

# Environmental variation has stronger effects than plant genotype on competition among plant species

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## Summary

1. Competition is a key factor affecting the performance and co-existence of species. Most ecological research on competition treats species' populations as phenotypically homogenous. However, plant populations typically contain genetic variation for multiple traits and have the potential to rapidly adapt to changing environments.
2. Recent theoretical and empirical research suggests that such variation and evolution may affect the ecological outcome of competitive interactions. We conducted a series of experiments to test the hypothesis whether genetic variation for competitive traits in a native plant (*Oenothera biennis*) affects the performance and diversity of competing plant species.
3. In greenhouse and field experiments, the presence of *O. biennis* reduced the performance and diversity of neighbouring plant species.
4. In greenhouse experiments, we detected heritable variation in *O. biennis* for above-ground and below-ground growth, and *O. biennis* varied genetically in response to competition, indicating the potential for adaptive evolution in response to selection by competitors.
5. Variation among *O. biennis* genotypes also affected the performance of neighbouring plants in the greenhouse, whereby genetic variation in *O. biennis* shoot : root ratio explained up to 41% of the variation in the performance of an exotic grass (*Bromus inermis*).
6. Despite effects of *O. biennis* genotype on *B. inermis* in the greenhouse, variable soil fertility had a much stronger effect on the grass's performance, and there were no effects of *O. biennis* genotype on neighbouring plants in the field.
7. *Synthesis*. Our results show that interspecific competition affected the biomass and diversity of plants, but heritable variation in competitive ability of *O. biennis* is not expected to affect short-term ecological dynamics in this system. Nevertheless, *O. biennis* has the potential to influence co-existence over longer timescales by adapting to competitors.

**Key-words:** character divergence, co-evolution, co-existence, community genetics, genetic correlation, plant ecology, species diversity

## Introduction

Interspecific competition frequently affects the performance and co-existence of species. Most models of competition assume that species' populations are phenotypically homogenous, such that species have fixed values for traits that influence competitive ability (Tilman 1982; Holt *et al.* 1994; Chesson 2000). In reality, single populations often exhibit variation for multiple traits, and a combination of conceptual and mathematical theory suggests that intraspecific variation

for competitive ability can affect species co-existence (Aarssen 1983; Abrams 2006; Urban 2006; Vellend 2006). Therefore, predicting the effects of competition on species interactions may rely on knowing the distribution of traits within species and the ecological effects of these traits on competitors. Here we describe a series of experiments that examined whether genetic variation in a native plant can affect competition with neighbouring plant species.

Recent research at the intersection of community ecology and evolutionary biology hypothesizes that heritable variation and evolution of ecologically important traits can affect ecological processes and patterns within communities (Whitham

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*et al.* 2003; Urban & Skelly 2006; Johnson & Stinchcombe 2007). Most research has focused on the 'bottom-up' effects of genetic variation in basal resource populations on consumer communities (Maddox & Root 1987; Dungey *et al.* 2000; Johnson & Agrawal 2005; Wimp *et al.* 2005), and how evolution in resource (Yoshida *et al.* 2003) and predator populations (Fussmann *et al.* 2003) shape predator–prey dynamics. Outside of extensive research on the evolution of character displacement (Schluter 2000), the effects of genetic variation and evolution on the ecology of competing species has received little attention compared to the study of multi-trophic effects.

Genetic variation is predicted to affect the ecological outcome of competition when: (i) a population genetically varies in response to competition, indicating the potential for an evolutionary response to selection by competitors (Shaw & Platenkamp 1993; Cahill *et al.* 2005); or, (ii) genetic variation in a species' traits affects the fitness of competing species by depleting or preventing access to shared resources (Aarssen 1989). Theoretical models indicate that these mechanisms can lead to greater or lesser co-existence among species compared to models that ignore genetic variation and evolution (Urban 2006; Vellend 2006). Most empirical evidence for the role of genetic variation and evolution in competitive interactions comes from plants. For example, *Trifolium repens* exhibits fine-scale adaptation to competition with specific plant species (Turkington & Harper 1979), and even to specific genotypes within competing species (Aarssen & Turkington 1985). Recent studies also show that variation among plant genotypes can influence the growth, biomass and density of competing plant species (Taylor & Aarssen 1990; Proffitt *et al.* 2005; Fridley *et al.* 2007; Lankau & Strauss 2007). Moreover, genetic variation in traits can lead to intransitive competitive interactions due to overlap in the competitive ability of co-existing plant species (Taylor & Aarssen 1990; Whitlock *et al.* 2007), which can increase co-existence within plant communities (Aarssen 1989; Laird & Schamp 2006; Lankau & Strauss 2007). Together, these results show that genetic variation in competitive ability can influence the performance of individual plants and potentially regulate diversity.

Further research is needed to understand whether genetic variation in competitive ability is ecologically important in nature. Most experiments have been conducted in pots where environmental variance is kept to a minimum to assess the potential effects of genetic variation on competitive interactions (Aarssen & Turkington 1985; Taylor & Aarssen 1990; Fridley *et al.* 2007). However, both the genotype and the environment determine a plant's phenotype, and environmental variation in the field may dampen the ecological effects of genetic variation. A combination of experiments from controlled growth environments and the field would provide the strongest test for the ecological importance of genetic variation. Studies that additionally manipulate ecological factors of known importance to competition (e.g. resource availability) provide further insight into the relative importance of genetic variation.

Here, we examine the hypothesis that genotype identity of a native plant (common evening primrose, *Oenothera biennis*) is an important factor affecting the performance and diversity of neighbouring plant species. Our first objective was to determine whether *O. biennis* competes with an exotic grass species *Bromus inermis*, with which it commonly co-occurs in nature. Once we established the nature of competitive interactions, we used greenhouse experiments to address three specific questions: (i) is there genetic variation in *O. biennis* for traits that can influence interspecific competitive interactions? (ii) Does genetic variation in plant traits of *O. biennis* affect the performance of *B. inermis*? and, (iii) what is the relative importance of plant genotype of *O. biennis* vs. variation in soil fertility in affecting the performance of *B. inermis*? Based on our results from pairwise competition greenhouse experiments, we then tested whether *O. biennis* genotype affects the community-wide performance and diversity of naturally occurring plants in the field.

## Methods

### STUDY SYSTEM

Our greenhouse experiments focused on interactions between two plant species common in open habitats of eastern North America, Common Evening Primrose (*O. biennis* L., Onagraceae) and Smooth Brome Grass (*Bromus inermis* Leyss., Poaceae). *Oenothera biennis* (hereafter '*Oenothera*') is a native facultative biennial that forms a single basal rosette prior to bolting into a 0.5–2.5-m flowering stalk. It occurs commonly in open habitats, such as old fields, mowed lawns, and disturbed soil. *Oenothera* has a reproductive system (permanent translocation heterozygosity) which renders it functionally asexual (Cleland 1972). This genetic system makes for a powerful experimental tool because single clonal genotypes can be grown from seed and replicated across treatments and experiments. *Bromus inermis* (hereafter '*Bromus*') is an exotic perennial grass that grows up to 1.5 m tall and produces tillers that form large clonal stands. *Bromus* and *Oenothera* often co-occur as they both colonize recently disturbed soil.

We also conducted a field experiment that examined competition between *Oenothera* and the diversity of plants that naturally colonize disturbed fields at University of Toronto's Koffler Scientific Reserve at Jokers Hill. All of the *Oenothera* genotypes used in experiments were collected as seed on or within 10 km of Jokers Hill.

### GREENHOUSE EXPERIMENTS

We conducted three greenhouse experiments that shared the same methods for planting, manipulation of competition, manipulation of soil fertility and measures of plant performance. Therefore, we provide a detailed description of the methods for 'experiment 1' and then highlight modifications to these methods for 'experiment 2' and 'experiment 3'.

#### *Greenhouse experiment 1*

Our first objective was to assess whether *Oenothera* and *Bromus* compete when growing in close proximity, and whether variation in soil fertility alters the strength of competition. *Oenothera* and *Bromus* plants were grown in the presence and absence of one

another, with and without fertilizer, in a fully factorial  $2 \times 2$  design with 10 replicate plants per treatment combination. To examine the average competitive interactions between *Oenothera* and *Bromus*, we used a single genotype of *Oenothera* that exhibited intermediate above-ground and below-ground growth when compared to 21 other genotypes used in subsequent experiments. Seeds of *Bromus* came from a monospecific stand at Jokers Hill and because of the clonality of *Bromus*, the seed stock was likely derived from a single maternal family. The same seed stock was used in all experiments.

Seeds of both species were germinated in petri dishes on wet filter paper placed in a windowsill. Following germination, we transplanted seedlings to 250 mL pots containing a 1 : 2 mixture of potting soil (Promix General Purpose BX soil, Premier Horticulture, Dorval, Canada) and coarse sand, respectively. In the absence of competition, a single *Oenothera* or *Bromus* plant grew alone. In the presence of a competitor, one *Oenothera* plant and one *Bromus* plant were planted 3 cm apart in a single pot. Fertilized plants received 5 mL of 200 p.p.m. 20 : 20 : 20, N : P : K, liquid fertilizer (Plant Prod, Brampton, ON, Canada) at the time of planting, and the same dose 1 week after planting. High soil fertility was maintained throughout the experiment by adding 0.23 g of slow release Nutricote pellets (13 : 13 : 13, N : P : K; Vicksburg Chemical, Vicksburg, MS, USA) to the soil surface. Control plants received an equal volume of water in place of fertilizer. The experiment was conducted in a single greenhouse set to 25 °C during the day and 20 °C at night. Natural light was supplemented with 400 W pressure sodium lamps set to a 16 : 8 h (day : night) cycle; plants were watered as needed. We completely randomized all pots at the beginning of the experiment and spaced plants to reduce shading between pots. The experiment ended after plants' roots filled the pot and growth stopped, which ranged from 6 to 9 weeks.

We determined the response of both *Oenothera* and *Bromus* to the factorial manipulation of competition and fertilizer by measuring six variables that depict plant performance: total plant biomass, above-ground biomass (shoot), below-ground biomass (root), shoot : root ratio, maximum plant growth rate and maximum plant size. The shoot : root ratio reflects the relative allocation plants make to their shoots vs. roots. Variation in this ratio need not be adaptive (Cahill 2003), but in conjunction with data on absolute allocation to shoots, roots, and total biomass, the shoot : root ratio can provide insight into how variation in allocation patterns within the plant influence neighbouring plant performance, as well as the response of a focal plant to competition. Biomass was determined by harvesting plants at the end of the experiment. We removed all soil from roots by gently shaking away loose soil followed by submersion in water where remaining soil was massaged free of the roots. Plants were separated into above-ground and below-ground portions and dried for 1 week at 60 °C and weighed to the nearest 0.001 g.

We measured plant size weekly as maximum rosette diameter (*Oenothera*) or maximum stem height (*Bromus*). These measures accurately predicted total plant biomass after 8 weeks of growth in both *Oenothera* ( $r = 0.89$ ,  $P < 0.001$ ,  $N = 28$ ) and *Bromus* ( $r = 0.72$ ,  $P < 0.001$ ,  $N = 32$ ). These data were used to estimate maximum plant growth rate and maximum plant size by fitting the weekly measures of plant size from each plant to the logistic growth equation in Proc NLIN of SAS 9.1 (SAS Institute, Cary, NC, USA), which typically explained over 95% of the variation in the growth of individual plants from both species. The logistic growth equation was depicted as  $\text{Size}_t = \text{Size}_{eq} / (1 + ze^{-gt})$ , where  $\text{Size}_t$  represents plant size at time  $t$ ,  $g$  represents the maximum rate of plant growth, and  $z$  is a plant-specific constant.  $\text{Size}_{eq}$  is the asymptotic size that

individual plants reached under our experimental conditions. Biologically, growth rate depicts the maximum rate ( $\text{cm day}^{-1}$ ) of growth experienced by a plant during its development, while maximum plant size estimates the maximum total biomass achieved by individual plants and also the potential competitive effect that a plant can have on its neighbours via shading.

We used Analysis of Variance (ANOVA) to assess the effects of interspecific competition, fertilizer and their interaction, on each of the six variables described above for *Oenothera* and *Bromus*, separately. Analyses were performed using Proc GLM in SAS. A square-root or log-transformation was used to improve normality and reduce heteroscedasticity when needed.

### Greenhouse experiment 2

The objectives of this experiment were to determine whether *Oenothera* exhibited genetic variation for traits that could influence interactions with neighbouring plant species, and whether variation among *Oenothera* genotypes affected the performance of *Bromus*. We used 22 genetic families (hereafter genotypes) of *Oenothera*, which were grown in a common environment for one generation to reduce maternal effects. Seeds were collected from these plants and used in the present experiment.

The experiment consisted of three treatments: (i) *Oenothera* grown alone, (ii) *Bromus* grown alone, and (iii) *Oenothera* and *Bromus* grown together. Each *Oenothera* genotype was replicated with 20 plants except one genotype with poor germination which had nine plants. Eight plants were randomly assigned to compete with *Bromus* and the remaining plants were grown in the absence of competition. Forty-two *Bromus* plants were also grown in the absence of competition. In total, there were 472 replicate pots containing 430 *Oenothera* and 210 *Bromus* plants.

To examine whether *Oenothera* genotype, competition with *Bromus*, and their interaction, affected the performance of *Oenothera*, we used restricted maximum likelihood (REML) in Proc Mixed of SAS. The significance of random effects (genotype and genotype X competition) was assessed using the log-likelihood ratio test (Littell *et al.* 1996). The significance of the fixed factor (competition) was calculated using an  $F$ -test where d.f. were adjusted according to Kenward & Roger (1997). Broad-sense heritabilities were calculated as  $H^2 = V_g/V_T$ , where  $V_g$  and  $V_T$  are the genetic and total components of variance, respectively. This equation is appropriate for *O. biennis* because it produces clonal progeny (Lynch & Walsh 1998).  $P$ -values for heritabilities were determined as the significance of plant genotype using untransformed data with the genotype X competition interaction excluded from the model. We assessed the effect of *Oenothera* genotype on *Bromus* performance using Proc Mixed, where only the effect of genotype was included in the model. One-way ANOVA was used to test how the presence/absence of *Oenothera* (irrespective of genotype) influenced *Bromus* performance.

We employed stepwise multiple regression to determine how genetic variation in the six traits measured from *Oenothera* predicted variation in the response of *Bromus*. To do this, we calculated the best-linear unbiased predictors (BLUPs; similar to genotype means) of trait values for each *Oenothera* genotype (Littell *et al.* 1996). We also calculated BLUPs for *Bromus* traits according to the *Oenothera* genotype against which the grass competed. Thus, there were 22 values for each *Bromus* and *Oenothera* trait, one for each *Oenothera* genotype. We then regressed each *Bromus* trait individually against all *Oenothera* traits; we did not include total *Oenothera* biomass into the model because it was highly correlated with above-ground and below-ground biomass. We then chose the best fitting

model using stepwise regression with an entry/exit value of  $P = 0.15$ . All analyses were performed in Proc REG of SAS.

### Greenhouse experiment 3

This experiment evaluated the relative importance of plant genotype, soil fertility, and their interaction on *Bromus* performance. All plants were grown in competition with a heterospecific neighbour and we used 21 of the 22 *Oenothera* genotypes from experiment 2. Each genotype was replicated with 14–16 plants, divided equally into the two fertilizer treatments. In total, there were 334 pots containing 668 plants. We again used REML in Proc Mixed for statistical analyses. The model was a fully factorial design, involving fertilizer as a fixed effect, plant genotype and genotype X fertilizer as random effects.

### FIELD EXPERIMENT

We conducted a field experiment at Jokers Hill during summer-2004 to investigate whether our results from greenhouse experiments translate to community-wide impacts on the performance and diversity of plants under more natural conditions. Our objective was to simulate the conditions plants experience when recruiting to freshly disturbed fields, which is the primary way in which *Oenothera* establishes new populations (Gross & Werner 1982), and to measure the effects of *Oenothera* genotype on the plant community that naturally establishes itself following disturbance. To do this we replicated the same experiment in two old fields (hereafter 'gardens') with contrasting productivity and plant cover. The 'Forest' garden was situated on a small hill where dry soil and gaps in the vegetation were common. The 'North 30' garden was an old field with dense vegetation and high productivity. At each garden, we ploughed a 700 m<sup>2</sup> area and delineated to two equal-sized blocks to account for environmental gradients.

This experiment used a larger number of genotypes (29), where thirteen of the genotypes overlapped with the greenhouse experiments. In early May, we germinated seeds and transplanted seedlings into 250 mL pots containing soil collected from the garden into which a given plant was later to be transplanted. Plants were then placed into outdoor meshed tents free of insects for 2 weeks, and during this time they were watered every second day but received no fertilizer. Plants were subsequently transferred to the field, removed from their pots and planted directly into the soil. All plants were planted into rows and columns separated by 1.5 m. In total, there were 638 *Oenothera* plants with 22 replicates per genotype, randomized and equally replicated within each block and garden. Plants received no additional water in the field and plants that died were not included in analyses.

To mimic natural processes of community establishment following disturbance, we allowed plants to recruit into the experimental fields throughout the summer. Plants rapidly colonized the fields and at the time of harvest, 19 species (5 native and 14 exotic) were growing in close proximity with *Oenothera*. We harvested both gardens in late-August by removing the above-ground portion of *Oenothera* plants; heavy soil and deep taproots precluded harvesting roots. We also collected above-ground portions of all plants within a 10 × 10 cm quadrat immediately adjacent to, but not including, the base of the focal *Oenothera* plant ('near quadrat'). A second 10 × 10 cm quadrat sample of the plant community was taken 30 cm away from the center of the focal *Oenothera* plant ('far quadrat'); this quadrat was beyond the longest leaves of the focal plant. The

location of quadrats was standardized by always sampling from the East side of focal plants. The strong competitive effects detected in this experiment (see Results) indicate that the size of quadrats was adequate to detect ecologically significant variation. Plants taken from each quadrat were sorted to species, dried at 60 °C and weighed to the nearest 0.1 g; plants were larger in this experiment and fine-scale measurements of biomass were not needed.

To assess the community-wide performance and diversity of plants growing close to focal *Oenothera* plants, we calculated total biomass, monocot biomass, dicot biomass, species richness, and the Shannon Index of diversity within each quadrat. Although many plants recruited to the gardens, no one species was common enough to measure individual responses.

We compared the vegetation between near and far quadrats to assess how proximity with *Oenothera* affected the performance and diversity of neighbouring plant species. This comparison reflects the local effects of competition by *Oenothera* because this species forms a taproot, so that most shading and below-ground competitive effects occurred within the limits of the outermost leaves of *Oenothera* and were likely negligible at a distance of 30 cm.

### Statistical Analysis

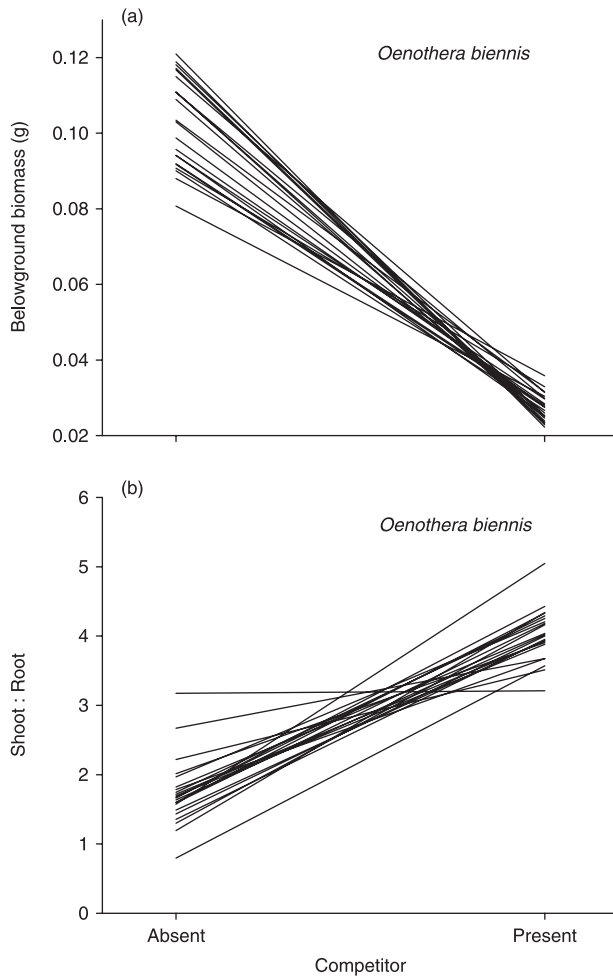
We used REML in Proc MIXED for all analyses. *Oenothera* biomass was analysed with garden as a fixed effect, plant genotype, block (garden), genotypeXgarden, and genotypeXblock (garden) as random effects. Analyses of plant community variables used a repeated measures design, where the full model was: Community variable = mean<sub>overall</sub> + garden + quadrat + gardenXquadrat + genotype + block(garden) + genotypeXgarden + genotypeXquadrat + genotypeXquadratXgarden + error. Samples taken from the paired near/far quadrats were given unique identifiers and treated as repeated subjects in analyses. Garden, quadrat and their interaction were fixed factors and all other factors were random effects. We deleted non-significant interactions and blocking factors using a backward selection procedure.

## Results

### GREENHOUSE EXPERIMENT 1

Overall, competition was asymmetric as *Oenothera* suppressed total biomass of *Bromus* by 57% ( $F_{1,30} = 106.8$ ,  $P < 0.001$ ), while *Bromus* did not significantly reduce total biomass of *Oenothera* ( $F_{1,24} = 1.9$ ,  $P = 0.18$ ). *Oenothera* caused a reduction in nearly all measures of *Bromus* performance (except growth rate) and caused a significant decrease in the shoot : root ratio ( $F_{1,30} = 23.2$ ,  $P < 0.001$ ). *Bromus* caused a significant reduction in *Oenothera* below-ground biomass ( $F_{1,24} = 11.3$ ,  $P = 0.003$ ) and maximum plant size ( $F_{1,33} = 50.2$ ,  $P < 0.001$ ), which resulted in an increase in the shoot : root ratio ( $F_{1,24} = 7.0$ ,  $P = 0.01$ ).

Increased soil fertility led to a significant increase in all measures of plant biomass for both species ( $P < 0.001$  for all biomass variables). Competition and fertilizer never interacted to affect significantly the performance of *Oenothera* ( $P \geq 0.07$  for all measures). In contrast, these factors interacted to affect significantly all measures of *Bromus* biomass ( $P < 0.01$  for all measures), which was due to stronger competitive effects in the presence of fertilizer.

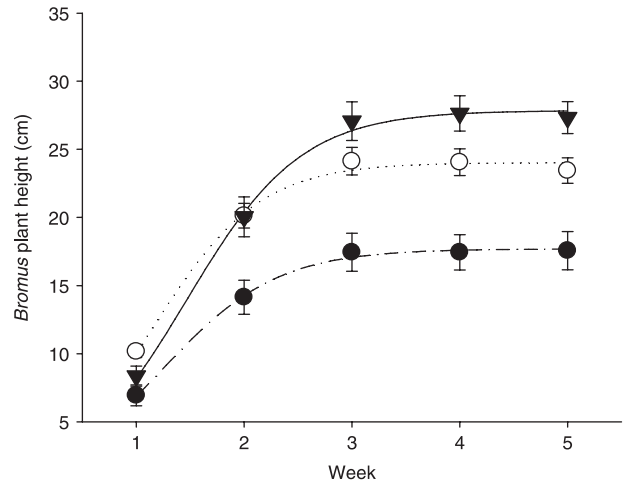


**Fig. 1.** Genetic variation in *Oenothera* in response to competition. (a) Below-ground biomass and (b) shoot : root ratio exhibited a significant genotype  $\times$  competition interaction (see Table S1).

#### GREENHOUSE EXPERIMENT 2

*Oenothera* exhibited significant heritable variation for all measures of plant performance with broad-sense heritability values ranging from 0.04 (shoot : root ratio) to 0.35 (growth rate) (specific heritabilities available from MTJJ). Therefore, there was variation among *Oenothera* genotypes for traits that could influence the performance of competing plants. Plant genotype and competition also interacted to affect below-ground biomass and the shoot : root ratio of *Oenothera* plants (Fig. 1, Table S1 in Supplementary Material), indicating the presence of genetic variation in response to competition with *Bromus*. *Bromus* also caused a reduction in all measures of *Oenothera* biomass and maximum plant size, as well as an increase in the shoot : root ratio (Supplementary Table S1).

Plant genotype of *Oenothera* significantly affected several measures of *Bromus* performance (Table S2). This effect is clearly seen for maximum plant size (Fig. 2), where the maximum height obtained by *Bromus* varied 33% between the most competitive *Oenothera* genotype and the least competitive genotype. In the absence of competition, maximum



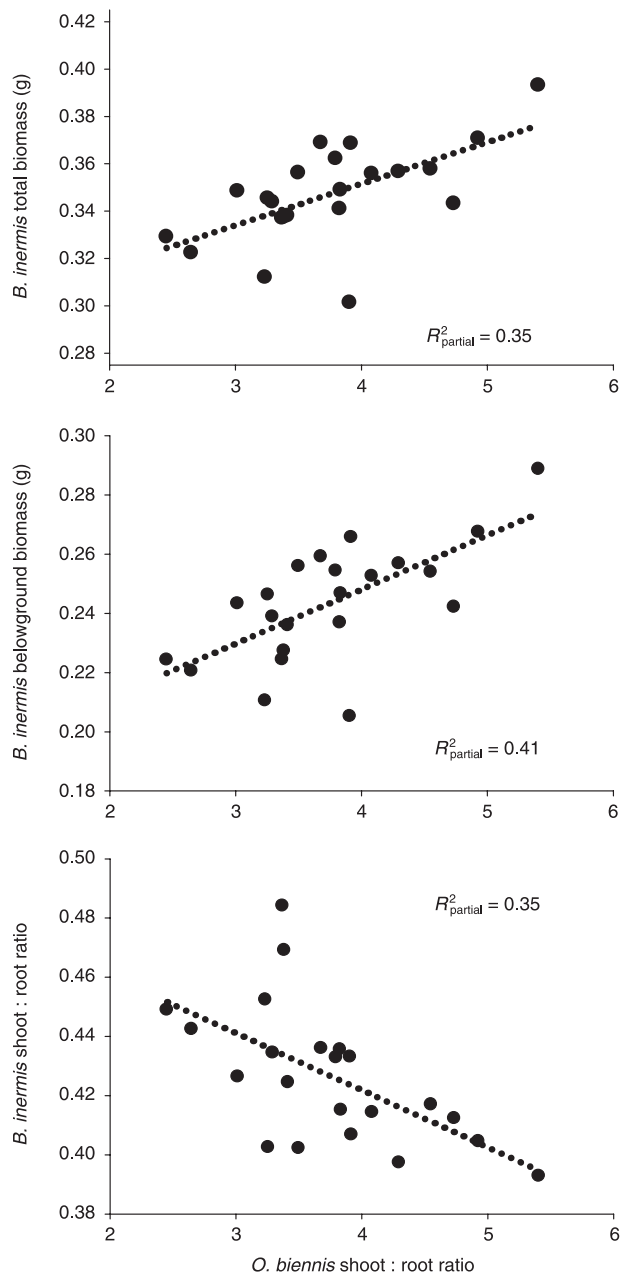
**Fig. 2.** The effect of *Oenothera* genotype on the growth of *Bromus* plants. The figure shows the mean height and best fitting growth curve of *Bromus* in the absence of competitors (triangles fitted by solid curve), and in the presence of the most competitive (solid circles fitted by dash-dot curve) and least competitive (open circles fitted by dotted curve) *Oenothera* genotypes. Growth curves of *Bromus* competing against other plant genotypes fell in between these extremes. Maximum growth rate and the maximum size of plants were estimated by fitting the logistic growth equation (best fitting curve shown) to the height of each *Bromus* plant measured over 5 weeks.

plant size of *Bromus* was only 17% greater than plants that had competed against the least competitive *Oenothera* genotype, while maximum size was 55% greater in the absence of competition compared to plants competed against the most competitive genotype (Fig. 2). Below-ground biomass and the shoot : root ratio of *Bromus* were also significantly affected by the identity of competing genotypes (Supplementary Table S2), as these variables varied by as much as 41% and 70% among *Oenothera* genotypes, respectively. Total biomass of *Bromus* showed a nearly significant response to plant genotype ( $P = 0.06$ ; Supplementary Table S2).

Multiple regression showed that genetic variation in the shoot : root ratio of *Oenothera* explained 35–41% of the variation in *Bromus* performance. As the shoot : root ratio of *Oenothera* increased, *Bromus* plants allocated more biomass to roots (slope =  $0.072 \pm 0.018$ ,  $P = 0.001$ , partial- $R^2 = 0.41$ ), which led to an increase in total plant biomass (slope =  $0.054 \pm 0.016$ ,  $P < 0.001$ , partial- $R^2 = 0.35$ ) and a decrease in the shoot : root ratio (slope =  $-0.177 \pm 0.050$ ,  $P = 0.001$ , partial- $R^2 = 0.34$ ) (Fig. 3). Although *Oenothera* genotype affected the maximum size of *Bromus*, none of the measured *Oenothera* traits were significant predictors, indicating that additional unmeasured traits also played a role.

#### GREENHOUSE EXPERIMENT 3

As observed in experiment 2, *Oenothera* exhibited genetic variation for all components of performance, except shoot : root ratio (Supplementary Table S3). Broad-sense



**Fig. 3.** The response of *Bromus* performance to genetic variation in shoot : root ratio of *Oenothera* biomass. *Oenothera* genotypes with higher shoot : root ratios caused *Bromus* plants to increase total biomass, primarily by increasing allocation to roots, which led to a decrease in the shoot : root ratio of *Bromus*. Each point represents the genotype mean of shoot : root ratio for an *Oenothera* genotype (x-axis) and the mean response of *Bromus* to that genotype (y-axis). Variables were back-transformed to their original units.

heritability ranged from 0.08 (below-ground biomass, no fertilizer) to 0.27 (maximum plant size, fertilizer), and the mean across all variables was 0.15 without fertilizer and 0.20 with fertilizer. Plant genotype and fertilizer interacted to affect total biomass, above-ground biomass and growth rate, indicating that *Oenothera* exhibited genetic variation in its response to soil fertility (Supplementary Table S3).

Surprisingly, there was no main effect of *Oenothera* genotype on *Bromus* performance and genotype did not interact with fertilizer to affect *Bromus* (Supplementary Table S4). When we analysed the influence of plant genotype for fertilized and unfertilized plants separately, we detected a significant effect of *Oenothera* genotype on the shoot : root ratio of *Bromus* in the presence of fertilizer ( $\chi_1^2 = 3.8$ ,  $P = 0.026$ ). In contrast to the weak effect of plant genotype, soil fertility had a large effect on *Bromus* performance. Fertilized plants showed significant increases for all measures of biomass, shoot : root ratio and maximum plant size (Table S4).

#### FIELD EXPERIMENT

*Oenothera* exhibited marginally non-significant genetic variation for above-ground biomass (Genotype effect: North 30:  $\chi_1^2 = 2.0$ ,  $P = 0.079$ ; Forest:  $\chi_1^2 = 2.3$ ,  $P = 0.065$ ). Heritability values were lower in both the North 30 ( $H^2 = 0.018$ ) and Forest ( $H^2 = 0.062$ ) gardens compared to heritabilities measured in the greenhouse (Above-ground biomass: Experiment 2,  $H^2 = 0.26$ ; Experiment 3,  $H_{\text{no fertilizer}}^2 = 0.23$ ,  $H_{\text{fertilizer}}^2 = 0.26$ ).

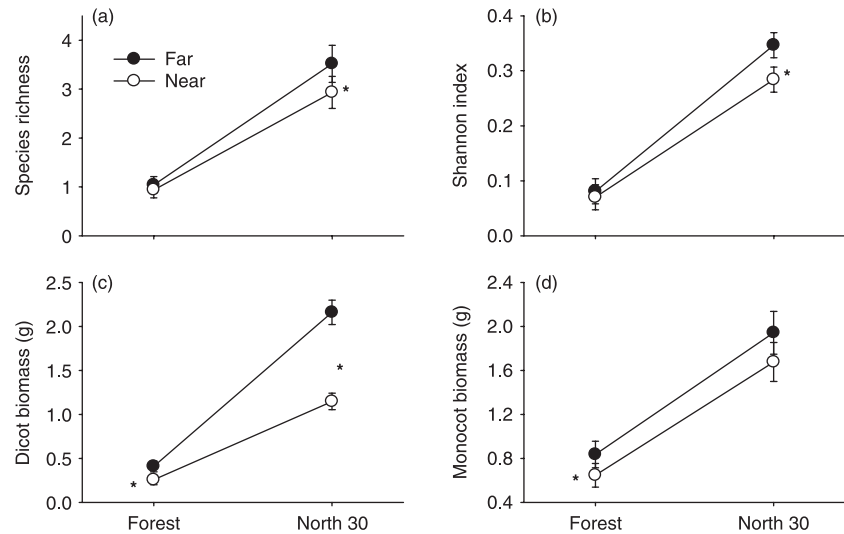
In contrast to the effects of *Oenothera* genotype in the greenhouse, genotype had no detectable effects on either the diversity or biomass of neighbouring plants in the field (Table S5). Proximity to *Oenothera* did suppress the species richness, Shannon diversity, and biomass of neighbouring plants, but these competitive effects varied between gardens (Fig. 4, Table S5). At the Forest garden, plant species richness and Shannon diversity were unaffected by proximity to *Oenothera*, while dicot and monocot biomass were reduced by 37% and 23%, respectively (Fig. 4). At the North 30 garden, the richness and Shannon diversity of plants were reduced by 17% and 18% by *Oenothera*, respectively (Fig. 4). Likewise, *Oenothera* reduced dicot biomass by 47%, while monocot biomass was unaffected by *Oenothera* (Fig. 4).

#### Discussion

The major findings of this study are that: (i) genetic variation in *Oenothera* traits affected the performance of competitors in controlled growth experiments, but (ii) this variation does not appear to be biologically important for the community-wide response of plants to *Oenothera* genotype in more natural environments. Several results support these conclusions. First, greenhouse and field experiments showed that *Oenothera* negatively affected the growth, biomass and diversity of neighbouring plant species, indicating that *Oenothera* competes with neighbouring species (Figs 2, 4). Second, *Oenothera* exhibited genetic variation for multiple traits (Appendices A, C; Fig. 1) and variation in the shoot : root ratio among *Oenothera* genotypes explained up to 41% of the variation in *Bromus* performance (Fig. 3). Third, the effects of plant genotype on competitors were inconsistent between experiments and variation in soil fertility had the largest effect on *Bromus* performance (Supplementary Table S4). Finally, our



**Fig. 4.** The effect of experimental garden and competition on the diversity and biomass of plants competing with *Oenothera* in the field. (a) Plant species richness, (b) Shannon diversity index, (c) dicot biomass, and (d) monocot (grass) biomass, were measured from 10 × 10 cm quadrats placed immediately adjacent to every *Oenothera* plant (near) and 30 cm away from the centre of *Oenothera* plants (far), in each of two experimental gardens (Forest and North 30). Asterisks indicate whether the community response significantly differed ( $P < 0.05$ ) between near and far quadrats at a given garden.



field experiment showed that heritable variation in *Oenothera* biomass was greatly reduced from that of the greenhouse, and there was no longer an effect of genotype on the performance of neighbouring plants. Therefore, our results suggest that it may not be necessary to investigate the effects of *Oenothera* genotype on its competitors in order to understand the short-term ecological consequences of competitive interactions in this community.

#### GENETIC EFFECTS ON THE PERFORMANCE AND CO-EXISTENCE OF COMPETITORS

For genetic variation in one plant species to affect the performance of another plant species there must be genetic variation in traits that influence competitive interactions. Consistent with this, we detected genetic variation in *Oenothera* for multiple traits that may play a role in above-ground and below-ground competition (Appendices A, C). Genetic variation in one of these traits, shoot : root ratio, strongly predicted variation in below-ground biomass of *Bromus* (Fig. 3), which also resulted in effects on the shoot : root ratio and total biomass of *Bromus*. Thus, although *Oenothera* competitively suppressed most aspects of *Bromus* performance (Appendices B, D), and there was genetic variation in above-ground and below-ground growth of *Oenothera* (Table S1), the effects of *Oenothera* genotype on *Bromus* performance were largely mediated by genetic variation in below-ground competitive ability.

Our results contribute to several recent studies that suggest genetic variation in plant competitive ability may be an important factor affecting the abundance and co-existence of competing plant species (Taylor & Aarssen 1990; Booth & Grime 2003; Proffitt *et al.* 2005; Fridley *et al.* 2007; Lankau & Strauss 2007; Whitlock *et al.* 2007). As with our experiment, greenhouse or outdoor competition experiments performed in pots typically find evidence for an effect of plant genotype on the performance of neighbouring plants. These results on

their own do not provide compelling evidence for the importance of plant genotype in affecting the performance of competitors in natural communities. In the field, *Oenothera* genotype did not significantly affect the community-wide biomass or diversity of co-occurring plants (Supplementary Table S5). Therefore conclusions derived from pot competition experiments might not translate to field experiments, where environmental variance is greater. This is not to say that *Oenothera* is not an important player in plant communities. On the contrary, *Oenothera* negatively affected the biomass and diversity of monocots and dicots that naturally colonized the gardens (Fig. 4).

It is possible that differences among *Oenothera* genotypes had undetected effects on the surrounding community in the field. A limitation of our field results was that we were unable to harvest the roots of plants, whereas our greenhouse results suggest that below-ground biomass are most strongly affected by genetic variation in plant traits (Fig. 3). It is also possible that *Oenothera* genotype influenced the performance of individual plant species in the field. However, any such effects were likely weak and inconsistent because the heritability of *Oenothera* biomass was much lower in the field and plant genotype typically explained zero variance in community-wide biomass and diversity (Table S5).

We found inconsistent effects of *Oenothera* genotype on *Bromus* performance between greenhouse experiments. This inconsistency was unexpected because our methods were identical between experiments in almost every way, including the same clonal genotypes of *Oenothera*, seed stock of *Bromus*, soil mixture, pot sizes, greenhouse space and experimenters. Furthermore, *Oenothera* genotypes exhibited significant heritable variation for most plant traits in both experiments. A likely explanation for the discrepancy is that although genotype means of shoot : root ratio were positively correlated between experiments ( $r = 0.53$ ,  $P = 0.01$ ), the correlation co-efficient was much less than 1, indicating a genotype X experiment interaction. The main difference

between experiments was time of year (fall vs. winter), which resulted in a different intensity and length of natural sunlight (despite light supplementation) and we believe this difference caused the interaction. Indeed, previous experiments demonstrated that genotype X environment interactions frequently affect the expression of multiple traits in *Oenothera* (Johnson & Agrawal 2005; Johnson 2007). Thus, our results indicate that the ecological effects of *Oenothera* genotype on competitors are inconsistent and plant genotype explains much less variation than environmental factors such as soil fertility.

#### EVOLUTION IN COMPETITIVE ABILITY AND THE CO-EXISTENCE OF SPECIES

Theoretical models predict that genetic variation in competitive ability facilitates evolution in response to selection by competitors, which can influence the probability of co-existence among species. For example, evolution can increase co-existence between two populations when they evolve to use different resources (Abrams 2006; Vellend 2006). By contrast, evolution can decrease the probability of co-existence when two populations utilize the same resource and one population evolves to persist on lower concentrations of the shared resource than its competitors (Levin 1971). For this to occur, populations must contain genetic variation in competitive ability. Consistent with this, we detected a significant genotype X competitor interaction that affected below-ground biomass and the shoot : root ratio of *Oenothera* (Fig. 1, Table S1), indicating the presence of genetic variation on which selection by competitors can act. Although the short-term effects of *Oenothera* genotype on neighbouring plants is expected to be negligible in nature, genetic variation within *Oenothera* populations may enable populations to adapt to competitors over multiple generations. Therefore, the ecological and evolutionary consequences of genetic variation in competitive ability may differ.

#### EFFECTS OF PLANT GENOTYPE WITHIN AND ACROSS TROPHIC LEVELS

This study expands on recent research that examines how genetic variation in traits of *Oenothera* shapes the ecological structure of multitrophic communities. We previously showed that genetic variation in *Oenothera* predicts variation in resistance to herbivory (McGuire & Johnson 2006), herbivore population dynamics (Johnson 2008), and the composition and diversity of over 100 arthropod species (Johnson & Agrawal 2005; Johnson & Agrawal 2007). In light of the present study, genetic variation in *Oenothera* appears to be more important in affecting species interactions between trophic-levels than within trophic-levels. The generality of this observation has not been explored, but the pattern is expected whenever herbivores impose weak and/or inconsistent selection on plant defense, and competitors impose strong selection towards an optimum, which erodes genetic variation in competitive ability.

#### WHEN TO EXPECT EFFECTS OF PLANT GENOTYPE ON COMPETITORS

Based on the results from field studies, we propose that preliminary predictions are emerging about when genotype identity is expected to affect the performance, abundance and diversity of competing plants. In one study, genotype identity of a coastal grass affected the growth and fine-scale abundance of two other plant species (Proffitt *et al.* 2005). Unlike *Oenothera*, the coastal grass formed clonal stands and exhibited genetic variation for stand size and senescence within stands. This difference in life-history likely contributed to the contrasting effects of plant genotype between studies, because genetic variation in the growth, biomass or senescence of vegetatively reproducing species affects the total area occupied by individuals, and therefore potential competitive effects. Genetic variation in secondary metabolites may also affect the structure of plant communities via allelopathic effects on neighbouring plants (Lankau & Strauss 2007). Evidence for this prediction comes from observational studies that show the diversity and composition of monoterpenes in the leaf litter of pine trees correlate with changes in soil chemistry and plant community structure (Iason *et al.* 2005; Pakeman *et al.* 2006).

There are still too few studies to perform meta-analyses on the effects of genetic variation in plant traits on competitive interactions. In *Oenothera*, genetic variation is unlikely to have strong effects on short-term ecological dynamics with competitors, but this is clearly not the case for all systems. Additional experiments on a diversity of species and systems will help us understand when intraspecific variation will be important in affecting the performance and co-existence of species.

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## Supplementary material

The following supplementary material is available for this article:

**Table S1** Effects of *Oenothera* genotype and interspecific competition on *Oenothera* performance in greenhouse experiment 2.

**Table S2** Effects of *Oenothera* genotype and interspecific competition on *Bromus* performance in greenhouse experiment 2.

**Table S3** Effects of soil nutrients and *Oenothera* genotype on *Oenothera* performance in greenhouse experiment 3.

**Table S4** Effects of *Oenothera* genotype and interspecific competition on *Bromus* performance in greenhouse experiment 3.

**Table S5** Effects of garden, competition and *Oenothera* genotype on the diversity and productivity of neighbouring plants in the field.

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