Note

Genetic Relatedness Influences Plant Biomass Accumulation in Eelgrass (*Zostera marina*)

Jay J. Stachowicz,[1,*,†] **Stephanie J. Kamel,**[1,†] **A. Randall Hughes,**[2] **and Richard K. Grosberg**[1]

1. Center for Population Biology, University of California, Davis, California 95616;   2. Northeastern University Marine Science Center, Nahant, Massachusetts 01908

abstract: In multispecies assemblages, phylogenetic relatedness often predicts total community biomass. In assemblages dominated by a single species, increasing the number of genotypes increases total production, but the role of genetic relatedness is unknown. We used data from three published experiments and a field survey of eelgrass (*Zostera marina*), a habitat-forming marine angiosperm, to examine the strength and direction of the relationship between genetic relatedness and plant biomass. The genetic relatedness of an assemblage strongly predicted its biomass, more so than the number of genotypes. However, contrary to the pattern observed in multispecies assemblages, maximum biomass occurred in assemblages of more closely related individuals. The mechanisms underlying this pattern remain unclear; however, our data support a role for both trait differentiation and cooperation among kin. Many habitat-forming species interact intensely with conspecifics of varying relatedness; thus, genetic relatedness could influence the functioning of ecosystems dominated by such species.

Keywords: genetic relatedness, biodiversity, productivity, community genetics, genetic diversity, intraspecific interactions.

Introduction

Biodiversity is now broadly appreciated to enhance numerous critical ecosystem functions, notably, productivity and stability (Cardinale et al. 2012). Underlying this relationship is the idea that the number of species present reflects functional diversity (Naaem and Wright 2003; Micheli and Halpern 2005) and that increases in functional diversity promote greater resource use and, ultimately, higher productivity (Tilman et al. 1997; Tilman 1999). Theory predicts that phenotypic diversity at any level should increase productivity (Norberg et al. 2001), and such diversity can certainly occur below the species level

(Bolnick et al. 2003). Indeed, a growing body of research demonstrates the community or ecosystem importance of genetic diversity within key species (Hughes et al. 2008).

Most empirical studies, however, consider only the number of genotypes in an assemblage, implicitly treating all genotypes as equally distinct from one another (e.g., Hughes and Stachowicz 2004; Reusch et al. 2005; Hughes et al. 2008). Such an approach fails to use information on quantitative genetic or functional differences among genotypes that could help predict their independent or combined effects on the community. Experiments linking species diversity to ecosystem functioning have used such information to sort species into discrete functional groups and to assess directly the effects of functional diversity on ecosystems (Naaem and Wright 2003). Such approaches, however, require trait data for all taxa, limiting the use of this approach for predicting effects of intraspecific diversity. Furthermore, even for assemblages of species with complete trait databases, defining relevant functional groups depends on the ecosystem function of interest (Hooper et al. 2005).

Reanalyses of species diversity manipulations reveal that phylogenetic diversity (e.g., total branch length in a phylogeny) of a species assemblage is often a better predictor of productivity than the number of species or functional groups (Cadotte et al. 2008, 2009; Flynn et al. 2011). More generally, the outcome of interspecific interactions can be predicted by the phylogenetic distinctiveness of the interacting species, with more closely related species competing more intensely, leading to lower group productivity and/or greater probability of competitive exclusion (Maherali and Klironomos 2007; Burns and Strauss 2011; Violle et al. 2011; Peay et al. 2012). These patterns putatively result from a relationship between evolutionary divergence and divergence in traits influencing the outcome of competition or ecosystem functioning (Violle et al. 2011). While not all such traits are evolutionarily conserved (Cavender-Bares et al. 2009; Best et al. 2013), at least among species,

* Corresponding author; e-mail: jjstachowicz@ucdavis.edu.

† These authors contributed equally to the work.

phylogenetic distance often provides an integrated measure of many traits that is positively correlated with ecological functioning or the outcome of interactions. This raises the unanswered question of whether genetic distance or relatedness among individuals within a species has comparable effects on ecosystem processes.

Several mechanisms could yield a relationship between relatedness of genotypes in an assemblage and the aggregate productivity of that assemblage. First, relatedness might serve as a proxy for differentiation of functional traits with a strong genetic component, just as in analyses above the species level. In assemblages of distantly related genotypes, this niche differentiation could reduce intraspecific competition, increase resource use, and lead to higher aggregate production relative to assemblages of close relatives, as appears to be the case in several studies of performance in groups of sessile marine invertebrates (Gamfeldt et al. 2005; Aguirre and Marshall 2011, 2012). Genetically diverse assemblages could also be more productive if highly productive genotypes are also genetically very different from others. These processes would be analogous to complementarity and sampling, respectively, in the species diversity–ecosystem functioning literature (Hooper et al. 2005; Stachowicz et al. 2007; Drummond and Vellend 2012). In contrast, strong local adaptation, combined with spatial genetic structuring, could result in assemblages of closely related genotypes outperforming diverse assemblages under conditions similar to their home site. However, other mechanisms, including inbreeding or outbreeding depression and self/nonself recognition, may also directly connect genetic relatedness and performance of mixtures in ways that have little to do with trait differences per se. Many vascular plants, for example, reduce belowground investment in response to the presence of related individuals, leading to increased allocation to aboveground productivity (File et al. 2011). In other cases, closely related genotypes are known to fuse, increasing the rate at which individuals achieve a critical size that renders them less vulnerable to biotic or abiotic stresses (Grosberg 1988). In either instance, assemblages of close relatives might outperform those consisting of unrelated individuals. Additionally, inbreeding or outbreeding depression could decrease vigor of sexual recruits and reduce population-level performance (e.g., Keller and Waller 2002), leading to optimal production at intermediate relatedness. Thus, a broad range of relationships between genetic relatedness and the productivity or functioning of an intraspecific group of individuals seems plausible.

As a first step toward assessing the potential for relatedness to predict biomass accumulation in mixed-genotype assemblages, we reanalyze published studies on the effects of genotypic richness on biomass accumulation in *Zostera marina*, a clonally spreading marine vascular plant.

Eelgrass occurs in dense stands in estuaries throughout the Northern Hemisphere, and its standing biomass and density directly contribute to a variety of estuarine ecosystem services, including primary production, nutrient cycling, habitat for fisheries species, and erosion control (Williams and Heck 2001). Our previous research (Hughes and Stachowicz 2004, 2009, 2011; Hughes et al. 2010) and that of others (Williams 2001; Reusch et al. 2005) shows that increasing the number of genotypes of eelgrass within experimentally planted assemblages increases plot biomass and shoot density. There is substantial variation within natural beds in both the number of genotypes per unit area (Hughes and Stachowicz 2009) and the relatedness of the genotypes that co-occur in a patch (Kamel et al. 2012), and these do not covary. Furthermore, eelgrass genotypes differ with respect to functionally important morphological and physiological traits (Hughes et al. 2009; Tomas et al. 2011). Here we use new estimates of genetic relatedness of the genotypes in our previous experiments to assess the relationship between relatedness and eelgrass abundance at the end of three experiments that manipulated the number of genotypes present (Hughes and Stachowicz 2004, 2011; Hughes et al. 2010) and one field survey of genotypic diversity in unmanipulated plots (Hughes and Stachowicz 2009). We also use a more limited set of trait data from eight genotypes to evaluate the relationship between genetic and ecological similarity and to identify mechanisms that could mediate effects of relatedness. For now, our analysis is limited to our own studies because reliable estimates of relatedness require data on the allelic frequencies in populations from which experimental genotypes are drawn, which are presently unavailable for most systems in which experiments have been conducted.

Methods

Experiments and Field Survey

All experiments were conducted in Bodega Harbor, California, and the survey included sites in both Bodega Harbor and nearby Tomales Bay. More detailed methods are available in the articles that present the analyses of the effects of genotypic richness alone on productivity. Here, we present only the directly relevant details (see also app. A).

In the first experiment (Hughes and Stachowicz 2004), we planted eight shoots belonging to two, four, or eight genotypes into 1-m^2 plots. Each combination of genotypes was unique (there were four plots with two genotypes, five with four genotypes, and nine with eight genotypes). We used the shoot density in each plot after 1 year as our response variable. In the second experiment (Hughes and

Stachowicz 2011), we planted eight different six-genotype mixtures (eight mixtures × three replicates per mixture) and measured shoot density and biomass in each plot at the end of 2 years as our response variable. The two field experiments were conducted at different sites, and neither site was the source of any of the genotypes, although all genotypes were collected from within 1 km of the planting site and little genetic structure exists at that scale (Kamel et al. 2012). The final experiment (Hughes et al. 2010) involved planting six unique combinations of four genotypes for each of four different grazer treatments (an isopod and amphipod and a snail, plus a no-grazer control) in outdoor flow-through mesocosms under natural light. A fifth grazer treatment that contained all grazer species was removed from the analysis in this article because of considerable variation among replicates in grazer species composition at the end of the experiment that confounded interpretation of relatedness effects. This experiment used a pool of six genotypes drawn from the same eight genotypes used by Hughes and Stachowicz (2011), and biomass was used as the response variable. All experiments also contained monocultures, but these were not analyzed here because shoots in those treatments have a relatedness of 1 by definition.

The field survey examined whether the correlation between genotypic richness and shoot density in naturally occurring beds matched the positive linear relationship observed in experiments (Hughes and Stachowicz 2009). We sampled 20 shoots per quadrat and measured shoot density in a 25 × 25-cm subplot within each 1-m² quadrat. There were a total of 84 quadrats from seven sites spread across two bays separated by 30 km. The few monocultures present were excluded from the analysis.

Estimating Relatedness

Using genotype data obtained in previous studies from five polymorphic loci (7–13 alleles per locus; see Hughes and Stachowicz 2004, 2009; Kamel et al. 2012), we used STORM, a regression-based measure of relatedness calibrated by the frequency of alleles in the population (Frasier 2008), to estimate the average pairwise relatedness (R) among genets within a group. For the experimental plots, we calculated R for the outplanted genets. For the field-surveyed plots, R was calculated for the sampled genets. Among both experiments and field surveys there was a significant negative relationship between the mean pairwise R of a plot and the standard deviation of all pairwise R values in that plot (experiments: $r^2 = 0.46$, $P = .0001$; survey: $r^2 = 0.37$, $P = .0001$). This suggests that plots with high average relatedness contain individuals that are all somewhat equally related to each other, whereas plots with lower R contain a mix of somewhat related and unrelated individuals.

Measuring Functional Diversity

We used 26 morphological and physiological traits of the genotypes used in two of the experiments (Hughes et al. 2010; Hughes and Stachowicz 2011) to calculate an index of functional diversity (FD) scaled to a minimum of 0 and a maximum of 1 (Petchy and Gaston 2002). These traits were related to root and shoot growth, nutrient uptake, clonal expansion, and ramet size; methods used to obtain trait data are reported elsewhere (Hughes et al. 2009; for a list of traits see app. B, available online). FD calculations were based on plot genotypic composition at the time of planting.

Statistical Analyses

Our analyses focused first on assessing the correlation between genetic relatedness (R) and eelgrass standing stock for all experiments and surveys. Secondarily, we compared the ability of relatedness versus other factors such as the number of genets (N_G) or FD to account for variation in eelgrass biomass or shoot density. Because of the different methods, purposes, and durations of each experiment, and because average R varied among experiments, we analyzed each experiment separately rather than in a single analysis. For each experiment we first ran single-variable mixed-effects models with shoot density or biomass as the response variable, with N_G, R, or FD as the predictor variable and with a spatial variable such as block or site as the random factor. For the experiment that included grazer manipulations, we included grazer treatment as an additional fixed factor. Second within each experiment, all potential predictors (R and FD or N_G) and their interactions were combined into a single mixed-effects model. We could not run a single model with N_G, R, and FD because genotypic richness did not vary among polycultures in the experiments for which we had trait data on the genotypes (Hughes et al. 2010; Hughes and Stachowicz 2011).

The field-surveyed assemblages covered a larger range of R than either experiment. We hypothesized that nonlinear optimum relationships may result and so included a quadratic term in the single mixed-effects model of R versus density, with the quadratic term recentered around its mean before squaring [$R^2 = (R - \overline{R})^2$] to avoid collinearity with the linear R term. We then evaluated the importance of N_G and R on shoot density in a single generalized additive model (GAM; Hastie and Tibshirani 1990). Additionally, we also ran single and multivariate

mixed-effects models on the subset of plots in which R <0.4 to allow for direct comparison with experiments that included only plots in this range of R. Mixed-effects models were run in JMP, version 8, and the GAM was run in SAS, version 9.1 (SAS Institute, Cary, NC).
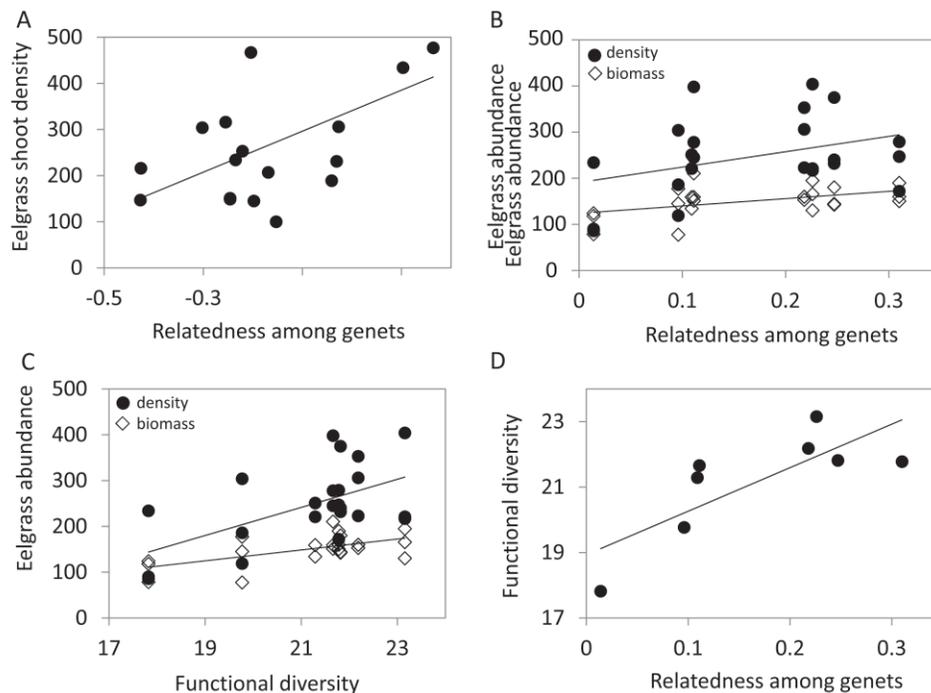
Results

The index of relatedness (R) among genotypes was a highly significant predictor of biomass and shoot density across experimental and natural assemblages of eelgrass. Among experimental assemblages that varied in both initial genetic richness and relatedness (Hughes and Stachowicz 2004), final shoot density was positively correlated with relatedness, but not richness, in both univariate and multivariate models (fig. 1*A*; table 1, pt. A). Richness and relatedness in this experiment were uncorrelated ($r^2 = 0.07$, $P = .28$), so the lack of significance of richness was not due to collinearity with relatedness.

In a separate field experiment in which all mixtures had equivalent richness (six genotypes) but varied in genotypic composition (Hughes and Stachowicz 2011), shoot density was also strongly positively correlated with relatedness (fig. 1*B*; table 1, pt. B1). In this experiment, functional trait diversity (FD) was also positively correlated with shoot density (fig. 1*C*; table 1, pt. B1). For the univariate regressions, the model including only FD was the strongest predictor of shoot density, and in the multiple regression, R was not a significant predictor when FD was included (table 1, pt. B2), presumably because of the strong positive correlation between these two predictors (fig. 1*D*; $r^2 = 0.61$, $P < .0001$). This collinearity limits our ability to assess whether trait diversity or relatedness is more important in explaining the variation in eelgrass shoot density in this experiment. In the mesocosm experiment (Hughes et al. 2010), grazer treatment had a strong effect on eelgrass biomass ($P < .0001$), but R explained additional variation ($P = .01$). Importantly, FD was uncorrelated with both eelgrass biomass ($P = .27$) and R ($P = .42$) in this experiment (table 1, pt. C).

In unmanipulated field plots (Hughes and Stachowicz 2009), R ranged from -0.3 to 0.9, which included much more highly related combinations than any of the exper-

Figure 1: Results of reanalysis of eelgrass genotypic richness manipulations showing consistent positive relationship between genetic relatedness and measures of eelgrass productivity. See table 1 for results of statistical analysis. *A*, Relatedness (and not richness) is positively associated with shoot density (shoots/m²) from the experiment in Hughes and Stachowicz (2004), in which genotypic richness and relatedness varied independently. Relatedness (*B*) and functional diversity (*C*) are positively correlated with eelgrass shoot density and biomass in mixtures of six genotypes from Hughes and Stachowicz (2011); shoot density is depicted as filled circles and biomass open diamonds. *D*, Functional diversity and relatedness are positively correlated in the experiment in Hughes and Stachowicz (2011) but not in Hughes et al. (2010).

Table 1: Statistical analysis of the relationship between eelgrass productivity and various metrics of biodiversity: genotypic richness, genetic relatedness, and functional diversity

Models and variables	F	P	df (den)	R^2
A. Field experiment: relatedness and richness (response = density):				
1. Single variable with site as random effect:				
Relatedness	5.38	.035	14.76	.45
No. genotypes	.41	.531	14.03	.30
2. Multivariable with site as random effect:				.46
Relatedness	4.57	.049	14.02	
No. genotypes	.05	.821	13.27	
B. Field experiment: relatedness and functional diversity (response = density):				
1. Single variable with site as random effect:				
Relatedness	5.77	.027	19.57	.46
Functional diversity	15.1	.001	18.11	.61
2. Multivariable with site as random effect:				.60
Relatedness	.13	.728	17.41	
Functional diversity	6.81	.018	17.17	
3. Single variable with site as random effect (response = mass):				
Relatedness	5.88	.025	20.71	.30
Functional diversity	4.23	.003	19.32	.18
4. Multivariable with site as random effect:				.40
Relatedness	.01	.941	18.59	
Functional diversity	4.03	.060	17.83	
C. Mesocosm experiment: relatedness and functional diversity (response = mass):				
1. Each diversity predictor tested separately with grazer as fixed effect in each model:				
Relatedness	6.04	.02	19	.87
Functional diversity	1.28	.27	19	.81
2. Multivariable with both diversity metrics:				.88
Grazer	40.7	<.001	19	
Relatedness	4.31	.05	19	
Functional diversity	.06	.81	19	
D. Field survey with site as random effect (response = density):				
1. Full data set, multivariable:				.35
Relatedness—quadratic model:				
R	.92	.346	33.88	
R^2	6.33	.017	33.73	
No. genotypes	5.40	.026	33.90	
2. Field survey: only plots with $R < .4$:				
Single variable with site as random effect:				
Relatedness	6.97	.018	15.63	.61
No. genotypes	3.51	.080	13.93	.55
3. Field survey: only plots with $R < .4$:				
Multivariable with site as a random effect:				.63
Relatedness	3.91	.049	15.24	
No. genotypes	.96	.334	13.93	

Note: Analysis is by general linear model or mixed model, except for part D1, which is a generalized additive model.

iments. Overall, N_G was a significant predictor of shoot density (table 1, pt. D1). R was also a significant predictor of density, but this relationship was nonlinear and was best described by adding a quadratic term to the regression equation (fig. 2; table 1, pt. D1). When both N_G and R were included in the model, only the nonlinear component of R was a significant predictor of shoot density ($\chi^2 = 8.73$, $P = .03$), and richness and relatedness were uncor-related here as well ($r^2 = 0.02$, $P = .46$). When we analyzed only field-surveyed plots in which R was within the range found in the three experiments (i.e., $R < 0.4$), we found a linear correlation between shoot density and R similar to that found in the experimental studies (fig. 2). In both single and multivariate analyses of this subset of the data, R was the only significant predictor of shoot density (table 1, pts. D2, D3).
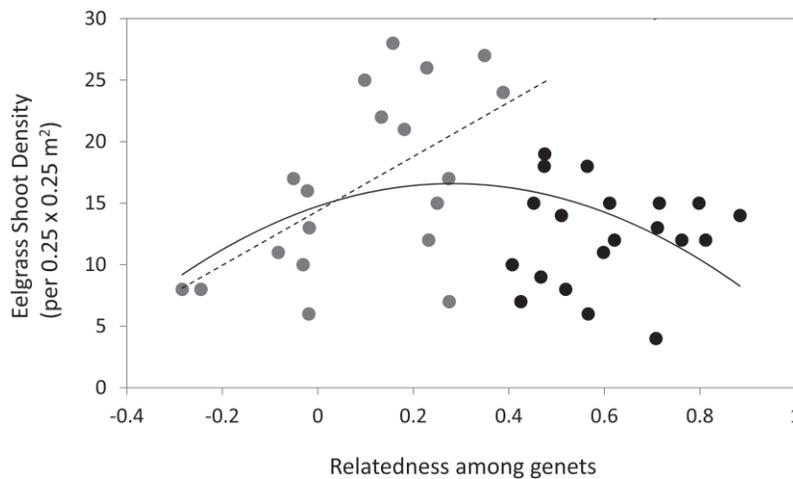
Figure 2: Shoot density in a plot is unimodally related to genetic relatedness of the genotypes in that plot. Note that this survey covers a much broader range of relatedness than the experiments in figure 1. Over the range of relatedness covered by the experiment, the survey also recovers a positive relationship between relatedness and shoot density (dashed line, gray circles only).

Discussion

Eelgrass occurs in dense meadows where it is the only vascular plant species and thus in natural settings will likely interact strongly with conspecifics of varying relatedness. We found that the average relatedness among individuals strongly predicted total biomass and/or density in both field plots and experimental assemblages across four different studies. In the studies in which the number and relatedness of genotypes varied independently (Hughes and Stachowicz 2004, 2009), relatedness was a better predictor of plant biomass than genotypic richness. These findings agree with those from meta-analyses of species diversity experiments (Cadotte et al. 2008, 2009), in that the richness of taxa (genotypes in our case) did not predict plant biomass as well as metrics that incorporated quantitative measures of functional or genetic distinctiveness among taxa. However, our results differ from those results and from those of some nonclonal animal studies (Gamfeldt et al. 2005; Aguirre and Marshall 2011, 2012) in that highest eelgrass biomass occurred in more closely related assemblages. This pattern was consistent across multiple experiments conducted in different locations with distinct pools of genotypes and in a field survey that included hundreds of genotypes from seven sites in two bays separated by 30 km (Kamel et al. 2012). Thus, at least for *Zostera marina* in Bodega Harbor and Tomales Bay, the correlation between the relatedness of individuals in an assemblage and the biomass achieved by that assemblage is robust.

The mechanisms linking genetic relatedness, functional diversity, and eelgrass production are, however, less clear.

The positive relationship between relatedness and biomass accumulation appears to contradict the idea that niche differentiation increases with genetic distance and suggests that cooperation among close relatives might play a role. Nonetheless, trait variation remains a viable explanation because in one of the experiments for which we had functional trait data, assemblages with higher relatedness also had greater trait diversity. We do not currently have a compelling explanation for this counterintuitive pattern, but the relationship is unlikely to be the effect of a particular genotype. This would require a genotype that was distantly related yet similar in trait values to most other genotypes, and such a genotype was not in the pool of eight used in these experiments. Such a relationship could also result from local selection for trait divergence among competing genotypes in a species with limited dispersal; however, the low degree of genetic structure of eelgrass across sites in Bodega Harbor (Kamel et al. 2012) argues against such a mechanism in our system. Moreover, we found no significant correlation between FD and R in a second experiment, suggesting that trait diversity is not a universal explanation for the correlation between relatedness and biomass. Given that there were only eight genotypes in our pool, the range of relatedness treatments in experiments for which trait data were available is relatively narrow, making it difficult to interpret the positive relationship (or lack thereof) between functional diversity, relatedness, and plant biomass. In particular, none of our experiments contained assemblages composed of closely related individuals ($R_{max} = 0.4$), yet such assemblages do occur in nature (Kamel et al. 2012; fig. 2).

Both experimental results and observational results produced a positive linear correlation for the range of relatedness over which they overlap (fig. 2). The correspondence between experiments and surveys for low values of R suggests that relatedness influences biomass in natural assemblages. Yet, field surveys involved a broader range of relatedness values, yielding a unimodal relationship between R and shoot density. Inbreeding and outbreeding depression could underlie this pattern (Willi and van Buskirk 2005) but would not contribute to patterns in our experiments because shoots in experimental plots were primarily a product of vegetative reproduction. However, the low shoot density in experimental monocultures ($R = 1$) relative to polycultures ($R < 0.4$; see Hughes and Stachowicz 2004, 2011; Hughes et al. 2010) is also consistent with the idea that at very high levels of relatedness, plot productivity suffers. Alternatively, the unimodal relationship between relatedness and plant biomass could reflect a balance between the benefits of trait differentiation among distantly related genotypes, leading to greater resource capture versus cooperation among close relatives. Unfortunately, because we lack trait data for genotypes in the survey, we cannot assess how trait diversity varies with relatedness over this full range of R. Additionally, it is possible that the interpretation of the relationship between R and shoot density in survey data could be confounded if additional factors (e.g., water flow) directly influence both local density and relatedness (e.g., by altering dispersal of seeds and gametes).

In contrast to our experiments with eelgrass, the performance of solitary invertebrates is often higher in groups of unrelated individuals compared to groups composed of siblings or half-sibs (Gamfeldt et al. 2005; Aguirre and Marshall 2011, 2012). At this point it remains difficult to explain why there is a positive relationship between relatedness and productivity in eelgrass and a negative relationship in sessile invertebrates. However, our experiments compare interactions among relatively unrelated individuals (low probability that any are sibs based on collection distances), whereas the invertebrate studies all used known sibling groups in their most related treatments. Thus, the direction of the relatedness-performance relationship might differ among studies because eelgrass and invertebrate experiments use relatedness values on the ascending and descending portions of the unimodal curve, respectively. Furthermore, species such as eelgrass that are highly clonal and compete intensely with conspecifics might be more likely to develop cooperative relationships with close relatives than sessile invertebrates that typically interact with a matrix of other species. The location of the peak in a unimodal relationship might then vary among taxa, depending on the relative strength of intraspecific niche differentiation, inbreeding depression, and cooperation among kin.

In light of the fact that functional traits will likely never be known for the vast majority of species, the increasing number of examples in which ecological outcomes in competition experiments can be predicted from phylogenetic relatedness (Burns and Strauss 2011; Violle et al. 2011; Peay et al. 2012) is encouraging (but see Best et al. 2013). Such approaches also hold promise for understanding the effects of genotypic diversity, as extending a purely functional trait-based approach is problematic, except in systems in which genotypic diversity is low and clonal reproduction dominates. However, differences in the mechanisms that can operate at the inter- and intraspecific levels mean that the slope and direction of the relationships may not be consistent across species and local levels of genetic diversity. Additionally, the slope and direction of the relationship between genetic differentiation and ecological functioning might depend on the genetic scale under consideration (fig. 2), as has been suggested for phylogenetic diversity among species (Peay et al. 2012). For example, within the same assemblage of species, a positive relationship between ecological and genetic distinctiveness can occur when a broad range of phylogenetic distances is considered, but the relationship becomes negative across a narrower range (Peay et al. 2012).

Currently, our evidence is consistent with both cooperation among relatives and functional diversity as factors contributing to the enhanced density and biomass of eelgrass. More definitive assessment awaits an experiment with a broader array of genotypes that can produce mixtures that decouple trait diversity and relatedness at the initiation of the experiment. Once the slope and direction of the relationship between relatedness and functionality across scales are more thoroughly understood, genetic relatedness might be useful in designing restoration or mitigation efforts for key foundation species such as sea grasses, mangroves, or some forest trees that occur in stands where species diversity is low and thus most individuals interact with conspecifics. We eagerly await additional data to evaluate the consistency of this relationship across taxa.

APPENDIX A

Table A1: Details of studies examining the effects of genotypic richness on biomass accumulation that are used to assess the effect of genetic relatedness on biomass in this study

Reference	Study type	Genotypic richness	No. unique combinations	Replicates of each combination	Total no. genotypes used	Trait data available for all genotypes?	Response variable	Duration
Hughes and Stachowicz 2004	Field experiment	1, 2, 4, 8	18	No	69	No	Density	1 year
Hughes and Stachowicz 2011	Field experiment	1, 6	8	Yes	8	Yes	Density and biomass	2 years
Hughes et al. 2010	Mesocosm experiment	1, 4	6	Yes	6	Yes	Density and biomass	10 weeks
Hughes and Stachowicz 2009	Field survey	2–15	NA	No	322	No	Density	NA

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QUERIES TO THE AUTHOR

q1.   When you say "the articles that present the analyses of the effects of the genotypic richness …," did you wish to provide any citations?

q2.   In table A1, does "NA" stand for "not available" or "not applicable"?

q3.   In table 1, (1) does "(den)" stand for "denominator"? If so, OK to remove (implied in degrees of freedom)? (2) What do the italic P values indicate? (3) Also, please note I have added the "response" imformation to individual stub cells (in parentheses) to more closely follow our table style and save space. OK?

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