

Genotypic diversity mitigates negative effects of density on plant performance: a field experiment and life cycle analysis of common evening primrose *Oenothera biennis*

Susan C. Cook-Patton¹, Amy P. Hastings² and Anurag A. Agrawal^{*,2,3}

¹AAAS Science & Technology Policy Fellow, 201 14th Street, SW, Washington, DC 20227, USA; ²Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA; and ³Department of Entomology, Cornell University, Ithaca, NY 14853, USA

Summary

1. Genotypic diversity in plant populations is known to enhance plant performance and ecosystem function. Nonetheless, the effect of genotypic diversity has rarely been examined across a population's lifecycle despite the expectation that changing conditions, such as population density, will alter the benefits of diversity.

2. We simultaneously manipulated a component of genotypic diversity (richness, the number of genotypes) and density of common evening primrose *Oenothera biennis* to address the consequences for herbivory and lifetime fitness in a 2-year field experiment that spanned seed germination to lifetime fruit production. We genotyped >1100 seedlings with microsatellite DNA markers to determine realized diversity and density in plots sown with *O. biennis* seeds. Our design achieved quantitative variation in plant density and diversity, with one to 44 individuals established in field plots and two to eight genotypes per polyculture plot (based on microsatellite analysis of reproductive plants).

3. We found a strong interaction between seed density and genetic diversity, with germination and establishment being 24% higher in genetic polycultures than monocultures, but only at low seed density. At high seed density, the opposite pattern emerged, with polycultures having 12% fewer individuals established than monocultures. Initial effects of emergence on plot density persisted through to the fruiting stage.

4. Higher plant densities resulted in increased mortality, decreased probability of reproduction, decreased plant height and lower levels of lifetime fruit production per plant. Increasing genotypic diversity increased the probability of reproduction overall and showed a significant interaction with plant density mitigating the negative effects of high density on individual height and lifetime fruit production.

5. *Synthesis*. Plant density and genotypic diversity interacted from the very early stages of seed germination and establishment of *Oenothera biennis*. This effect persisted over the 2-year life cycle of plants, and genotypic diversity buffered against the negative fitness consequences of high plant density. These results imply a dynamic interplay between the long-held paradigm of density effects in plant ecology and the genetic structure of populations.

Key-words: biodiversity–ecosystem function, complementarity, density dependence, dominance effect, plant–insect interactions, seed addition

Introduction

Classic studies have demonstrated that plant genotypic diversity within a species can alter competitive dynamics, plant–

animal interactions and ultimately the fitness of individuals in a population (Antonovics & Ellstrand 1984; Power 1988; Hughes *et al.* 2008). More recent work shows that genotypic diversity can also impact community and ecosystem properties, for example arthropod communities and nutrient cycling, respectively, with the magnitude of effect rivalling that of

*Correspondence author. E-mail: aa337@cornell.edu

species diversity (Crutsinger *et al.* 2006; Cook-Patton *et al.* 2011). Nonetheless, we still have a limited understanding of the context dependency of such diversity effects as well as the mechanisms that contribute to enhanced components of performance in diverse mixtures, especially in genotypically diverse communities (Pachepsky *et al.* 2007; Bolnick *et al.* 2011; Zupping-Dingley *et al.* 2014).

One potentially important, but infrequently evaluated mechanism for enhanced performance in genotypically diverse communities may be differential plant density among plant populations (Antonovics & Ellstrand 1984; He *et al.* 2005; Cardinale *et al.* 2011). If diversity manipulations are established from seed, which is important to understand diversity effects across the life cycle, plot density may vary from very early stages due to variation in seed number, even with attempts to standardize treatments by seed size or mass (Guo 2011). Moreover, seed density and diversity can impact germination and establishment (Murray 1998; Tielbörger & Prasse 2009; Crawford & Whitney 2010). Thus, with potential differences in seed number, germination and establishment among diversity treatments, unanticipated density differences may arise in studies of plant biodiversity. This makes it important to consider the direct and indirect (via density) effects of diversity from the very early stages of plant community establishment.

Even when biodiversity plots are established with uniform seedling densities (Hughes & Stachowicz 2004; Crutsinger *et al.* 2006; Johnson, Lajeunesse & Agrawal 2006), indirect effects of diversity may cascade to impact plant density and competitive interactions. For example, competition, herbivory and disease are expected to be higher in monocultures than polycultures (Root 1973; Keesing, Holt & Ostfeld 2006). Schnitzer *et al.* (2011) observed higher pathogen loads in species monocultures than polycultures, and Parker, Salminen & Agrawal (2010) documented higher vole herbivory in genotypic monocultures than polycultures. Thus, the expectation is that even with uniform starting conditions, plant density may decline more strongly in monocultures than polycultures. If monocultures have higher mortality or lower population growth than polycultures, then impacts on plot-level performance in polycultures may result from differences in density as well as diversity (Weiner & Freckleton 2010). Accordingly, a critical frontier in biodiversity research is examining the direct effect of diversity on plant performance as well as the indirect effect via density, especially when considering diversity effects throughout the life cycle.

The literature on plant density dependence is classic and voluminous (Antonovics & Levin 1980; Goldberg & Barton 1992). For example, it is well established that seedling recruitment is often limited by high intraspecific densities and this promotes species coexistence (Harms *et al.* 2000). Similarly, seedlings are more likely to persist at higher densities when surrounded by diverse neighbours (Metz, Sousa & Valencia 2010). However, it is not clear whether these interactions between diversity and density hold true within a species. Only a few studies have simultaneously examined plant genotypic diversity and density, and these found variable

results (Franks & Peterson 2003; Crawford & Whitney 2010; Erfmeier, Hantsch & Bruelheide 2013; Firestone & Jasieniuk 2013). Moreover, none of these examined the entire life cycle of plants in a field experiment.

To examine the potential feedbacks between plant density and diversity, we experimentally generated a gradient of plant genotypic diversity and density in field plots of the monocarpic plant *Oenothera biennis* (Common Evening Primrose). We followed a cohort of seeds from germination to final fruiting (a measure of lifetime fitness) over 2 years. Changes in plant density are reported, and we used microsatellite DNA markers to track quantitative variation in genotypic diversity. We also quantified insect attack to address the impacts of plant density and genotypic diversity on species interactions. Impacts of plant density and genotypic diversity on such interactions have been previously reported and are hypothesized to feedback onto diversity effects (Schmitt & Antonovics 1986; Power 1988; Andow 1991; Hughes & Stachowicz 2004; Crutsinger *et al.* 2006; Johnson, Lajeunesse & Agrawal 2006).

Specifically, we predicted that competition and herbivory would increase with plant density, but would decline with increasing genotypic diversity. We conducted analyses of independent and interactive effects of plant density and genotypic diversity at the level of individual plants as well as at the level of plots. In terms of individuals, we expected plant performance to decline with density, but to increase with diversity. At the plot level, we predicted that performance would increase with genotypic diversity due to niche complementarity, density-related mechanisms and reduced herbivory.

Materials and methods

PLANT AND INSECT NATURAL HISTORY

We studied *Oenothera biennis* L. (Onagraceae), a native herbaceous plant common to old fields and disturbed areas in eastern North America. Several independent experiments have previously demonstrated community and ecosystem consequences of genotypic diversity in *O. biennis* (Johnson, Lajeunesse & Agrawal 2006; Parker, Salminen & Agrawal 2010; Cook-Patton *et al.* 2011), but none have followed the plants through an entire life cycle or manipulated plant density. *Oenothera biennis* produces genetically identical seeds via a permanent translocation heterozygosity mating system (Cleland 1972), which enables the acquisition and maintenance of distinct genotypes. *Oenothera biennis* is monocarpic and genotypes vary from an annual to a biennial life history, and this strategy is known to plastically respond to environmental conditions (Johnson 2007). We thus ran the experiment through two field seasons to determine lifetime fruit production for all plants that emerged as a cohort in 2010.

We used 12 of 40 genotypes which were all grown in a common garden in 2006 to reduce the impact of maternal environment effects (Johnson *et al.* 2009). The 40 original collections were all from early successional habitats, which are typical of this species (one per site, average distance between sites 12 km; all from Tompkins County, New York, USA). Although our field experiment was also located in

Tompkins County, the closest collection site was ≈ 2 km away. Because of the wide sampling of the genotypes, we acknowledge that the effects of genotypic diversity observed here may be greater than would be expected in a more natural setting (Tack, Johnson & Roslin 2012). We used seeds from the common garden in the current experiment. To prevent outcrossing (which occurs occasionally in this species), we collected seeds from bagged flowering stalks. Microsatellite data showed that the 12 *O. biennis* genotypes used in this experiment are unique and identifiable using four markers (Larson *et al.* 2008; Agrawal *et al.* 2013).

In our study area, three specialist seed predator moths – *Mompha brevivittella*, *Schinia florida* and *Mompha stellella* – attack *O. biennis* reproductive structures and strongly impact plant fitness (Agrawal *et al.* 2012). They are easily distinguished by their damage marks. *Mompha brevivittella* leave small exit holes in the walls of the fruit, *S. florida* consumes the fruit walls and contents, and *M. stellella* gall flower buds, which then appear to be inflated.

PLOT ESTABLISHMENT

We established plots in a mesic, abandoned agricultural field in Dryden, NY (42°27'49" N, 76°26'19" W), which was fenced to exclude deer. In the fall of 2009, we sprayed herbicide, removed large rocks, tilled the field to remove resident vegetation and tamped the soil to improve uniformity. In February 2010, we sprinkled *O. biennis* seeds evenly with a salt shaker onto bare soil in each of the 15.2 cm \times 15.2 cm experimental plots. All the seeds allocated to a single plot were added to the shaker and dispersed before refilling for the next plot. Winter sowing permitted natural cold stratification and emergence in the spring. After sowing, we left the soil undisturbed except to weed out heterospecific seedlings in the spring of 2010 and 2011.

We sowed 100 polyculture plots in accordance with the range of genotypic diversity observed in natural populations (typically one to a few genotypes per population, with a maximum of 14 reported) (Steiner & Levin 1977). Specifically, we sowed 50 polyculture plots with 40 seeds – eight each of five different *O. biennis* genotypes. We generated polyculture compositions randomly (out of the 12 total genotypes), but adjusted compositions slightly so that genotypes occurred with roughly equal frequency. An additional 50 polyculture plots received 80 seeds, with 10 seeds each of eight different genotypes. Note that the lower density plots with 40 seeds also had fewer genotypes than the higher density plots with 80 seeds. We deliberately took this approach to achieve a gradient of realized density and diversity among polycultures (post-germination and establishment). We successfully created such a gradient (see Results), which allowed us to conduct quantitative analyses of the effects of density and diversity (and their interaction). High-density recruitment is common in the field, in part because of the lack of a dispersal mechanism of *O. biennis* fruits (e.g. Agrawal *et al.* 2012).

An additional 120 plots were sown as monocultures, each containing a single *O. biennis* genotype. We planted 10 monocultures for each of the 12 genotypes, five with 40 seeds and five with 80 seeds. Monocultures were planted to assess the impacts of density variation within a genotype (10 plots per genotype) to assess the null expectation of density effects in polycultures. All plots were arrayed in a randomized complete block design with each plot type present in each of five spatial blocks.

For 2 years, we followed a cohort of seeds that emerged in 2010. As such, we hand-collected all fruits from any plants that bolted in 2010 to prevent them from dispersing new seeds. Thus, the only plants remaining in the second year of the experiments were those

from the original cohort that did not bolt in 2010 and lived through the winter.

GENOTYPING

In both 2010 and 2011, we collected leaf tissue from every bolting plant in the polycultures to determine its genotypic identity (but not from monocultures because we knew the genotypic identity of all individuals in those plots). Across the 2 years, we genotyped 1102 plants). In the field, we collected a leaf piece (≈ 1 cm²) directly into a 96-well plate on dry ice. We stored the tissue at -80 °C and freeze-dried it prior to DNA extraction. DNA was extracted from freeze-dried tissue, and PCRs were performed as described in Agrawal *et al.* (2013), using the same four microsatellite loci.

Multiplex PCR products were diluted 1:3 with water and mixed with Hi-Di formamide and Genescan LIZ-500 size standard (Applied Biosystems, Foster City, CA, USA). We analysed samples on a 3730xl DNA Analyzer (Applied Biosystems) at the Cornell University Life Sciences Core Laboratories Center. We determined allele sizes using Genemapper version 3.5 software (Applied Biosystems) and checked all calls by eye. We assigned a plant to a particular genotype only if its alleles at all four loci were representative of that genotype. Among our 1102 total samples genotyped, only three samples showed differences from the 12 original multilocus genotypes, which each showing a change in a single allele. These changes are likely the result of mutation and not PCR error, as extraction and amplification were repeated to give the same results. We excluded these three samples from the analyses.

DATA COLLECTION

During the 2010 field season, we periodically recorded the number of seeds that had germinated in each plot (April 16th, May 14th, June 1st, August 6th) and measured maximum germination as the highest seedling count per plot observed across all sampling dates. We also recorded the number of bolting plants (June 22nd, July 20th and August 6th) and measured maximum bolting as the highest number of plants that bolted per plot across sampling dates. During the 2011 field season, we counted winter survivors on May 11th and number of bolting plants and rosettes on August 30th. Data were recorded for all 220 plots.

In addition to genotypic identity, we recorded three additional pieces of information on the bolting plants: fruit production, height and attack by specialist seed predators. We estimated total fruit production, for each plot, as the total number of fruits produced in both 2010 and 2011. Mean fruit production was estimated as total fruit production divided by the number of bolting individuals in that plot from both 2010 and 2011. We assessed height (as a proxy for vigour and to assess responses to the competitive environment) in June 2011. We recorded attack by specialist herbivores in late summer 2011 by recording damage caused by three different specialist seed predators. Note that we collected the latter two measures in 2011 only because the vast majority of plants in the experiment (>80%) followed a biennial life cycle and thus did not bolt in 2010.

ANALYSIS OVERVIEW

We conducted all analyses in R (version 3.0.1, R Foundation for Statistical Computing). Because we tracked seeds through their entire life cycle, we had three different measures of plot density: sown density (40 vs. 80 seeds), maximum realized density (all germinated seedlings

in 2010) and 2011 realized density (all germinated seedlings minus those that had died over the winter or completed their life cycle in 2010). Similarly, we had three measures of plot diversity: sown diversity (one, five or eight genotypes), maximum realized diversity (all plants that bolted in 2010 and 2011) and 2011 realized diversity (all plants that bolted in 2011). We only used sown density and diversity for the germination analyses, and primarily realized density and diversity thereafter. Realized density and diversity were somewhat correlated ($n = 98$ plots, $r = 0.69$, $P < 0.001$); accordingly, we examined the variance inflation factors (VIFs) in our statistical models to ensure that collinearity was not an issue in statistical models (Zuur, Ieno & Elphick 2010). VIFs were consistently well under a value of 3, indicating no major problem of collinearity.

We also include a traditional biodiversity analysis of diversity effects (separately at low density and high density) that do not account for changes in density. This analysis, in contrast to our main analysis, shows what conclusions we might have drawn by not accounting for the indirect effects of genetic diversity via impacts on plant density.

Emergence

Because we sowed plots with a different number of seeds (40 or 80 seeds), we parsed out plots for the emergence analyses. To examine the independent effect of density, we used monoculture plots ($N = 120$). To examine the independent effect of diversity, we compared monocultures and polycultures for the low seed density ($N = 110$) and high seed density plots ($N = 110$) separately. We used mixed effects logistic regression with sown density or sown diversity, respectively, as the fixed effect, and block and genotype as random factors [*glmer* in R package *lme4* (Bates, Maechler & Bolker 2013)]. Here and for all statistical tests, we used likelihood ratio tests to compare nested models and determine the significance of the individual factors (a single degree of freedom test of the difference in likelihoods based on a chi-square distribution).

Proportion bolting

To examine the independent effect of density on the proportion of plants that bolted vs. persisted as a rosette, we again used monoculture plots. We employed mixed effects logistic regression with density as a fixed effect, and block and genotype as random factors. To examine the effect of diversity and its interactions with density, we took advantage of the quantitative variation in density and diversity in polycultures ($N = 98$, two plots were excluded because they only had one genotype establish in 2010). We used mixed effects logistic regression with diversity and density, plus their interaction as a fixed effect, and block and genotype as random factors. We conducted these analyses for both 2010 and 2011 using the appropriate density and diversity measure.

Survival

We counted survival as either successful completion of a life cycle (bolting and fruiting) or persistence as a rosette until the end of the experiment. To examine the independent effect of density on the likelihood that an individual would survive or die, we used monoculture plots. To examine the independent effect of diversity and its interactions with density, we used polycultures. We used the same general statistical models as for bolting, with maximum density and maximum diversity as predictors.

Growth and reproduction

We had three measures of plant performance: plant size (height), fruit production per individual and total fruit production per plot. We transformed ($\ln+1$) the latter two to improve the normality of the residuals. To examine the independent effect of density on each of the three measures, we applied mixed effects linear models with density as a fixed factor, and genotype and block as random factors to the monoculture data [*lmer* in R package *lme4* (Bates, Maechler & Bolker 2013)]. To examine the effect of diversity and its interaction with density, we used the polyculture data and a mixed effects linear models with density and diversity as fixed effects plus their interaction, and block as a random factor.

Contribution of different genotypes to diversity effects

Plant performance is frequently higher in polycultures than monocultures (Hooper *et al.* 2005; Cardinale *et al.* 2011), and there are two general ways this might occur. On average, genotypes/species might perform better in polyculture than monoculture (i.e. complementarity) or specific genotypes/species might drive the gains (i.e. selection) (Loreau & Hector 2001). Fox (2005) further partitioned the selection effect to determine whether the genotype/species that benefitted the most in polyculture did so without suppressing neighbours (i.e. trait-dependent complementarity, TDC) or by dominating others (i.e. dominance effect, DE). He also renamed the complementarity effect, trait-independent complementarity (TIC), to emphasize that TIC does not depend on the characteristics of genotypes/species. We used this approach (see Appendix S1, Supporting Information) to examine how different genotypes contributed to the number of bolting individuals and total fruit production in the low seed density and high seed density polycultures, respectively.

Because this method does not account for any potentially confounding differences in density between monocultures and polycultures (i.e. plots are assumed to be at constant final yield), we further refined the calculations to account for variation in density. Although this approach did not produce compelling results with our data, we believe the methods represent a valuable contribution to the field, and accordingly, we fully describe the methods in Appendix S1.

Attack by specialist herbivores

Each *M. brevivittella* hole typically represents 20% seed loss per fruit (Agrawal *et al.* 2012). Each flower bud galled by *M. stellerella* produces no seeds. Seed consumption by *S. florida* equals the proportion of the fruit consumed (Parker, Salminen & Agrawal 2010). We therefore calculated the total number of fruits consumed in each plot as $0.2 \times (\text{number of } M. brevivittella \text{ holes}) + \text{number of fruits attacked by } M. stellerella + S. florida \text{ damage}$. We transformed ($\ln+1$) total fruit consumed to improve the normality of the residuals and analysed these data with mixed effects linear model. For monocultures, we included 2011 realized density as a fixed effect, and genotype and block as random factors; fruit number per plot was added to this model subsequently to address the relative role of plant vs. fruit density. For polycultures, we included 2011 realized density, realized diversity and density \times diversity in the model, plus block as a random factor; again fruit number was added subsequently.

Results

SEED GERMINATION AND ESTABLISHMENT

Of the 13 200 seeds sown in the field plots, one quarter germinated (3353 individuals) and 81% of these survived beyond

the emergence stage. Seeds in high-density monocultures had 24% greater odds of germinating than in low-density monocultures ($\chi^2 = 13.8$, $P = 0.0002$). With these increased odds that an individual seed would germinate and the greater total number of seeds sown, high-density monocultures had 135% more seedlings than low-density monocultures (21.5 vs. 9.2 individuals on average, respectively). Seeds were also 24% more likely to germinate and establish in polycultures than monocultures, but only at low seed density ($\chi^2 = 17.3$, $P < 0.0001$; Fig. 1a). At high seed density, the opposite pattern emerged, with polycultures having 12% fewer individuals than monocultures ($\chi^2 = 11.6$, $P = 0.0007$; Fig. 1a).

After germination, density of established plants ranged from one to 44 individuals in monocultures, and three to 37 individuals in polycultures. Based on microsatellite analysis of bolting plants, genotypic diversity in polycultures ranged from two to eight genotypes. Thus, we achieved the desired gradient of variation in plant density and diversity and could subsequently assess their direct and interactive effects on plant performance and herbivore colonization. Initial effects of emergence on plot density persisted through to the fruiting stage (Fig. 1b). Only 16% bolted (and completed their life cycle) in their first growing season (2010) and an additional 72% bolted in 2011. The remainder (12%) failed to bolt, either eventually dying (10%) or persisting as tiny rosettes (2%).

PLANT SURVIVAL AND BOLTING

Across the 2 years of the experiment, survival of established plants declined with density in monoculture and polyculture plots (monoculture: $\chi^2 = 46.5$, $P < 0.0001$, odds ratio = 0.94; polyculture: $\chi^2 = 55.2$, $P < 0.0001$, odds ratio = 0.93), and neither genotypic diversity nor an interaction of density and diversity impacted subsequent survival (Fig. 2a; diversity: $\chi^2 < 0.1$, $P = 0.848$; diversity \times density: $\chi^2 = 0.6$, $P = 0.430$).

The likelihood that an individual would bolt also declined with density in both years for monocultures and polycultures (monocultures: 2010; $\chi^2 = 48.8$, $P < 0.0001$, odds ratio = 0.93; 2011; $\chi^2 = 70.7$, $P < 0.0001$, odds ratio = 0.88;

polycultures: 2010: $\chi^2 = 16.3$, $P < 0.0001$, odds ratio = 0.96; 2011: $\chi^2 = 54.2$, $P < 0.0001$, odds ratio = 0.85; Fig. 2b). Increasing genotypic diversity, however, increased the likelihood of bolting (2011: $\chi^2 = 4.3$, $P = 0.036$, odds ratio = 1.21, Fig. 2c), although this effect only appeared in 2011 when the majority of plants (72%) bolted (2010: $\chi^2 = 0.9$, $P = 0.333$). Density and diversity did not interact to impact the probability of bolting (2010: $\chi^2 = 2.7$, $P = 0.096$; 2011: $\chi^2 = 1.5$, $P = 0.210$).

PLANT GROWTH AND REPRODUCTION

Plant height and fruits per individual declined with density in monocultures (height: $\chi^2 = 7.1$, $P = 0.007$, $\beta = -1.10$; fruits per individual: $\chi^2 = 68.5$, $P < 0.0001$, $\beta = -0.05$) (see Fig. S2). A different dynamic occurred in polycultures. Plant height and mature fruits per bolting individual also declined with density, but genotypic diversity mitigated the negative impact of density (height: Fig. 3a, diversity \times density: $\chi^2 = 6.7$, $P = 0.009$; fruit production per individual: Fig. 3b, diversity \times density: $\chi^2 = 11.6$, $P = 0.0006$). Traditional analyses that do not account for differences in density and simply compared monocultures to polycultures did not reveal effects of genotypic diversity on plant performance (Table S1).

There was no overall signal of total plot-level fruit production declining across the 40-fold gradient in plant density in monocultures ($\chi^2 = 0.3$, $P = 0.557$; Fig. 4). Nonetheless, total plot-level fruit production did show a decline with density in polycultures and was marginally improved by increasing genotype diversity in polycultures (density: $\chi^2 = 5.3$, $P = 0.021$, diversity: $\chi^2 = 3.0$, $P = 0.080$, diversity \times density: $\chi^2 = 1.2$, $P = 0.273$; Fig. 4).

CONTRIBUTION OF DIFFERENT GENOTYPES TO DIVERSITY EFFECTS

When we partitioned diversity effects into their respective components (TDC, TIC and DE) and identified the contributions of specific genotypes (Fox 2005), we found strong effects on survival to the reproductive stage in low-density plots (mean diversity effect \pm 95% CI; TIC: 2.62 ± 1.28 ;

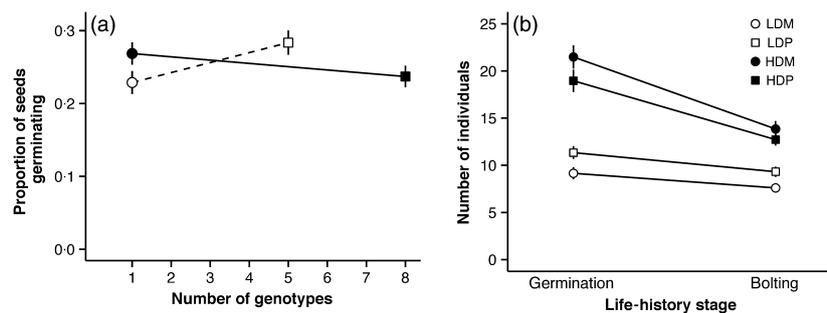


Fig. 1. Genotypic diversity enhances germination only at low seed density. Seed density and diversity affected (a) the proportion of seeds that germinated, and (b) the resulting differences in density persisted through the entire life cycle of the plants (mean \pm SEM). Low-density monocultures ('LDM') and low-density polycultures ('LDP') are depicted with open circles and squares, respectively. High-density monocultures ('HDM') and high-density polycultures ('HDP') are depicted with closed circles and squares, respectively.

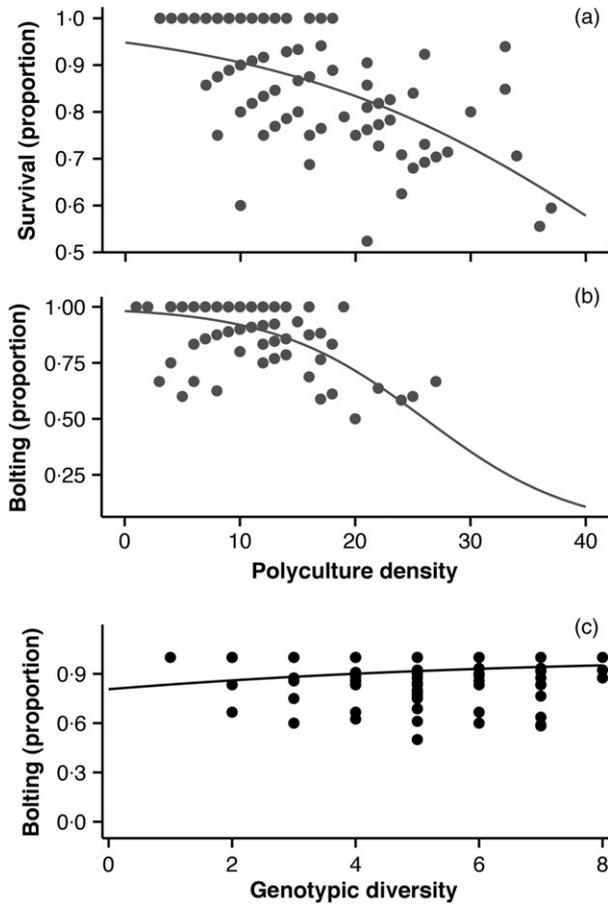


Fig. 2. Effects of plot density and diversity on survival and bolting. Plant density reduced (a) survival and (b) probability of bolting in 2011. Genotypic diversity only impacted (c) probability of bolting, and there were no interactive effects between plant density and genotypic diversity. Model-fits with back-transformed data are shown. The single plot that had one genotype (shown in panel C) had two genotypes in the previous year (2010).

TDC: -0.34 ± 0.18 ; DE: -0.47 ± 0.18). In polycultures, there were more bolting individuals per genotype than would be expected from monoculture performance (i.e. significant positive TIC). In addition, we also found that the genotypes that produced the fewest bolting individuals in monoculture benefitted the most from growing in polyculture (i.e. significant negative TDC) and that this benefit came by dominating genotypes that produced more individuals in monoculture (i.e. DE). However, we only saw significant effects in low seed density plots, and none were significant at high seed density (TIC: -1.08 ± 1.28 ; TDC: -0.02 ± 0.12 ; DE: -0.22 ± 0.31).

Concordant with no overall diversity effect on total fruit production, we did not find significant complementarity or selection effects when we calculated them with traditional methods or with our modification to account for differences in density (see Appendix S1). Nonetheless, our modifications of the method did reveal that the magnitudes of complementarity and selection effects were inflated when density was not accounted for (Appendix S1).

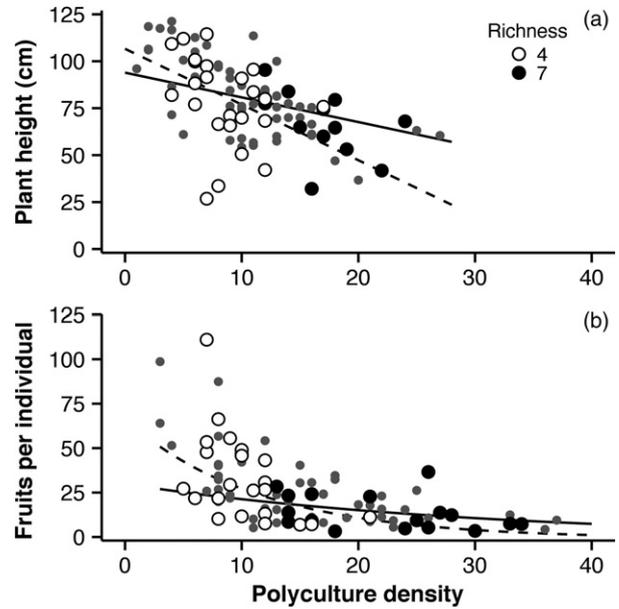


Fig. 3. Genotypic diversity of *Oenothera biennis* dampens the negative effect of plant density on (a) plant height and (b) production of mature fruits per bolting plant in polyculture. For ease of visualization, we illustrate the interaction between density and diversity by showing how the relationship between density and each performance trait differs in plots with four (open circles, dashed lines) or seven genotypes (filled circles, solid line). Note that we highlight plots with four or seven genotypes for illustrative purposes only, and the remaining polycultures are shown as grey dots in the figure. We included all data in the statistical model (with genotypic richness as a continuous variable). Model-fits with back-transformed data are shown.

ATTACK BY SPECIALIST HERBIVORES

Specialist herbivores attacked almost all of the plots (93% of monocultures, 99% of polycultures). In monocultures, herbivore damage increased with plant density ($\chi^2 = 6.01$, $P = 0.013$), but this effect was explained by the number of fruits present ($\chi^2 = 50.6$, $P < 0.0001$, plant density no longer significant with fruit number in the model: $\chi^2 = 2.1$, $P = 0.148$). Similarly, in polycultures only fruit number impacted damage, with greater damage in plots with more fruits (fruit number: $\chi^2 = 7.6$, $P = 0.006$; density: $\chi^2 < 0.1$, $P = 0.807$; diversity: $\chi^2 = 1.4$, $P = 0.233$; fruit number \times diversity: $\chi^2 = 0.5$, $P = 0.485$; density \times diversity: $\chi^2 = 0.2$, $P = 0.698$). The patterns were the same for each of the three specialist herbivores (Fig. 5).

Discussion

We followed a cohort of *O. biennis* from germination to senescence in the field to examine the relative importance of density vs. genotypic diversity on herbivory, plant life-history traits and lifetime reproduction. Natural populations of *O. biennis* range tremendously from one to thousands of plants (personal observations), and with up to 14 genotypes (Steiner & Levin 1977). We found that although there were positive effects of genotypic diversity on plant performance, these effects generally emerged as mitigating effects with respect to

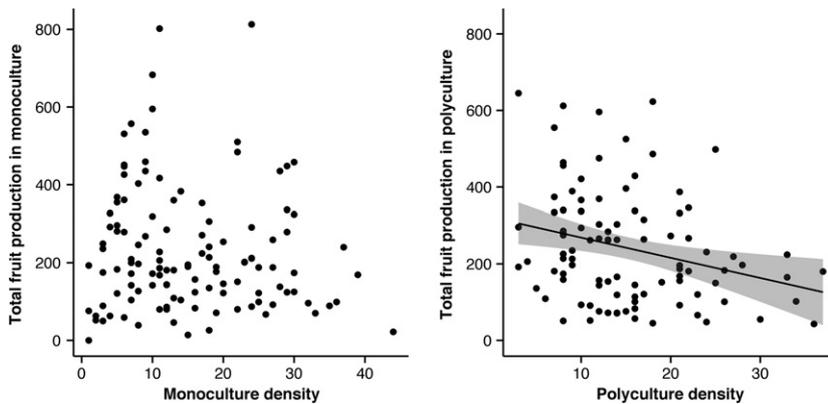


Fig 4. The impact of *Oenothera biennis* plant density on total plot-level fruit production for monocultures and polycultures. Best-fit and 95% confidence interval are shown for the statistically significant relationship.

the stronger negative effects of density. Had we not accounted for differences in density and simply compared monocultures to polycultures, we would have mistakenly concluded that increasing genotypic diversity had little effect on plant performance (see Table S1). This work thus contributes to a growing number of studies that show positive effects of genotypic diversity on communities of interacting plants (Crutsinger *et al.* 2006; Johnson, Lajeunesse & Agrawal 2006; Kotowska, Cahill & Keddie 2010; Cook-Patton *et al.* 2011), but indicates the context dependency of these effects.

Perhaps our most striking result was the interactive effect of density and genotypic diversity on germination and initial establishment. At low seed density, germination was higher in polyculture than monocultures. At high seed density, the opposite effect occurred (Fig. 1). While we did not genotype individuals at this stage, our analyses of how different genotypes contributed to the number of bolting plants in each plot showed that there were more individuals than predicted from monoculture in the low-density polycultures (i.e. positive TIC). We also observed negative TDC and DEs in these plots, indicating that genotypes with the fewest bolting plants in monoculture benefited the most. Accordingly, genotypic diversity effects emerged at the early stages of plant establishment and persisted through to reproductive maturity.

Several experiments have shown density-dependent germination, with results ranging from positive to negative effects of intraspecific density on germination (Waite & Hutchings 1978; Murray 1998; Dyer, Fenech & Rice 2000; Yin *et al.* 2009). Germination is predicted to decline with intraspecific density if the species occupies disturbed habitats or has highly persistent seeds, because this strategy allows the species to wait for opportune growing conditions (Linhart 1976). As a ruderal species with seeds that can persist for 80 years (Darlington & Steinbauer 1961), *O. biennis* is predicted to show reduced germination with increasing seed density. Indeed, this is what we observed when we compared low- vs. high-density polycultures (Fig. 1). Those studies that previously observed negative density-dependent germination also presumably employed multiple genotypes in their seed mixes (Linhart 1976; Murray 1998; Dyer, Fenech & Rice 2000).

Among our genotypic monocultures, however, we observed positive density-dependent germination and establishment.

This result is more difficult to interpret, as very few other studies have simultaneously manipulated density and genotypic diversity. Erfmeier, Hantsch & Bruelheide (2013) found that germination rates increased with intraspecific density of *Senecio vernalis*, but observed no impact of genotypic diversity. In contrast, Crawford & Whitney (2010) found that increasing genotypic diversity enhanced germination of *Arabidopsis thaliana*, whereas density had no effect. Thus, our study is unique in its observation of an interactive effect between density and diversity on seed germination. Seeds and seedlings are known to communicate with each other via chemical cues (Bergelson & Perry 1989; Murray 1998; Tielbörger & Prasse 2009), so it may be that seeds differentially sense the chemical cues of genetically identical or distinct neighbours at different densities. One possibility is that germination rate responds in a concave manner to seed cues, with low seed density triggering greater germination and higher densities suppressing germination. If the chemical signal from the same genotype is sensed less effectively, this would result in low seed densities triggering greater germination in polyculture (while the suppressive effect of high density being weaker in monoculture). Density-dependent effects of kin recognition have been observed at later stages of plant development, where plants can increase reproductive allocation (or reduced competitive traits) in the presence of kin (Lepik *et al.* 2012; Dudley, Murphy & File 2013). Kin recognition during the germination phase holds promise for future study.

Beyond the germination stage, density proved to be the most important explanation of variation in plant performance (Fig. 2, S2). In polycultures, density negatively impacted plant height and fruit production per individual. Self-thinning and density-dependent growth is well established in plant ecology, especially in annual plants with minimal seed dispersal (Antonovics & Levin 1980; Schmitt, Eccleston & Ehrhardt 1987). Genotypic diversity, in contrast, had less of an effect, although it did ameliorate the negative relationship between density and several measures of plant performance, including probability of bolting in 2011, plant height and fruit production per individual (Figs 2 and 3). Competition is thought to be especially intense among genetically identical individuals, presumably because they share the same

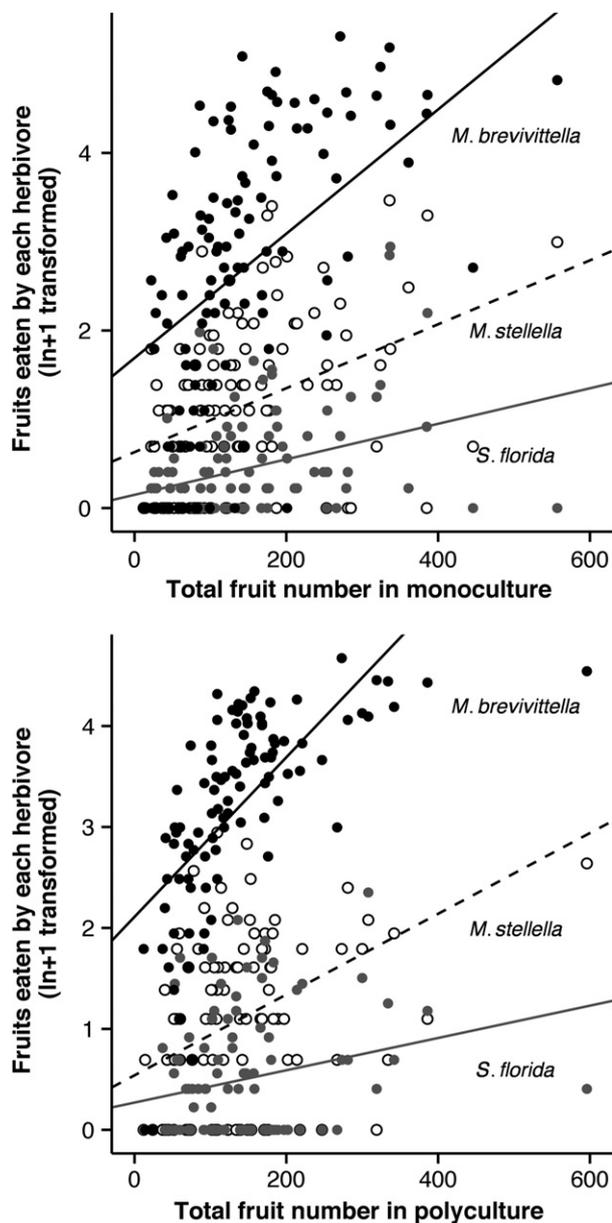


Fig. 5. Number of *Oenothera biennis* fruits consumed by each specialist herbivore in monocultures (top panel) and polycultures (bottom panel). We depict *Mompha brevivittella* data with black circles and a black best-fit line, *Mompha stellella* with open circles and a dashed best-fit line and *Schinia florida* with grey circles and a grey best-fit line. We show the $\ln+1$ -transformed data for number of fruits consumed for ease of comparison.

way of interacting with the environment and acquiring resources (Cook-Patton *et al.* 2011). Increasing genotypic diversity may thus be especially important to alleviate intense intraspecific competition.

Fruit density in plots increased absolute attack rates by specialist herbivores. Because the specialist herbivores of *O. biennis* consume reproductive structures (Dickerson & Weiss 1920), these results are generally consistent with the 'resource concentration hypothesis' (Root 1973), which posits that herbivores are more likely to locate or stay in dense patches of their preferred food source. Increasing genotypic diversity, in

contrast, did not impact the herbivores. Other genotypic diversity manipulations have found an increase in herbivore abundance with increasing genotypic diversity (Crutsinger *et al.* 2006; Cook-Patton *et al.* 2011; Utsumi *et al.* 2011; McArt & Thaler 2013), and some of these effects may be driven by increased plant density or biomass.

Conclusions

Results generally support our first prediction that plant competition should increase with plant density, but decline with increasing genotypic diversity (effects on herbivory were less strong). We further expected plant density to decline over time due to competition, but more precipitously in low genetic diversity plots (again due to higher competition). In contrast, we found that while plant density did decline over time, plant survival depended primarily on plot density and differences in density were established by interactions between density and genetic diversity at the very early stages of plant development. If we had not employed a life cycle approach and used molecular markers to account for quantitative variation in realized diversity and density, we would not have deciphered the key mitigating impact of genetic diversity on plant performance. While it is possible that diversity effects are less important than density effects in short-term experiments before constant yield is reached, even long-term and large-scale experiments show evidence that density drives productivity gains in polyculture (Marquard *et al.* 2009). Such effects may be especially acute if monocultures thin over time due to disease or herbivory (Parker, Salminen & Agrawal 2010; Schnitzer *et al.* 2011).

It is clear from current work that genotypic diversity profoundly affects the performance of individual plants and communities (Crutsinger *et al.* 2006; Johnson, Lajeunesse & Agrawal 2006; Parker, Salminen & Agrawal 2010; Cook-Patton *et al.* 2011). The current study demonstrates that the effect of genotypic diversity may extend from early germination to final fruit production. Nonetheless, community interactions that impact plant density, such as competition and herbivory, are critical for understanding the impacts of genotypic diversity on plant performance. Our results imply a dynamic interplay between the long-held paradigm of density dependence in plant populations with the emerging picture of ecological consequences of genetic structure at a local scale.

Authors' contributions

All authors designed the experiment; S.C.C. and A.P.H. established the field experiment and collected the data; A.P.H. conducted all genotyping; S.C.C. and A.A.A. conducted analyses; S.C.C. wrote the first draft of the manuscript. All authors contributed substantially to revisions and gave final approval for publication.

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Data accessibility

Data supporting the results in this study are archived in Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.h2162> (Cook-Patton, Hastings & Agrawal 2016).

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Supporting Information

Details of electronic Supporting Information are provided below.

Appendix S1. Complementarity, TDC, and dominance effects.

Fig. S2. Density dependence of plant performance in monocultures.

Table S1. Effect of genotypic richness on plant performance at low and high seeding density based on traditional biodiversity analyses.