothein B and its isomer were conducted by observing UV spectra and *m/z* values of 783.1 and 1567.1 via triple quadropole HPLC-MS-MS in tandem with referencing previous literature on this compound in *O. biennis* (Johnson et al. 2009a, Karonen et al. 2010). Characterization of the oxidized derivative of oenothein A and its isomer were conducted by a combination of UV spectroscopy and ESI-TOF mass spectrometry (Karonen et al. 2010). In short, the compound had a molecular weight of 2366.21 g/mol and produced a characteristic water fragment during ESI-MS analysis. These were evidenced by the following *m/z* values: *m/z* 1182.108 ($[M-2H]^{2-}$), 1173.107($[M-2H-H_2O]^{2-}$), 1576.477 ($[2M-3H]^{3-}$), 1570.475 ($[2M-3H-H_2O]^{3-}$), and 1583.815 ($[2M-4H+Na]^{3-}$). This ellagitannin had 14 Da higher molecular weight than the trimeric oenothein A (2352 g/mol). The oxidation of phenolic HHDP group of oenothein A into a DHHDP group would increase oenothein A's molecular weight by 16 Da, not by 14 Da (=> 2368 g/mol). Such a compound, oenotherin T1, has been earlier identified from other *Oenothera* species (Taniguchi et al. 2002). The DHHDP group typically yields a water fragment in ESI-MS due to the presence of two OH-groups attached to the same carbon atom; this was evidenced for

the new ellagitannin trimer. Its UV spectrum was slightly altered from that of oenothein A, suggesting a higher HHDP to galloyl load in the molecule (*cf.* (Salminen et al. 2011)). Indeed, the formation of one HHDP group from two galloyl groups decreases the molecular weight of the ellagitannin by 2 Da. Thus, the new ellagitannin was characterized as a trimeric oenothein A derivative having one HHDP group oxidized into DHHPP group and two galloyl groups joined to form a HHDP group (*i.e.,* 2352 Da + 16 Da – 2 Da = 2366 Da). This type of oligomeric ellagitannin structure was further supported by its behavior during Sephadex LH-20 gel chromatography (Salminen et al. 2011). The full identification of the molecule and the unmasking of the exact positions of the galloyl, HHDP and DHHDP groups in the three glucoses of this new ellagitannin would require compound purification followed by detailed NMR and hydrolysis product analysis; this is the target of upcoming studies. We verified the identities of oenothein B and the oxidized derivative of oenothein A between instruments and labs by purifying each compound and comparing retention time, UV spectroscopy, and mass spectrometry data.

Population manipulations

In early July 2010 we visited seven populations of *O. biennis* in Tompkins Co. and, corresponding to the time when natural colonization of *O. biennis* by *P. japonica* occurred in each population, we controlled whether plants received beetle leaf herbivory (induced) or not (control) via identical methods outlined for the choice experiment. We maintained these treatments for 5-7 days in each population, then removed bags and allowed natural colonization of herbivores and other animals such as pollinators for the rest of the season. In mid-September we revisited each population, counted the number of fruits on each plant, and quantified seed predation using the same methods outlined in the observational study. We tested for differences

in number of fruits and percent seed predation between treatments via ANOVA with population as a random effect.

Isolated fitness impacts of P. japonica

Ten replicate plants from each of fourteen *O. biennis* genotypes were germinated in pots in September 2010, grown for two months in a greenhouse until mature rosettes, then placed outside in a cold frame to overwinter. In July 2011, we controlled whether plants received beetle leaf herbivory (induced) or not (control) via identical methods outlined for the choice experiment and population manipulations. Bags were removed after 7 days and in mid-September (when all fruits had developed) we counted the number of fruits on each plant. We removed two fruits located 15 cm below the apex of each bolting stalk and counted and weighed (to the nearest 0.1 mg) all seeds from these fruits. This fruiting position on the plant was chosen to subsample because it was where flowers and fruits developed in the week following the induction treatment. Plant height ($F_{1,69} = 0.31$, $P = 0.58$) and length of the fruiting stalk ($F_{1,69} = 0.09$, $P = 0.77$) did not differ between treatments, suggesting this method of subsampling fruits was unbiased. We tested for differences in the number of fruits, number of seeds per fruit, and average seed weight on control vs. beetle-induced plants via ANOVA with *O. biennis* genotype as random effect.

Results

We first investigated the potential for plant-mediated interactions on *O. biennis* by surveying animal abundance and damage patterns in four *O. biennis* populations. We found fewer *M. stellella* ($F_{1,140} = 6.8$, $P < 0.001$) and *S. florida* ($F_{1,140} = 2.0$, $P = 0.045$), the two seed predators present during our initial survey, on *O. biennis* plants with greater amounts of *P. japonica* leaf herbivory (Fig. 3.1).

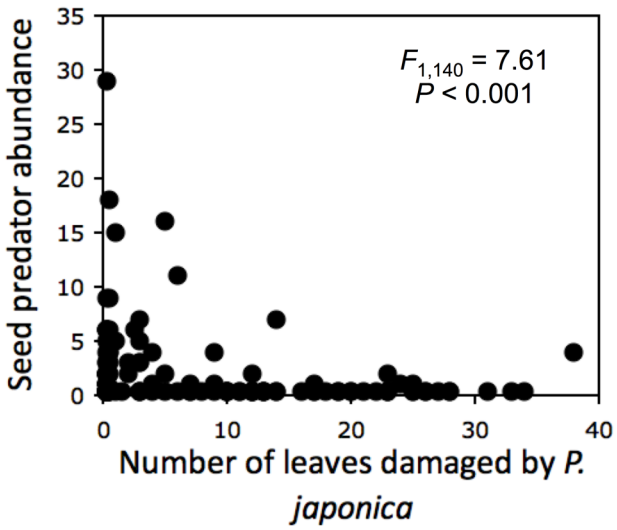

Figure 3.1: Relationship between number of leaves damaged by Japanese beetles (*Popillia japonica*) and abundance of adult and larval *Schinia florida* and *Mompha stellella* in four evening primrose (*Oenothera biennis*) populations surveyed in Tompkins Co., NY (USA). Individual responses of each seed predator: *M. stellella*: $F_{1,140} = 6.77$, $P < 0.001$; *S. florida*: $F_{1,140} = 2.00$, $P = 0.045$.

At the end of the season, we found less damage by the two seed predators that consume flower buds (*M. stellella*: $F_{1,104} = 6.1$, $P = 0.015$, and *S. florida*: $F_{1,104} = 6.5$, $P = 0.012$), while there was no relationship between leaf herbivory and seed predation by *M. brevivittella* ($F_{1,104} = 0.5$, $P = 0.50$), the herbivore that preys exclusively on maturing seeds (Fig. 3.2). Overall, total seed predation by all three Lepidoptera was greatly reduced on plants that received more beetle leaf damage ($F_{1,104} = 16.1$, $P < 0.001$).

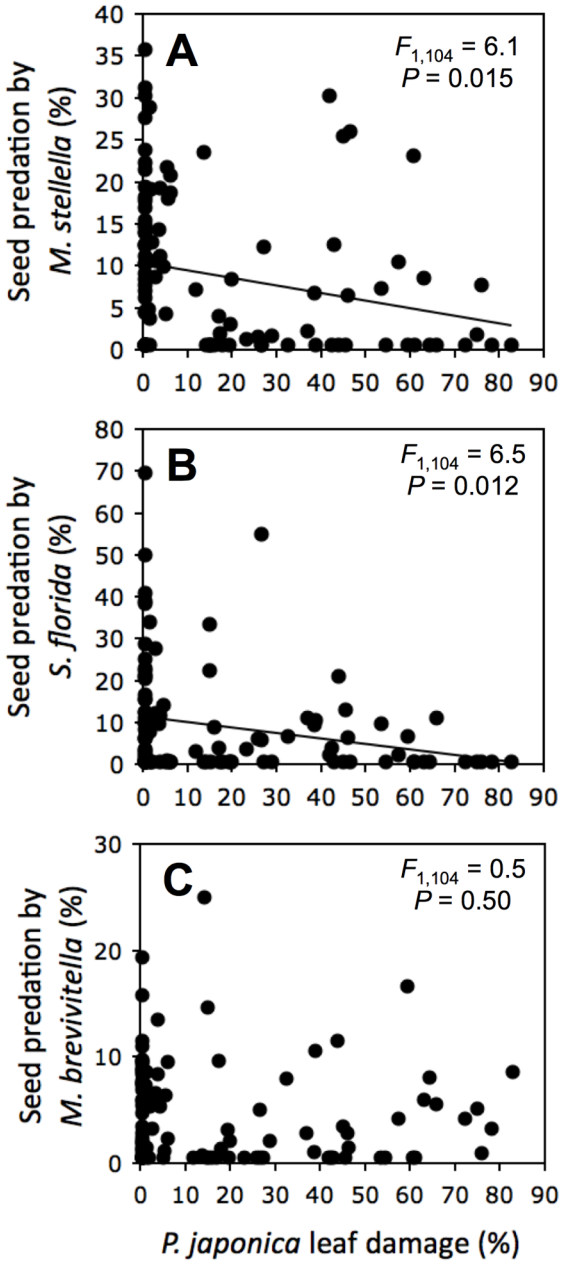

Figure 3.2: Relationship between percent leaf damage by *P. japonica* and percent of seeds consumed by *M. stellella* (***A***), *S. florida* (***B***), and *M. brevivittella* (***C***) in four *O. biennis* populations surveyed in Tompkins Co., NY (USA).

The jasmonic acid (JA) signaling cascade is the major wound-induced mediator of plant defensive responses to herbivores (Creelman and Mullet 1997). Concentration of JA was

elevated in both leaves ($F_{1,18} = 8.6$, $P = 0.009$, Fig. 3.3*A*) and flower buds ($F_{1,18} = 5.0$, $P = 0.039$, Fig. 3.3*B*) but not maturing fruits ($F_{1,14} = 0.5$, $P = 0.49$, Fig. 3.3*C*) of plants with beetle leaf damage in our observational survey. Furthermore, the extent of beetle leaf damage was positively associated with concentration of JA in both leaves ($R^2 = 0.57$, $P < 0.001$, Fig. 3.3*A* inset) and flower buds ($R^2 = 0.22$, $P = 0.038$, Fig. 3.3*B* inset), suggesting a JA-mediated response to *P. japonica* may have altered seed predator behavior.

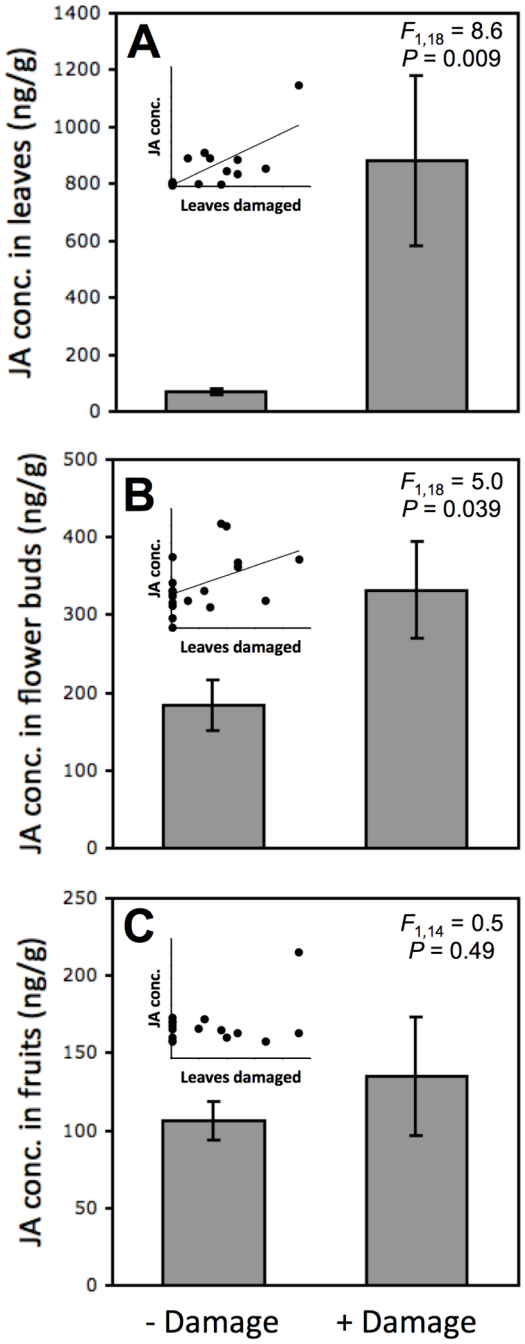

Figure 3.3: Concentration of jasmonic acid (JA) in leaves (***A***), flower buds (***B***), and fruits (***C***) of plants with *P. japonica* leaf damage (+ Damage) or without leaf damage (- Damage) from the observational survey of four *O. biennis* populations (ng/g tissue, mean ± s.e.m.). Insets show relationship between the number of leaves damaged by *P. japonica* and JA concentration in each respective tissue.

To more directly test whether these correlated patterns of leaf herbivory and seed predation were due to induced plant resistance, we manipulated *P. japonica* leaf herbivory and measured induction of defensive chemicals and oviposition preference of *S. florida*, the dominant seed predator on *O. biennis*. Plant height ($F_{1,233} = 0.4$, $P = 0.55$) and the number of *O. biennis* flower buds ($F_{1,233} = 1.1$, $P = 0.30$) and fruits ($F_{1,233} = 0.1$, $P = 0.72$) – the two primary oviposition sites utilized by *S. florida* adults – were similar on control vs. induced plants immediately following induction, suggesting that *P. japonica* leaf herbivory did not alter plant growth or reproductive phenology.

Instead, we found a 42% higher concentration of ellagitannins in the flower buds of plants that received *P. japonica* leaf damage ($F_{1,57} = 8.8$, $P = 0.005$, Table 3.1). This result was primarily driven by the induction of two compounds: the dimeric ellagitannin oenothein B – the most abundant phenolic compound in *O. biennis* tissues – was 45% more concentrated ($F_{1,57} = 6.0$, $P = 0.017$), and an oxidized derivative of trimeric oenothein A was 81% more concentrated in the flower buds of beetle-induced compared with control plants ($F_{1,57} = 8.1$, $P = 0.006$).

Table 3.1: Concentration of ellagitannins in flower buds of control plants vs. plants induced with *P. japonica* leaf herbivory.[†]

Compound	Percentage of total ellagitannins	Control mean (mg/g)*	Induced mean (mg/g)*	*P*
Oenothein B	57.4	138.8	191.5	**0.018**
Oenothein A	26.5	64.3	88.5	0.25
Oxidized oenothein A derivative	5.1	11.1	18.1	**0.015**
Oenothein B (isomer)	4.6	11.4	15.3	**0.025**
Ellagitannin 1	2.5	6.9	7.3	0.86
Ellagitannin 2	1.5	4.8	4.0	0.39
Ellagitannin 3	0.6	1.4	2.0	0.058
Oxidized oenothein A derivative (isomer)	0.5	1.0	2.1	**0.045**
Ellagitannin 4	0.5	1.1	1.5	0.061
Ellagitannin 5	0.4	1.0	1.3	0.25
Ellagitannin 6	0.4	0.9	1.3	0.19
Total oenothein B	62.0	150.2	206.8	**0.017**
Total oxidized oenothein A derivative	5.6	12.1	20.2	**0.006**
Total individual ellagitannins	100	242.8	332.8	**0.005**

[†]For each ellagitannin quantified, we tested for differences between treatments via ANOVA with cage and analysis date as random effects (*P* values shown).
[*]Least square means expressed in pentagalloyl glucose equivalents (mg/g dry tissue).

Next, via the choice experiment, we found that *P. japonica* leaf herbivory induced resistance to *S. florida*, causing adult moths to oviposit 62% fewer eggs on induced vs. control plants ($F_{1,233} = 7.4$, $P = 0.007$, Fig. 3.4*A*). This difference in oviposition choice caused *S. florida* larvae to consume less than half the number of flower buds on induced vs. control plants ($F_{1,233} = 19.7$, $P < 0.001$, Fig. 3.4*B*) and there was a trend for 30% fewer seeds consumed on induced plants ($F_{1,233} = 2.4$, $P = 0.12$). Thus, seed predation on *O. biennis* was suppressed by an induced response to leaf herbivory.

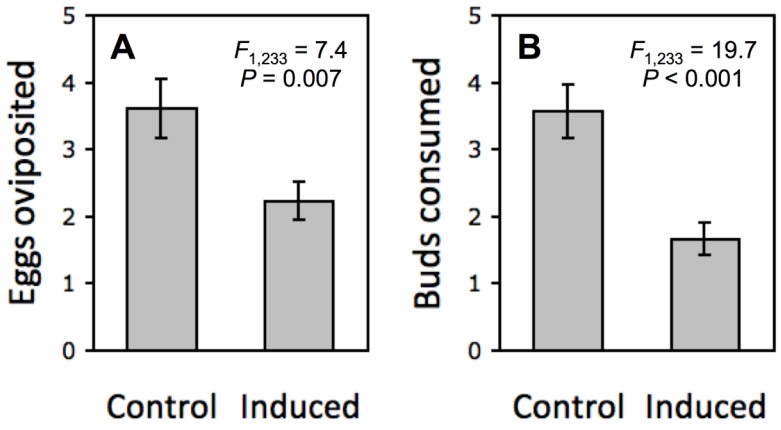

Figure 3.4: Induced resistance to seed predators via leaf herbivory. *Schinia florida* adults oviposited fewer eggs (***A***) and larvae consumed fewer flower buds (***B***) on plants with *P. japonica* leaf herbivory compared to control plants in the manipulative choice experiment (mean ± s.e.m.).

In order to measure the fitness outcomes of these plant-mediated interactions in nature, we conducted a second manipulative experiment where plants either received *P. japonica* leaf herbivory (induced) or not (control) in seven naturally occurring *O. biennis* populations. At the end of the growing season, the number of seeds remaining unconsumed by seed predators was enhanced by 7% due to induced resistance from *P. japonica* leaf herbivory ($F_{1,344}$ = 23.2, P < 0.001, Fig. 3.5). Because *O. biennis* is monocarpic, the number of seeds produced that escape predation is a strong indicator of lifetime fitness. This fitness benefit from induction was due to two factors. First, seed predators consumed 77% fewer seeds on beetle-induced compared to control plants ($F_{1,344}$ = 23.2, P < 0.001), which was primarily driven by the most abundant seed

predators in these populations, *S. florida* ($F_{1,344}$ = 21.2, P < 0.001) and *M. brevivittella* ($F_{1,344}$ = 12.0, P = 0.001). Second, *O. biennis* tolerated *P. japonica* folivory; leaf herbivory by itself did not affect the number of fruits ($F_{1,69}$ = 0.1, P = 0.88), number of seeds per fruit ($F_{1,69}$ = 2.0, P = 0.16), or the mass of individual seeds ($F_{1,69}$ = 0.1, P = 0.71) produced by *O. biennis*.

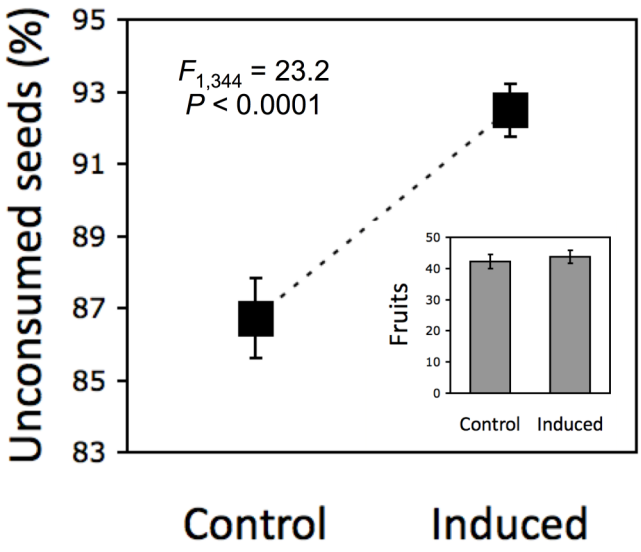

Figure 3.5: Fitness benefits of leaf-to-reproductive tissue induction in nature. The percent of seeds remaining after naturally occurring seed predation on control plants vs. plants experimentally induced with *P. japonica* leaf herbivory in seven *O. biennis* populations (mean ± s.e.m.). The number of fruits produced on control vs. induced plants did not differ ($F_{1,344}$ = 0.30, P = 0.59, inset), and a supplemental experiment with identical amounts of leaf herbivory found the number of fruits, number of seeds per fruit, and average seed mass did not differ between control vs. induced plants (P > 0.05 in all cases).

Discussion

In this study we demonstrate a direct mechanistic link between leaf herbivory and seed predation by showing how jasmonate-mediated leaf-to-reproductive tissue induction of defenses alters seed predator behavior. Furthermore, because *O. biennis* experiences a fitness benefit via induced resistance against its native seed predators, we show that leaf-to-reproductive tissue

induction can be an adaptive trait in plants. Our results broaden the finding of two previous studies that showed how induction of defenses in vegetative tissues can be adaptive (Agrawal 1998, Baldwin 1998) and therefore may evolve as a plant defense strategy.

Our results also show how a native plant (*O. biennis*) benefits from being consumed by *P. japonica* – a highly invasive herbivorous pest (Potter and Held 2002). Numerous previous studies have found little or no cost of leaf herbivory on correlates of plant fitness (Hawkes and Jon 2001), and when induced resistance to herbivores does not itself incur a fitness cost, it is considered a form of plant vaccination (Kessler and T. Baldwin 2004). Because vaccination occurs in plant-herbivore systems comprised entirely of endemic species (Kessler and T. Baldwin 2004, Halitschke et al. 2011) there is little reason to suspect that vaccination of *O. biennis* by *P. japonica* occurs due to the ecological novelty of their interaction. However, while invasive species occasionally facilitate endemic species (Rodriguez 2006), to our knowledge this is the first example of an invasive species indirectly facilitating an endemic species by consuming it.

Links between the production of secondary metabolites in plant vegetative and reproductive tissues may occur for numerous reasons. Perhaps the most commonly hypothesized mechanism for the presence of toxins in reproductive tissues is pleiotropy (Eriksson and Ehrlen 1998, Adler 2000), and a handful of studies support this hypothesis via genetic correlations between secondary metabolites in plant vegetative and reproductive tissues (Adler et al. 2006, Irwin and Adler 2006, Kessler and Halitschke 2009). Indeed, the biosynthetic pathways involved in the production of metabolites in vegetative and reproductive tissues may exhibit some degree of overlap. For example, Fineblum and Rausher (Fineblum and Rausher 1997) hypothesized that resistance to herbivory may be related to flower color since pigment

compounds (anthocyanins) and defensive compounds (flavonoids and tannins) are both produced via the flavonoid pathway.

In our study we found that ellagitannins – compounds produced via the flavonoid biosynthetic pathway – were induced in the flower buds of *O. biennis* via leaf herbivory. Importantly, we found higher levels of jasmonic acid in the flower buds of plants that received leaf herbivory, suggesting induction occurred via a jasmonate-mediated response in these reproductive tissues and was not simply a consequence of vascular transport from leaves. The high oxidative activity of ellagitannins is revitalizing interest in the defensive capabilities of tannins for insect herbivores (Barbehenn et al. 2006, Salminen and Karonen 2011, Salminen et al. 2011). Suggestive of a potent induced defense against the seed predators of *O. biennis*, oenothein B and other ellagitannins are known to have high oxidative activity in the alkaline gut conditions common to Lepidoptera (Barbehenn et al. 2006).

Plants that rely on pollinators and/or seed dispersers to promote reproductive success may be under strong selection to avoid deterring these mutualists, and therefore leaf-to-reproductive tissue induction of defenses is predicted to be ecologically costly (Strauss et al. 2002, Kessler and Halitschke 2009, Kessler et al. 2011, Whitehead and Poveda 2011). However, *O. biennis* is functionally asexual via its permanent translocation heterozygote genetic system (Johnson 2011a), lacks any biotic seed dispersal agent, and therefore may escape the possible ecological costs of induced leaf-to-reproductive tissue defenses. Whether related plant species that show high levels of outcrossing lack such induction (or experience attenuated induction) remains to be tested (Johnson et al. 2009b). However, we predict that leaf-to-reproductive tissue induction is common since seed predation is ubiquitous in nature (Crawley 1992), imposes direct fitness costs, and can be a stronger agent of natural selection on plant reproductive traits than

mutualistic interactions such as pollination (Parachnowitsch and Caruso 2008). Due to the potentially direct fitness impacts of pollinators, seed dispersers, florivores, and seed predators, leaf-to-reproductive tissue induction is likely a common but understudied mechanism by which leaf herbivory impacts plant fitness and contributes to natural selection on plant traits.

Acknowledgements

We thank N. Andrew, I. Betancourt, and S. Nelson for assistance with fieldwork, B. Blossey for generously providing large mesh field cages for the choice experiment, and A. Agrawal, B. Blossey, M. Johnson, A. Kessler, K. Poveda, and R. Raguso for helpful comments on the manuscript. The NSF (IGERT small grant in Biogeochemistry and Environmental Biocomplexity to S.H.M.), Grace Griswold Endowment (S.H.M.), USDA-NRI (2006-35302-1731 to J.S.T.) and Academy of Finland (119659 to J.P.S.) supported this work.

REFERENCES

Adler, L. S. 2000. The ecological significance of toxic nectar. Oikos **91**:409-420.

Adler, L. S. and R. E. Irwin. 2005. Ecological Costs and Benefits of Defenses in Nectar. Ecology **86**:2968-2978.

Adler, L. S., M. Wink, M. Distl, and A. J. Lentz. 2006. Leaf herbivory and nutrients increase nectar alkaloids. Ecology Letters **9**:960-967.

Agrawal, A. A. 1998. Induced responses to herbivory and increased plant performance. Science **279**:1201-1202.

Agrawal, A. A., M. T. J. Johnson, A. P. Hastings, and J. L. Maron. 2012. Experimental evolution of plant life-history traits and its eco-evolutionary feedback to seed predator populations. In press at American Naturalist.

Baldwin, I. T. 1998. Jasmonate-induced responses are costly but benefit plants under attack in native populations. Proceedings of the National Academy of Sciences **95**:8113-8118.

Baldwin, I. T. and M. J. Karb. 1995. Plasticity in allocation of nicotine to reproductive parts in *Nicotiana attenuata*. Journal of Chemical Ecology **21**:897-909.

Barbehenn, R., C. Jones, A. Hagerman, M. Karonen, and J.-P. Salminen. 2006. Ellagitannins have greater oxidative activities than condensed tannins and galloyl glucoses at high pH: Potential impact on caterpillars. Journal of Chemical Ecology **32**:2253-2267.

Burd, M. 1994. Bateman's principle and plant reproduction: The role of pollen limitation in fruit and seed set. Botanical Review **60**:83-139.

Cipollini, M. L. and D. J. Levey. 1997. Secondary metabolites of fleshy vertebrate-dispersed fruits: Adaptive hypotheses and implications for seed dispersal. The American Naturalist **150**:346-372.

Cleland, R. E. 1972. Oenothera: cytogenetics and evolution. Academic Press, New York.

Crawley, M. J. 1992. Seed predators and plant population dynamics. Pages 157-191 *in* M. Fenner, editor. Seeds: the ecology of regeneration in plant communities. CAB International.

Creelman, R. A. and J. E. Mullet. 1997. Biosynthesis and action of jasmonates in plants. Annual Review of Plant Physiology and Plant Molecular Biology **48**:355-381.

Eriksson, O. and J. Ehrlen. 1998. Secondary metabolites in fleshy fruits: Are adaptive explanations needed? The American Naturalist **152**:905-907.

Euler, M. and I. T. Baldwin. 1996. The chemistry of defense and apparency in the corollas of *Nicotiana attenuata*. Oecologia **107**:102-112-112.

Fineblum, W. L. and M. D. Rausher. 1997. Do floral pigmentation genes also influence resistance to enemies? The W locus in *Ipomoea purpurea*. Ecology **78**:1646-1654.

Fleming, W. E. 1976. Integrated control of the Japanese beetle - a historical view. **1545**:65 pp.

Fritz, R. S. and E. L. Simms. 1992. Plant resistance to herbivores and pathogens: ecology, evolution, and genetics. 1 edition. University of Chicago Press.

Gegear, R. J., J. S. Manson, and J. D. Thomson. 2007. Ecological context influences pollinator deterrence by alkaloids in floral nectar. Ecology Letters **10**:375-382.

Halitschke, R., J. G. Hamilton, and A. Kessler. 2011. Herbivore-specific elicitation of photosynthesis by mirid bug salivary secretions in the wild tobacco *Nicotiana attenuata*. New Phytologist **191**:528-535.

Halpern, S., L. Adler, and M. Wink. 2010. Leaf herbivory and drought stress affect floral attractive and defensive traits in *Nicotiana quadrivalvis*. Oecologia **163**:961-971-971.

Hardwick, D. F. 1970a. A generic revision of the North American Heliothidinae (Lepidoptera: Noctuidae). Memoirs of the Entomological Society of Canada **Number 73**.

Hardwick, D. F. 1970b. The life history of *Schinia florida* (Noctuidae). Journal of the Lepidopterists' Society **24**:282-287.

Hawkes, C. V. and J. S. Jon. 2001. The impact of herbivory on plants in different resource conditions: A meta-analysis. Ecology **82**:2045-2058.

Herrera, C. M. 1982. Defense of ripe fruit from pests: Its significance in relation to plant-disperser interactions. The American Naturalist **120**:218-241.

Howe, H. F. and J. Smallwood. 1982. Ecology of seed dispersal. Annual Review of Ecology and Systematics **13**:201-228.

Irwin, R. E. and L. S. Adler. 2006. Correlations among traits associated with herbivore resistance and pollination: implications for pollination and nectar robbing in a distylous plant. American Journal of Botany **93**:64-72.

Johnson, M. 2011a. The contribution of evening primrose (*Oenothera biennis*) to a modern synthesis of evolutionary ecology. Population Ecology **53**:9-21.

Johnson, M. T. J. 2011b. The contribution of evening primrose (*Oenothera biennis*) to a modern synthesis of evolutionary ecology. Population Ecology **53**:9-21.

Johnson, M. T. J., A. A. Agrawal, J. L. Maron, and J. P. Salminen. 2009a. Heritability, covariation and natural selection on 24 traits of common evening primrose (*Oenothera biennis*) from a field experiment. Journal of Evolutionary Biology **22**:1295-1307.

Johnson, M. T. J., S. D. Smith, and M. D. Rausher. 2009b. Plant sex and the evolution of plant defenses against herbivores. Proceedings of the National Academy of Sciences **106**:18079-18084.

Karban, R., A. A. Agrawal, J. S. Thaler, and L. S. Adler. 1999. Induced plant responses and information content about risk of herbivory. Trends in Ecology & Evolution **14**:443-447.

Karban, R. and I. T. Baldwin. 1997. Induced responses to herbivory. University of Chicago Press, Chicago.

Karonen, M., J. Parker, A. Agrawal, and J.-P. Salminen. 2010. First evidence of hexameric and heptameric ellagitannins in plants detected by liquid chromatography/electrospray ionisation mass spectrometry. Rapid Communications in Mass Spectrometry **24**:3151-3156.

Kessler, A. and R. Halitschke. 2009. Testing the potential for conflicting selection on floral chemical traits by pollinators and herbivores: predictions and case study. Functional Ecology **23**:901-912.

Kessler, A., R. Halitschke, and K. Poveda. 2011. Herbivory-mediated pollinator limitation: negative impacts of induced volatiles on plant-pollinator interactions. Ecology **92**:1769-1780.

Kessler, A. and I. T. Baldwin. 2004. Herbivore-induced plant vaccination. Part I. The orchestration of plant defenses in nature and their fitness consequences in the wild tobacco *Nicotiana attenuata*. The Plant Journal **38**:639-649.

Kinsman, S. 1982. Herbivore responses to *Oenothera biennis* (Onagraceae): Effects of the host plant's size, genotype, and resistant conspecific neighbors. Cornell University, Ithaca, NY.

Louda, S. M. and M. A. Potvin. 1995. Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant. Ecology **76**:229-245.

Mauricio, R. 1998. Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana*. The American Naturalist **151**:20-28.

McCall, A. and R. Karban. 2006. Induced defense in *Nicotiana attenuata* (Solanaceae) fruit and flowers. Oecologia **146**:566-571.

McCall, A. C. and R. E. Irwin. 2006. Florivory: the intersection of pollination and herbivory. Ecology Letters **9**:1351-1365.

McCune, B. and J. B. Grace. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, Oregon, USA.

Ohnmeiss, T. E. and I. T. Baldwin. 2000. Optimal defense theory predicts the ontogeny of an induced nicotine defense. Ecology **81**:1765-1783.

Parachnowitsch, A. L. and C. M. Caruso. 2008. Predispersal seed herbivores, not pollinators, exert selection on floral traits via female fitness. Ecology **89**:1802-1810.

Potter, D. A. and D. W. Held. 2002. Biology and management of the Japanese beetle. Annual Review of Entomology **47**:175-205.

Powell, J. A. 1980. Evolution of larval food preferences in microlepidoptera. Annual Review of Entomology **25**:133-159.

Rodriguez, L. 2006. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. Biological Invasions **8**:927-939-939.

Rosenthal, G. A. and M. R. Berenbaum. 1992. Herbivores: Their interactions with secondary plant metabolites, Vol. II: Evolutionary and ecolological processes. Academic Press, New York.

Salminen, J.-P. and M. Karonen. 2011. Chemical ecology of tannins and other phenolics: we need a change in approach. Functional Ecology **25**:325-338.

Salminen, J.-P., M. Karonen, and J. Sinkkonen. 2011. Chemical ecology of tannins: Recent developments in tannin chemistry reveal new structures and structure–activity patterns. Chemistry – A European Journal **17**:2806-2816.

Strauss, S. Y., R. E. Irwin, and V. M. Lambrix. 2004. Optimal defence theory and flower petal colour predict variation in the secondary chemistry of wild radish. Journal of Ecology **92**:132-141.

Strauss, S. Y., J. A. Rudgers, J. A. Lau, and R. E. Irwin. 2002. Direct and ecological costs of resistance to herbivory. Trends in Ecology & Evolution **17**:278-285.

Taniguchi, S., Y. Imayoshi, R. Yabu-uchi, H. Ito, T. Hatano, and T. Yoshida. 2002. A macrocyclic ellagitannin trimer, oenotherin T1, from *Oenothera* species. Phytochemistry **59**:191-195.

Tewksbury, J. J. and G. P. Nabhan. 2001. Seed dispersal: Directed deterrence by capsaicin in chillies. Nature **412**:403-404.

Thaler, J. S., A. A. Agrawal, and R. Halitschke. 2010. Salicylate-mediated interactions between pathogens and herbivores. Ecology **91**:1075-1082.

Whitehead, S. R. and K. Poveda. 2011. Herbivore-induced changes in fruit–frugivore interactions. Journal of Ecology **99**:964-969.

Wittstock, U. and J. Gershenzon. 2002. Constitutive plant toxins and their role in defense against herbivores and pathogens. Current Opinion in Plant Biology **5**:300-307.

CHAPTER 4

Plant genotypic diversity reduces the efficiency of consumer resource utilization

Introduction

Human alterations of the environment are accelerating the loss of biodiversity (Pimm et al. 1995), which can have profound impacts on communities and ecosystem functioning (Chapin et al. 1997, Hooper et al. 2005). A handful of studies have begun to address how plant species diversity impacts resource utilization by consumers (Duffy et al. 2007) and the strength of consumer control over plant communities (Hillebrand and Cardinale 2004, Edwards et al. 2010). However, while several studies have now shown that plant genotypic diversity alters consumer communities (Wimp et al. 2004, Crutsinger et al. 2006, Cook-Patton et al. 2010), little work has assessed how genotypic diversity alters the utilization of resources by consumers.

At least three formal hypotheses have been suggested for how plant species diversity may impact patterns of resource utilization by herbivores. First, the *variance in edibility hypothesis* (Leibold 1989, Duffy 2002) posits that a resource base with more species is more likely to contain at least one species that is resistant to consumption, which will dominate in the presence of consumers. This is analogous to the selection effect (Loreau and Hector 2001) at the resource rather than consumer level. Second, the *resource concentration hypothesis* (Root 1973) posits that fewer specialist herbivores will accumulate in diverse plant assemblages due to reduced plant apparency, herbivore residence time, and/or herbivore reproductive output. Third, the *enemies hypothesis* (Root 1973) posits that both generalist and specialist natural enemies will be more abundant in diverse plant assemblages and therefore suppress herbivore populations in polycultures more than monocultures.

In addition to these hypotheses, arguments have been made for how plant diversity may result in greater consumption from generalist herbivores via complementary acquisition of deficient nutrients (Tilman 1982) or physiological limits of detoxifying the particular secondary compounds found in individual plant species (Freeland and Janzen 1974, Marsh et al. 2006). Alternatively, consumption may increase on simple vs. diverse diets via compensatory feeding due to sub-optimal nutrient ratios present in a single diet (Raubenheimer and Simpson 1993, Simpson and Raubenheimer 1993).

While some experimental support exists for each of these hypotheses (*e.g.*, (DeMott 1998, Steiner 2001, Singer et al. 2002, Marsh et al. 2006, Haddad et al. 2009), by far the most comprehensive evidence supports the *resource concentration* and *enemies* hypotheses. In a review of 209 studies of 287 herbivorous and 130 predatory arthropod species, Andow (1991) found that 51.9% of the herbivorous species examined had lower population densities on plants in polycultures than monocultures (compared to 15.3% of species having lower densities on monocultures). Furthermore, 52.7% of predator species had higher population densities in polycultures (compared to 9.3% having higher densities in monocultures). While the *resource concentration* and *enemies* hypotheses are not mutually exclusive, Andow determined that *resource concentration* was somewhat better at explaining these results. Regardless, it is important to note that most data regarding these hypotheses solely considers the population responses of animals to plant diversity and does not explicitly link animal abundance to plant damage (Andow 1991, Duffy et al. 2007).

Whether hypothesized mechanisms regarding herbivore consumption dynamics in response to plant species diversity carry over to plant genotypic diversity is virtually untested (Hughes et al. 2008, Utsumi et al. 2011). In addition, fundamentally different mechanisms have

been found responsible for shaping consumer community structure in response to plant genotypic vs. species diversity (Cook-Patton et al. 2010), suggesting mechanisms governing consumption dynamics may differ as well. In this study we test how genotypic diversity of the common evening primrose (*Oenothera biennis*) influences the abundance and impact of one of its dominant herbivores, the Japanese beetle (*Popillia japonica*). Japanese beetles consume 15% of the leaf area of *O. biennis* on average in Tompkins Co., NY, USA (McArt et al. 2012), and it is not uncommon for plants in field populations to be completely defoliated. We address two main questions in this study: 1) How are consumption dynamics by *P. japonica* altered in response to plant genotypic diversity? 2) What mechanisms explain differences in consumption in response to plant genotypic diversity?

Materials and Methods

Study system, plant propagation, and field establishment

We manipulated genotypic richness of *Oenothera biennis* L (Common evening primrose, Onagraceae), a native herbaceous plant that is common to old-fields and disturbed areas in eastern North America. *O. biennis* reproduces via a permanent translocation heterozygosity genetic system, which results in seeds that are genetically identical to each other and the parent (Cleland 1972, Johnson 2011). We collected *O. biennis* seeds from individual plants in 20 distinct populations around Ithaca, NY. Each genotype used in this experiment was determined to be unique using nine polymorphic microsatellite loci developed for *O. biennis* (Larson et al. 2008). To reduce maternal effects, we first grew the seeds in a common garden in 2007, which was sprayed with insecticide at regular intervals throughout the growing season, and we used seeds collected from these plants (20 genotypes) for our experiment.

We cold stratified (4°C, four days) all seeds for the field experiment in April 2010, sowed them into 96-well trays filled with soil (Pro-mix "BX" with biofungicide, Premier), and thinned germinated seedlings to a single individual per well. Plants were watered *ad libitum* and fertilized weekly (21-5-20 NPK, 150 ppm) while in the greenhouse (14:10 hour light:dark cycle, 5 weeks) and then field-hardened in an outdoor mesh cage (one week) prior to planting in the field.

In May 2010, we established the field experiment in an abandoned agricultural field near Ithaca, NY where the soil was plowed, but otherwise untreated. Using our pool of 20 *O. biennis* genotypes, we constructed two treatments: genotypic monocultures (one *O. biennis* genotype) and genotypic polycultures (seven *O. biennis* genotypes). All plots contained seven equally spaced individual plants arrayed in a ring 0.5 m in diameter, and plots were separated by 1.5 m. We clipped encroaching weeds by hand every 2-3 weeks to ensure treatments remained consistent throughout the summer. The original design included 120 plots, but due to the loss of individuals within plots, we restricted our analyses to the 109 plots that experienced no mortality (monocultures: $n = 55$; polycultures: $n = 54$). Every genotype appeared in ~19 polycultures and there were three monocultures of each genotype (except for five *O. biennis* genotypes that had two monocultures each due to mortality). Due to its large size, we divided our experiment into four spatial blocks where each block contained the same proportion of monocultures and polycultures.

Herbivory surveys and plant productivity

We conducted two censuses of Japanese beetles (*Popillia japonica*) at the peak of their abundance (once in late July and again in early August) by visually surveying every plant in the experiment ($n = 840$ plants). Japanese beetles are the dominant folivore on *O. biennis* at our

sites and are responsible for >95% of the leaf area consumed on this plant species in Tompkins Co., NY, USA (McArt et al. 2012). In early September, when all beetles were gone but leaves were still on plants, we surveyed the quantity of beetle leaf damage. We placed an acetate sheet printed with a 1 cm^2 grid over each leaf of every plant in the experiment, quantifying leaf area consumed on each plant. In early October, when plants stopped producing new fruits, we counted the number of fruits and collected the above-ground biomass for each plant in the experiment. We dried all plant material for 5 days at 40°C and then weighed to the nearest 0.1 g.

We tested for differences in plot-level beetle abundance, leaf area consumed, above-ground plant biomass, and number of fruits produced between diversity treatments via ANOVA with spatial block as a random effect (JMP Pro 9.0.2). We log transformed beetle abundance data to improve normality of the residuals. To compare the amount of leaf area consumed between *O. biennis* genotypes in each treatment we used ANOVA and tested for the effect of genotype, treatment, and the interaction between genotype and treatment. While genotypes occurred only once in each polyculture plot, numerous plants from a single genotype comprised each monoculture. Therefore we used mean plot-level damage values as replicates for monocultures and individual plants as replicates in polycultures.

To test whether plot-level differences in herbivory and fruit production were due to complementarity or selection among plant genotypes we followed the methods of Loreau and Hector (2001). For plant productivity, positive complementarity implies that increases in polyculture yield are due to resource partitioning or facilitation among plant genotypes, whereas negative selection implies that smaller genotypes grow proportionally better in polycultures than monocultures compared to larger genotypes. Similarly, for herbivory negative complementarity implies that decreases in herbivore damage in polycultures are due predominantly to beneficial

associational effects among plant genotypes, whereas positive selection implies that genotypes that received the greatest amount of damage in monoculture receive proportionally more damage in polyculture than low-damage genotypes. We tested whether complementarity and selection effects were positive or negative by observing whether 95% confidence intervals overlapped zero.

Beetle movement

In order to quantify beetle movement within and among *O. biennis* patches, we replicated the 2010 field establishment protocols for a follow-up experiment in 2011 containing 17 of the original 20 genotypes. Equal numbers of monocultures and polycultures were planted where each genotype occurred in 7 polycultures and one monoculture (n = 34 patches total). Similar to the previous field experiment, monocultures were spatially alternated with polycultures such that two monocultures and two polycultures were present in four-patch groups. In late-July we observed beetle movement in each four-patch group for 15 min periods. Each time a beetle moved off a plant we recorded whether it moved to a neighboring plant within a patch or anywhere outside the patch (including outside the four-patch group). We repeated these observations for each group of patches such that every patch in the experiment was surveyed for 15 minutes per day, and we repeated this observation protocol for three successive days. We tested whether overall beetle movement within vs. between patches differed from a 50-50 expectation, and whether within vs. between patch movement differed between diversity treatments, via Pearson Chi-square analysis.

Sequential beetle bioassay

We conducted a two-part bioassay to test the resistance of *O. biennis* genotypes to *P. japonica* grown in monocultures vs. polycultures. During the peak of *P. japonica* abundance in

late-July 2010, we collected individual leaves from six replicate plants of each genotype in each treatment (6 leaves per genotype × 20 genotypes × 2 treatments = 240 bioassays). The first fully expanded leaf from each plant was cut at its petiole, placed in a Petri dish (9 cm diameter) containing a moist sheet of filter paper, and immediately transported back to the lab. One *P. japonica* adult (collected from the wild) was placed in each dish and allowed to feed for 24 hrs at 20°C. At the end of 24 hrs each *P. japonica* adult was removed and leaf area consumed (mm^2) was assessed on leaves. In order to assess resistance of individual plants grown in monocultures vs. polycultures, we tested for differences in leaf area consumed between treatments via ANOVA with genotype as a random effect. Forty-one beetles did not initiate feeding or died during this assay (n = 22 monoculture, n = 19 polyculture), however whether or not we include these zero values in our analysis did not alter the direction or significance of results. Therefore, we present the data excluding beetles for consistency with our sequential feeding assay (see below).

In order to test whether sequential feeding on the same vs. different genotypes altered beetle consumption we continued this initial bioassay. All beetles from the first assay were immediately transferred to a new leaf in a new Petri dish and allowed to feed for an additional 24 hrs. Leaves for the second feeding assay were obtained from the field experiment in an identical manner to the first collection. In order to mimic the way sequential feeding might occur within patches, beetles assigned to a monoculture sequential-feeding treatment were transferred to a leaf from a different plant of the same genotype, while beetles assigned to a polyculture sequential-feeding treatment were transferred to a leaf from a different genotype. Leaf area consumed during the second assay was assessed at the conclusion of 24 hrs, and this design was repeated for one additional 24-hr period (three Petri dish assays per beetle in sequence over 72 hrs). Thus,

beetles in the monoculture sequential-feeding treatment consumed three leaves of the same genotype, while beetles in the polyculture sequential-feeding treatment consumed three leaves from three different genotypes.

We tested for differences in leaf area consumed over all three assays by analyzing the effect of treatment, assay, and treatment × assay interaction via ANOVA, including genotype as a random effect. Twelve beetles died over the course of this experiment and 83 beetles stopped feeding during the second or third assays ($n = 45$ monoculture sequence, $n = 50$ polyculture sequence). Whether or not we include these zero values did not alter the direction or significance of any results (treatment and assay always $P > 0.05$, treatment × assay interaction always $P < 0.05$). Therefore, to most completely assess the effects of sequential feeding, here we present only the data where full sequential feeding for all three assays occurred.

Results

We found 28% more Japanese beetles in polycultures vs. monocultures during our surveys of beetle abundance ($F_{1,103} = 4.9$, $P = 0.029$, Fig. 4.1A). In stark contrast to these results, we found a 24% decrease in the amount of leaf area consumed in *O. biennis* polycultures ($F_{1,103} = 4.0$, $P = 0.044$, Fig. 4.1B). Because plants tended to be larger in polycultures (see productivity results below), the percent of leaf area consumed was likely even further reduced in polycultures compared to this absolute measure of herbivory.

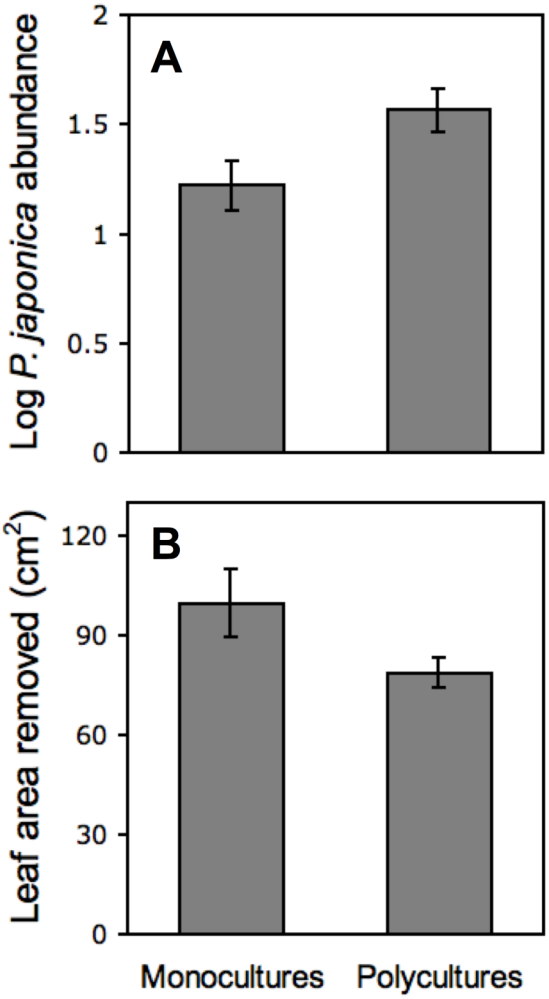

Figure 4.1: (**A**) The abundance of Japanese beetles (*Popillia japonica*) was greater in *Oenothera biennis* genotypic polycultures vs. monocultures, while (**B**) the amount of leaf damage was less in polycultures vs. monocultures (mean ± SEM). Beetle abundance log transformed to improve normality.

While genotypes differed in resistance over 12 fold from lowest to highest ($F_{19,389} = 14.3$, $P < 0.001$), the consistent effect of associational resistance in polycultures was evident via the reduced magnitude of damage on 16 out of 20 genotypes and no genotype × treatment interaction ($F_{19,389} = 0.7$, $P = 0.85$, Fig. 4.2). When we partitioned the mechanisms for reduced herbivory in polycultures, we found further evidence for the consistency of associational resistance: we found strong complementarity among genotypes for reduced damage (95%

confidence = -21.4 ± 8.3), while there was much weaker positive selection (95% confidence = 4.1 ± 3.2, Fig. 4.2 inset). This latter result indicates that genotypes that received the greatest amount of damage in monoculture receive proportionally more damage in polyculture than low-damage genotypes.

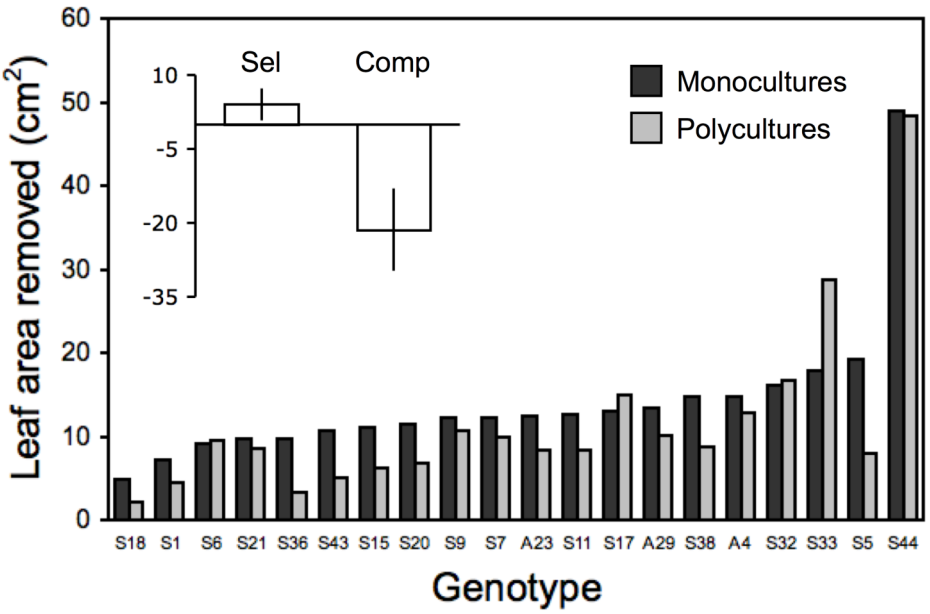

Figure 4.2: Mean values of damage on 20 *O. biennis* genotypes grown in monocultures vs. polycultures (error bars not shown for figure clarity). Associational resistance in polycultures resulted from negative complementarity (Comp) and positive selection (Sel) among *O. biennis* genotypes for the amount of damage received (inset). Complementarity and selection analyses performed following the methods outlined in Loreau and Hector (2001), mean ± 95% confidence intervals shown.

Given the strong complementarity and associational resistance in *O. biennis* polycultures, we hypothesized that more beetles could do less damage for two possible reasons. First, because plant phenotypic traits such as biomass (Tilman et al. 1996, Cardinale et al. 2007), C:N ratio (van Ruijven and Berendse 2005, Fargione et al. 2007), and chemical defenses against herbivores

(Mraja et al. 2011) can change when plants are grown in diverse mixtures, individual plant resistance could be greater for genotypes when grown in polyculture. When we performed a bioassay allowing beetles to consume leaf tissue from the same set of genotypes grown in monoculture vs. polyculture, we found no difference in consumption between treatments ($F_{1,178}$ = 1.9, P = 0.17, Fig. 4.3 assay #1).

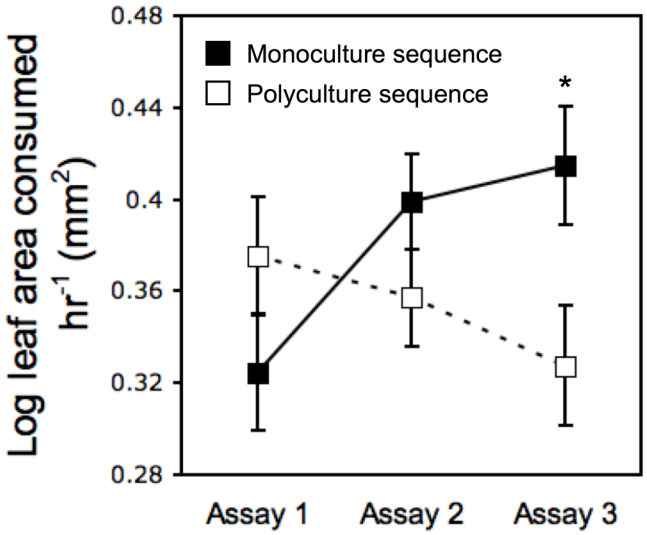

Figure 4.3: Leaf area consumed by *P. japonica* (log transformed to improve normality) when fed leaves from different plants of the same genotype (black squares, solid line) or leaves from different genotypes (open squares, dashed line) in sequence (LS mean ± SE). Individual beetles from each treatment were fed leaves in sequence such that each beetle consumed one leaf in each assay. Assay #1 tested the effect of treatment on individual plant resistance to *P. japonica*, while Assays 1-3 tested sequential resistance. * $P < 0.05$ post-hoc independent contrast between treatments for individual assays.

Alternatively, we observed that beetles were more than twice as likely to move between plants within a patch compared to leaving a patch to feed elsewhere. This pattern differed

significantly from a 50-50 expectation (within-patch $n = 144$, between-patch $n = 67$: Pearson $\chi^2 = 28.1$, $P < 0.001$), and was similar for beetles moving from plants in monocultures ($n = 91$) vs. polycultures ($n = 120$) (Pearson $\chi^2 = 0.001$, $P = 0.98$). Thus, we hypothesized that sequential feeding on different genotypes in polyculture compared to sequential feeding on different plants of the same genotype in monoculture could result in reduced feeding. Consistent with this form of associational resistance, we found that beetles reduced consumption when fed leaves from three different genotypes in sequence compared to when fed leaves from the same genotype in sequence over a period of 72 hrs (treatment × assay interaction: $F_{2,404} = 4.2$, $P = 0.015$, post-hoc contrast assay #3: $P = 0.010$, Fig. 4.3).

In order to contrast herbivore damage with plant productivity, we quantified above-ground plant biomass and the number of fruits produced in each treatment. While we did not find an increase in plant biomass ($F_{1,103} = 1.3$, $P = 0.26$), we found an 11% increase in the number of fruits produced in *O. biennis* genotypic polycultures compared to monocultures ($F_{1,103} = 5.9$, $P = 0.017$, Fig. 4.4). Increased fruit production was a result of strong positive complementarity (95% confidence = 158.0 ± 63.0), while there was weak but significant negative selection (95% confidence = -9.2 ± 7.5, Fig. 4.4 inset).

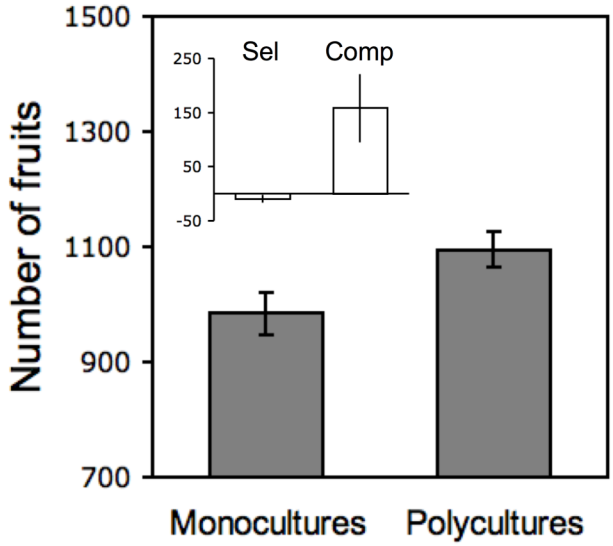

Figure 4.4: The number of fruits produced by *Oenothera biennis* increased in polycultures vs. monocultures (mean ± SEM). Increased productivity resulted from positive complementarity (Comp) and negative selection (Sel) in polycultures. Complementarity and selection analyses performed following the methods outlined in Loreau and Hector (2001), mean ± 95% confidence intervals shown.

Discussion

We found the abundance of a dominant folivore of *O. biennis*, the Japanese beetle (*P. japonica*), increased abundance in response to plant genotypic diversity. This result is similar to numerous previous studies that have manipulated plant genotypic diversity and found an increased abundance of herbivore populations and communities in this system (Cook-Patton et al. 2010) and in others (Reusch et al. 2005, Crutsinger et al. 2006, Hughes et al. 2008, Utsumi et

al. 2011). Importantly, in this study we link patterns of herbivore abundance with plant damage, showing that despite an increase in numbers of *P. japonica*, the amount of damage incurred by plants was reduced in an absolute and proportional sense in genotypic polycultures compared to monocultures. Furthermore, we provide a mechanistic link for this pattern by showing how sequential consumption of different plant genotypes can cause associational resistance in genotypically diverse plant patches.

While the impact of herbivores is typically reduced in response to plant species diversity (Hillebrand and Cardinale 2004, Duffy et al. 2007, Edwards et al. 2010), numerous mechanisms may be responsible for this effect. Our results do not provide strong support for any of the three main hypotheses posed for a reduction in herbivory in response to plant species diversity. Because we found greater numbers of *P. japonica* in polycultures, our data does not support the *resource concentration hypothesis* (Root 1973). Because *P. japonica* adults are relatively resistant to predation (Potter and Held 2002) (we never observed a predation event on adult beetles in this study), and we did not find and increase in predator abundance or richness in response to *O. biennis* genotypic diversity in a previous study in this system (Cook-Patton et al. 2010), we find no conclusive evidence for the *enemies hypothesis* (Root 1973). Finally, we found there was significant positive selection for plant damage in polycultures (Fig. 4.2), meaning that the most resistant genotypes in monoculture were even more resistant in polyculture. While this result is consistent with the *variance in edibility hypothesis* (Leibold 1989, Duffy 2002), the ability of resistant *O. biennis* genotypes to dominate in polycultures (*i.e.*, positive selection) was weak compared to the strong overall associational resistance (*i.e.*, negative complementarity for plant damage) in genotypic polycultures (Fig. 4.2 inset). This

result suggests that mechanisms other than *variance in edibility* were the primary drivers of reduced herbivore damage in response to *O. biennis* genotypic diversity.

In our bioassay with *P. japonica* (Fig. 4.3), we found that individual plant resistance did not differ between genotypes grown in monocultures vs. polycultures. This suggests that individual plant quality traits that can change when plants are grown in diverse species mixtures (van Ruijven and Berendse 2005, Fargione et al. 2007, Mraja et al. 2011) were either not affected by plant genotypic diversity or unimportant. Instead, we found that sequential feeding on different *O. biennis* genotypes resulted in reduced damage compared to sequential feeding on the same genotype (Fig. 4.3). Because *P. japonica* preferentially moves and feeds on plants within patches compared to between patches, reduced consumption via sequential feeding provides a mechanistic link between the opposing patterns of increased *P. japonica* abundance and reduced damage we observed in *O. biennis* polycultures during the field experiment.

While sequential feeding on different plants has not previously been considered as a mechanism of associational resistance to herbivores (Atsatt and O'Dowd 1976, Barbosa et al. 2009), this may be a common response of mobile herbivores that feed on multiple neighboring plants. Compensatory feeding can occur when animals are restricted to diets suboptimal for their target intake of different nutrients, such as suboptimal protein:carbohydrate ratios (Raubenheimer and Simpson 1993, Simpson and Raubenheimer 1993). Because the genotypes used in this experiment are known to differ significantly in nutritional characteristics such C:N ratio (Johnson et al. 2009), individual *P. japonica* beetles may have compensated for suboptimal nutrition in single-genotype monocultures by consuming more leaf tissue compared to mixed-genotype polycultures (where dietary mixing among genotypes occurred). Alternatively, the genotypes we used in this experiment are also known to differ substantially in the abundance of

particular secondary compounds such as ellagitannins and flavonoids (Johnson et al. 2009). Although dietary mixing among plant species that contain different toxins is predicted to allow increased consumption in generalist herbivores (Freeland and Janzen 1974), there is remarkably little experimental evidence supporting this detoxification limitation hypothesis (Marsh et al. 2006). Furthermore, we are aware of no studies that have tested the hypothesis for different plant genotypes, which may differ more in the abundance of different toxins as opposed to their qualitative presence or absence. Understanding how primary and secondary metabolites interact to affect consumption is an important but understudied area of chemical and nutritional ecology (Behmer et al. 2002, Steppuhn and Baldwin 2007), and likely underlies the specific physiological mechanism responsible for our results.

A handful of studies now show that plant species diversity can alter the strength of consumer impacts on plant communities (Hillebrand and Cardinale 2004, Duffy et al. 2007, Edwards et al. 2010), and these diversity-mediated feedbacks have recently been extended to plant genotypic diversity (Parker et al. 2010). However, because it is rare for herbivore abundance, plant damage, and plant productivity to be simultaneously assessed in the same study, the specific mechanisms for how consumption is reduced in response to plant diversity and therefore feeds back into productivity is poorly understood (Andow 1991, Duffy et al. 2007). Although we did not explicitly manipulate herbivores in our field experiment, we observed that while plant damage decreased in *O. biennis* polycultures, the number of fruits produced in polycultures increased. We also observed strikingly similar yet opposing patterns of negative complementarity for herbivore damage (Fig. 4.2 inset) and positive complementarity for the production of fruits (Fig. 4.4 inset). These results are consistent with the notion that the specific

mechanism we found for reduced herbivore consumption in genotypic polycultures may feed back to affect plant productivity.

In summary, we have shown that plant genotypic diversity decreases herbivore consumption efficiency by increasing the abundance of herbivores but reducing the amount of damage in genotypic polycultures. Thus, opposing forces (herbivore abundance vs. consumption efficiency) can mediate the strength of top-down control in response to plant genotypic diversity. We also found that sequential feeding by *P. japonica* on different plant genotypes reduced intake, likely due to the nutritional or physiological constraints imposed by a mixed-genotype diet. Thus, by linking behavioral observations with animal abundance and damage patterns we were able to gain remarkable insight into the mechanism for how plant genotypic diversity resists herbivory. Overall, our results suggest that different mechanisms are responsible for patterns of consumer resource utilization in response to plant genotypic vs. species diversity, which has direct implications for how trophic dynamics affect ecosystem functioning (Duffy et al. 2007).

Acknowledgements

We thank A. Agrawal and A. Kessler for helpful comments on the manuscript. The NSF (IGERT small grant in Biogeochemistry and Environmental Biocomplexity to S.H.M.), Grace Griswold Endowment (S.H.M.), and USDA-NRI (2006-35302-1731 to J.S.T.) supported this work.

REFERENCES

Andow, D. A. 1991. Vegetational diversity and arthropod population response. Annual Review of Entomology **36**:561-586.

Atsatt, P. R. and D. J. O'Dowd. 1976. Plant defense guilds. Science **193**:24-29.

Barbosa, P., J. Hines, I. Kaplan, H. Martinson, A. Szczepaniec, and Z. Szendrei. 2009. Associational resistance and associational susceptibility: Having right or wrong neighbors. Annual Review of Ecology, Evolution, and Systematics **40**:1-20.

Behmer, S. T., S. J. Simpson, and D. Raubenheimer. 2002. Herbivore foraging in chemically heterogeneous environments: Nutrients and secondary metabolites. Ecology **83**:2489-2501.

Cadotte, M. W., B. J. Cardinale, and T. H. Oakley. 2008. Evolutionary history and the effect of biodiversity on plant productivity. Proceedings of the National Academy of Sciences **105**:17012-17017.

Cardinale, B. J. 2011. Biodiversity improves water quality through niche patitioning. Nature **472**:86-89.

Cardinale, B. J., J. P. Wright, M. W. Cadotte, I. T. Carroll, A. Hector, D. S. Srivastava, M. Loreau, and J. J. Weis. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Sciences **104**:18123-18128.

Chapin, F. S., B. H. Walker, R. J. Hobbs, D. U. Hooper, J. H. Lawton, O. E. Sala, and D. Tilman. 1997. Biotic control over the functioning of ecosystems. Science **277**:500-504.

Cleland, R. E. 1972. Oenothera: cytogenetics and evolution. Academic Press, New York.

Cook-Patton, S. C., S. H. McArt, A. L. Parachnowitsch, J. S. Thaler, and A. A. Agrawal. 2010. A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function. Ecology **92**:915-923.

Crutsinger, G. M., M. D. Collins, J. A. Fordyce, Z. Gompert, C. C. Nice, and N. J. Sanders. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. Science **313**:966-968.

DeMott, W. R. 1998. Utilization of a cyanobacterium and a phosphorus-deficient green alga as complementary resources by daphnids. Ecology **79**:2463-2481.

Duffy, J. E. 2002. Biodiversity and ecosystem function: the consumer connection. Oikos **99**:201-219.

Duffy, J. E., B. J. Carinale, K. E. France, P. B. McIntyre, E. Thebault, and M. Loreau. 2007. The functional role of biodiversity in ecosystems: Incorporating trophic complexity. Ecology Letters **10**:522-538.

Edwards, K. F., K. M. Aquilino, R. J. Best, K. L. Sellheim, and J. J. Stachowicz. 2010. Prey diversity is associated with weaker consumer effects in a meta-analysis of benthic marine experiments. Ecology Letters **13**:194-201.

Fargione, J., D. Tilman, R. Dybzinski, J. H. R. Lambers, C. Clark, W. S. Harpole, J. M. H. Knops, P. B. Reich, and M. Loreau. 2007. From selection to complementarity: Shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. Proceedings of the Royal Society B **274**:871-876.

Freeland, W. J. and D. H. Janzen. 1974. Strategies in herbivory by mammals: The role of plant secondary compounds. The American Naturalist **108**:269-289.

Haddad, N. M., G. M. Crutsinger, K. Gross, J. Haarstad, J. M. H. Knops, and D. Tilman. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. Ecology Letters **12**:1029-1039.

Hillebrand, H. and B. J. Cardinale. 2004. Consumer effects decline with prey diversity. Ecology Letters **7**:192-201.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setala, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs **75**:3-35.

Hooper, D. U. and P. M. Vitousek. 1998. Effects of plant composition and diversity on nutrient cycling. Ecological Monographs **68**:121-149.

Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood, and M. Vellend. 2008. Ecological consequences of genetic diversity. Ecology Letters **11**:609-623.

Johnson, M. 2011. The contribution of evening primrose (*Oenothera biennis*) to a modern synthesis of evolutionary ecology. Population Ecology **53**:9-21.

Johnson, M. T. J., A. A. Agrawal, J. L. Maron, and J. P. Salminen. 2009. Heritability, covariation and natural selection on 24 traits of common evening primrose (*Oenothera biennis*) from a field experiment. Journal of Evolutionary Biology **22**:1295-1307.

Johnson, M. T. J., M. J. Lajeunesse, and A. A. Agrawal. 2006. Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. Ecology Letters **9**:24-34.

Larson, E. L., S. M. Bogdanowicz, A. A. Agrawal, M. T. J. Johnson, and R. G. Harrison. 2008. Isolation and characterization of polymorphic microsatellite loci in common evening primrose (*Oenothera biennis*). Molecular Ecology Resources **8**:434-436.

Leibold, M. A. 1989. Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. The American Naturalist **134**:922-949.

Loreau, M. and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature **412**:72-76.

Marsh, K., I. Wallis, R. Andrew, and W. Foley. 2006. The detoxification limitation hypothesis: Where did it come from and where is it going? Journal of Chemical Ecology **32**:1247-1266.

McArt, S. H., R. Halitschke, J. P. Salminen, and J. S. Thaler. 2012. Invasive herbivore increases plant fitness via induced resistance to seed predators. Submitted to PNAS.

Mraja, A., S. B. Unsicker, M. Reichelt, J. Gershenzon, and C. Roscher. 2011. Plant community diversity influences allocation to direct chemical defence in *Plantago lanceolata*. PLoS ONE **6**:e28055.

Parker, J. D., J.-P. Salminen, and A. A. Agrawal. 2010. Herbivory enhances positive effects of plant genotypic diversity. Ecology Letters **13**:553-563.

Pimm, S. L., G. J. Russell, J. L. Gittleman, and T. M. Brooks. 1995. The future of biodiversity. Science **269**:347-350.

Potter, D. A. and D. W. Held. 2002. Biology and management of the Japanese beetle. Annual Review of Entomology **47**:175-205.

Raubenheimer, D. and S. J. Simpson. 1993. The geometry of compensatory feeding in the locust. Animal Behaviour **45**:953-964.

Reusch, T. B. H., A. Ehlers, A. Hammerli, and B. Worm. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proceedings of the National Academy of Sciences of the United States of America **102**:2826-2831.

Root, R. B. 1973. Organization of a plant-arthropod association in simple and diverse habitats - fauna of collards (*Brassica oleracea*). Ecological Monographs **43**:95-120.

Schweitzer, J. A., J. K. Bailey, S. C. Hart, and T. G. Whitham. 2005. Nonadditive effects of mixing cottonwood genotypes on litter decomposition and nutrient dynamics. Ecology **86**:2834-2840.

Simpson, S. J. and D. Raubenheimer. 1993. A multi-level analysis of feeding behaviour: The geometry of nutritional decisions. Philosophical Transactions: Biological Sciences **342**:381-402.

Singer, M. S., E. A. Bernays, and Y. Carriere. 2002. The interplay between nutrient balancing and toxin dilution in foraging by a generalist insect herbivore. Animal Behaviour **64**:629-643.

Steiner, C. F. 2001. The effects of prey heterogeneity and consumer identity on the limitation of trophic-level biomass. Ecology **82**:2495-2506.

Steppuhn, A. and I. T. Baldwin. 2007. Resistance management in a native plant: nicotine prevents herbivores from compensating for plant protease inhibitors. Ecology Letters **10**:499-511.

Tilman, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, New Jersey, USA.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature **379**:718-720.

Utsumi, S., Y. Ando, T. P. Craig, and T. Ohgushi. 2011. Plant genotypic diversity increases the population size of a herbivorous insect. Proceedings of the Royal Society B **278**:3108-3115.

van Ruijven, J. and F. Berendse. 2005. Diversity-productivity relationships: Initial effects, long-term patterns, and underlying mechanisms. Proceedings of the National Academy of Sciences of the United States of America **102**:695-700.

Wimp, G. M., W. P. Young, S. A. Woolbright, G. D. Martinsen, P. Keim, and T. G. Whitham. 2004. Conserving plant genetic diversity for dependent animal communities. Ecology Letters **7**:776-780.