Functional trait differences and the outcome of community assembly: an experimental test with vernal pool annual plants

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Functional trait-based approaches have seen rapid development in community ecology and biogeography in recent years, as they promise to offer a better mechanistic and predictive understanding of community structure. However, several key challenges remain. First, while many studies have explored connections between functional traits and abiotic gradients, far fewer have directly tested the common assumption that functional trait differences influence interspecific interactions. Second, empirical studies often ignore intraspecific trait variation within communities, even though intraspecific variation has been known to have substantial impacts on community dynamics. Here we present an experiment designed to assess the role of functional trait differences in predicting the outcome of interspecific species interactions among a suite of California vernal pool annual plants. Eight species were grown in pairwise combinations in two levels of inundation in a greenhouse and functional traits were measured on all individuals. Nested models predicting focal plant performance were fit to the data. For seven of the eight species in the experiment, the best model included a functional trait difference term that was consistent with a competitive hierarchy, indicating that focal species tended to do better when they had larger leaf size, lower specific leaf area, and greater investment in lateral canopy spread than their neighbors. Models that included individually measured trait values generally performed better than models using species trait averages. We tested if the same trait measurements predicted tolerance of inundation (a feature of vernal pool habitats), and species depth distributions from extensive field surveys, though we did not find strong relationships. Our results suggest that functional traits can be used to make inferences about the outcome of interspecific interactions, and that greater predictive power can come from considering intraspecific variation in functional traits, particularly in low diversity communities.

Functional trait-based approaches have seen rapid development in recent years and are widely used for studying community structure and assembly at local (Kraft et al. 2008, Angert et al. 2009, Weiher et al. 2011, Gotzenberger et al. 2012) and biogeographic scales (Swenson and Weiser 2010, Siefert et al. 2013). Trait-based approaches promise to alleviate challenges with contingency and generality that community ecology often suffers from (Lawton 1999). In particular, shifting the focus from analyses of species per se and onto functional components of the phenotype can lead to a more mechanistic understanding of the forces shaping communities and to a greater ability to generalize results across organisms and systems (McGill et al. 2006, HilleRisLambers et al. 2012). Trait-based approaches have been particularly helpful in situations where experimental approaches are intractable, such as at broad geographic scales or in high-diversity communities.

Despite the widespread adoption of functional trait and related phylogenetic community structure (Webb et al. 2002) approaches, several key issues currently limit their utility. First, it has become increasingly difficult to confidently map functional trait patterns (or related phylogenetic patterns) to a single ecological process, as many processes are predicted to produce similar patterns within communities (reviewed by Cavender-Bares et al. 2009). In the case of competitive exclusion, recent developments even suggest that two distinct patterns can be produced by a single process depending the nature of the trait that is considered (Mayfield and Levine 2010). These findings indicate that more detailed experimental work is needed in order to better understand the connections between functional trait differences, community assembly processes, and the resulting patterning within communities, particularly in relation to interspecific interactions.

Many trait and phylogenetic-based community assembly analyses typically assume that coexistence between competitors is promoted by trait differences, such that competitive exclusion can result in a pattern of high phenotypic disparity between co-occurring species (MacArthur and Levins 1967, Webb et al. 2002, Stubbs and Wilson 2004).

In this hypothesis, the intensity of competition is predicted to decrease for both species as trait disparity increases (referred to here as the 'trait distance' hypothesis). However, if traits instead indicate the position of a species along a competitive hierarchy, then the negative effects of competition should be directional – as trait distance increases, the competitive superior species should do relatively better and the inferior should do worse (Uriarte et al. 2010, Kunstler et al. 2012). In this hypothesis, both the distance between competitors in trait space in addition to the direction are predicted to be important in understanding the outcome of competition (the 'trait hierarchy' hypothesis). In this scenario, competitive exclusion is predicted to lead to phenotypic clustering of species within communities (Mayfield and Levine 2010). While these two alternatives have very different implications for the phenotypic (and phylogenetic) structure of communities, there have been relatively few studies that have attempted to distinguish between the prevalence of these two phenomena across communities. This is particularly important as many observational studies report phenotypic (or phylogenetic) clustering and interpret the pattern as the result of habitat filtering. For example, recently Kunstler et al. (2012) report evidence for plant traits driving competitive hierarchies in vegetation plot analyses. Likewise, competition studies in plant communities have reported patterns consistent with traits predicting competitive hierarchies (Gaudet and Keddy 1988, Goldberg and Landa 1991), and experimental studies considering phylogenetic distance have reported patterns consistent with trait distance decreasing competition (close relatives compete more than distant relatives: Burns and Strauss 2011), and of no relationship (Cahill et al. 2008, Bennett et al. 2013). Despite the importance of this issue, to date little effort has been made to distinguish between the 'trait distance' and the 'trait hierarchy' hypotheses by directly measuring the outcome of species interactions in an experimental context (but see Fort et al. 2014).

A second challenge facing trait- based ecology is the issue of intraspecific variation (Violle et al. 2012). Many early studies, noting that at broad taxonomic scales intraspecific trait variation is typically modest relative to interspecific differences, have focused solely on analyses of species means. However, intraspecific variation can be considerable in some species, and this variation is predicted to be a particularly important influence on species interactions in low diversity communities dominated by a one or a few species (Crutsinger et al. 2006, Hughes et al. 2008). Even in more diverse systems, recent studies have shown that incorporating intraspecific trait variation into analyses can lead to different, often stronger, inferences about the underlying drivers of community assembly (Cornwell and Ackerly 2009, Jung et al. 2010, Paine et al. 2011, Dwyer et al. 2014). Despite these findings and calls for better incorporation of intraspecific variation into trait-based approaches (Violle et al. 2012), to date relatively few studies have incorporated intraspecific variation into analyses.

Vernal pool plant communities are an outstanding system for experimentally investigating the role of inter- and intraspecific trait differences in driving community assembly processes. Vernal pools are seasonal wetlands that annually cycle between aquatic and terrestrial conditions (Keeley and Zedler 1998). In the Central Valley of California, USA, vernal pools fill with water during the cool, wet winter months, rapidly dry each spring as temperatures rise and precipitation declines, and remain dry through the hot, dry summer. The annual accumulation and recession of water generates a steep ecological gradient along vernal pool sideslopes in both biotic and abiotic conditions, with flooding depth and duration being greatest at the bottoms of pool depressions, and productivity being greatest at the terrestrial edges (Holland and Dains 1990, Emery et al. 2009). California vernal pools support diverse plant communities dominated by endemic annual herbs, many of which belong to a handful of phylogenetically and functionally distinct lineages (Stone 1990). Members of this community are ideally suited for experimental work due to their small stature and annual life history, making it possible to conduct highly replicated experiments in compact areas over relatively short timeframes. In their native habitat, the relative abundances of vernal pool species are clearly influenced by hydrological processes, as plant density and distribution patterns vary within pools across inundation gradients (Barbour et al. 2003, Emery et al. 2009), among pools with different hydrological regimes (Holland and Dains 1990), and among years with different precipitation patterns (Bauder 2000, Collinge et al. 2011). Furthermore, experimental work has found that competitive interactions can interact with hydrological dynamics to drive plant distribution patterns across vernal pool inundation gradients (Gerhardt and Collinge 2007, Emery 2009). However, these studies have not directly investigated the role of functional trait variation in driving these responses, limiting our ability to generalize these results to other communities and environmental contexts.

Here we present a study with vernal pool plants designed to experimentally assess the role of trait differences in driving the outcome of species interactions. Specifically, we ask three questions. First, do trait differences between a focal species and a neighbor predict variation in performance, and if so, does the direction of the trait difference matter ('trait hierarchy' hypothesis) or just the absolute value of the trait difference ('trait distance' hypothesis)? Second, do analyses incorporating intraspecific trait variation differ from analyses using species trait means? Finally, in order to place our experimental results in a broader geographic context we examine whether the competitive response and trait differences in the experiment relate to distribution patterns of the focal species in field surveys.

Methods

Species selection and seed collection

We selected eight vernal pool annual plant species (Table 1) that co-occur in vernal pools at Mather Regional Park in the Central Valley of California, USA. This site is in the southeastern Sacramento Valley Vernal Pool Region and the vernal pools are classified the Northern Hardpan type (Keeler-Wolf et al. 1998). These species belong to six taxonomic families

Table 1. Eight species selected for the experiment.

Species	Family	Code
Downingia bicornuta	Campanulaceae	DOWBI
Lasthenia fremontii	Asteraceae	LASFR
Lasthenia glaberrima	Asteraceae	LASGL
Layia fremontii	Asteraceae	LAYFR
Limnanthes alba	Limnathaceae	LIMAL
Navarretia leucocephala	Polemoniaceae	NAVLE
Plagiobothrys stipitatus var. *micranthus*	Boraginaceae	PLAST
Pogogyne douglasii	Lamiaceae	POGDO

that each contain numerous vernal pool endemics (Baldwin et al. 2012). Collectively, these eight species make up the vast majority of cover and biomass in many vernal pools at this site, with individual species reaching up to 100% cover in some microhabitats and years (Emery unpubl.). The majority of seed collected for our experiment was from the Mather Field site, where seed heads were collected two years prior to the experiment in the late spring and early summer and stored in envelopes in the laboratory prior to germination for the experiment. Seed collections for three species were supplemented with collections from additional vernal pools sites in the Central Valley of California (Supplementary material Appendix 1) one and two years prior to the experiment.

Experimental design and harvest

To assess the role of inter- and intra-specific trait differences in determining species interactions, we established a competition experiment under two abiotic conditions in the greenhouse in the late spring of 2009. Species were planted in all pairwise combinations in two abiotic treatments designed to capture conditions deep in a vernal pool as well as at the edge of a pool.

To trigger germination, seeds were planted in flats filled with potting mix and were placed in growth chambers that simulated the cool temperatures and reduced daylight hours experienced by these species during their natural autumn germination period. The chamber was programmed to impose a light/temperature cycle of 10 h of daylight at 15°C for and 14 h of darkness at 5°C, which had been shown to stimulate germination in pilot studies. Plants were bottom-watered using alternating wet and dry periods of four and three days, respectively, which maintained high soil moisture but minimized the accumulation of algae. One week after seedling emergence, seedlings were transplanted into 3.8×21 cm 'cone-tainer' pots containing a soil mix of one part generic fine-grain sand, one part fine sandy loam and one part potting soil mix.

Pairs of seedlings were transplanted into pots in all pair-wise combinations of the eight species, for a total of 36 unique combinations of inter and intra-specific pairs. In addition, one individual of each species was planted alone in order to assess tolerance of the inundation treatment, discussed below, for a total of 44 planting combinations. Ten sets of the planting combinations (440 pots total) were placed in racks in the greenhouse, and racks were placed in large plastic tubs and filled with water. To simulate variation in depth in the vernal pools, half of the plants

($n = 5$ replicates per planting combination) were subjected to inundation for the first six weeks of the experiment, mimicking conditions at deeper positions in the vernal pool depressions ('deep' treatment). During the inundation treatment, 2–3 cm of water was maintained above the surface of the soil, and the water was replaced every week to reduce algal growth. The other set of five replicates was assigned to a 'shallow' treatment designed to mimic conditions far up the sides of a vernal pool with no inundation. In this treatment the bottoms of the pots were submerged in water in the tub, but the surface of the soil in the pots was above water. Following the six weeks of inundation, water in the 'deep' treatment was lowered to the level in the 'shallow' treatment and maintained for the duration of the experiment, mimicking the natural drying down of vernal pools in the late spring. Plants were grown under standard greenhouse conditions (25°C, 12 h light/dark cycle) and harvested at peak biomass before senescence began, approximately three months after initial planting. At harvest, plants were sampled for functional traits. Subsequently, the aboveground portion of each plant was collected, dried to a constant mass in a 60°C oven and weighed. Pots in which one or both plants died prior to harvest were excluded from further analysis.

Trait sampling

Vegetative traits were sampled on all individuals at harvest following established protocols (Cornelissen et al. 2003). As many vernal pool species exhibit changes in leaf form and function throughout their lifecycle (heterophylly) corresponding to the shift from submerged juvenile to emergent adult growth form, at harvest we sampled two leaves from each individual that spanned the ontogenetic changes seen in each species. Length and width were measured for each leaf, and fresh area was determined by scanning each leaf on a flatbed scanner in the greenhouse at the moment of harvest and calculating laminar area using ImageJ (Abramoff et al. 2004). Harvested leaves were placed into coin envelopes, dried to a constant mass in a 60°C oven and weighed on semi-analytic balance to determine specific leaf area (cm^2 fresh area g^{-1} dry mass). Canopy shape was quantified by dividing average leaf length by plant height. As branching along stems was minimal in the species included in the experiment, this yields an index that ranges from close to 0 for plants that primarily invest in vertical growth to values >1 for plants that invest more in lateral growth than vertical growth. In addition to these individual-level traits, we also measured average seed mass for species from the seeds collected for the experiment, as well as maximum height of species in the experiment, assessed by taking the 95th quantile of the adult height distribution for each species. Traits were not sampled from individuals or leaves in poor condition – in these rare cases species averages from the same experimental replicate were substituted in analyses.

Analyses

Following harvest, we tested for effects of species, depth treatment, and neighbor size on final target plant biomass

using analysis of covariance. Results from the ANCOVA were used to inform the models used to test for trait effects, described below. Biomass measures were log10 transformed prior to analysis.

Next, to quantify the extent and source of intraspecific variation in functional traits in the experiment we partitioned variance in the functional traits sampled in the experiment into nested depth treatment, species, individual, and leaf levels following the analysis approach of Messier et al. (2010). Specifically, we used the 'lme' function in R (ver. 2.15.1) to fit a general linear model of variance across the nested scales and the 'varcomp' function to perform variance components analysis. We performed this analysis for $\log_{10}$ transformed measurements of leaf size and SLA. An additional analysis was performed for canopy shape, omitting the effect at the 'leaf' level of the analysis. We also tested for trait differences across the abiotic treatments using analysis of variance with species and treatment as factors. As seed size and maximum height were not measured at the individual level they could not be subject to these analyses.

As we were primarily interested in the overall role of trait similarity in shaping interspecific interactions, we used species scores along the first axis of a principle components analysis of the trait data as an integrated trait measurement. Separate principle components analyses were performed at the level of species trait averages as well as the level of individuals for use in the model selection procedure described below. In the individual-level analysis, conspecifics were all assigned identical species average seed size and species maximum height values averaged from the corresponding inundation treatment, as these traits could not be measured at the individual level.

Trait effects on experimental outcomes: model selection

To distinguish between our alternative hypotheses about the role of trait differences in driving the outcome of species interactions, we used a model selection approach following from neighborhood modeling approaches in forest ecology (Uriarte et al. 2010) where growth and mortality are modeled as functions of neighbor distance, size and (more recently) functional identity. For each species, we modeled final biomass using six alternative linear models. The first simply includes an intercept term and a lognormal error distribution, with no effect of neighbor size or functional traits. The second model adds an effect of neighbor biomass to the first model, but still lacks an effect of functional traits. The remaining four models add a measure of trait similarity of the neighbor to the second model, calculated either using species trait averages or traits measured on the individual plants, and calculating the disparity between the trait (T) of focal individual and the neighbor either as a trait difference (consistent with the trait hierarchy hypothesis):

$$T_{\text{focal}} - T_{\text{neighbor}}$$

or as a trait distance, using the absolute value of the difference (consistent with the trait difference hypothesis):

$$|T_{\text{focal}} - T_{\text{neighbor}}|$$

Thus the final four models differed in using either species average or individual traits and in using either trait hierarchy or trait distance measures. As our focus is on the aggregate effects of trait similarity, we used scores along axis 1 of the principle components analyses described above, performed either at the species or individual level, as the trait measure.

Model parameters were estimated using maximum likelihood, and the six alternative models for each species were compared using the Akaike information criterion corrected for small sample size (AICc) from which we calculated Akaike weights, where the best candidate model has the lowest AICc and the highest weight (Burnham and Anderson 2002). We assessed goodness of fit for the models using conventional regression statistics (R^2). All model fitting and selection analyses were conducted in R.

Connecting experimental results to landscape-level patterns

Finally, we used a recent, extensive sampling effort in natural vernal pool communities to test the extent to which our experimental results captured patterns seen in vernal pool communities across the California Floristic Province. A total of 43 pools spanning 19 sites were sampled for vernal pool plant composition across inundation gradients (see Appendix A in Emery et al. 2012). In each sampled pool, the percent cover of the focal species (and other congeners) was estimated in 0.25×0.25 m plots placed at regular intervals (every 0.5 m for all pools except exceptionally large pools at one site, where 2-m intervals were used) along transects that spanned the inundation gradient. The elevation of each plot relative to the pool edge was measured to the nearest centimeter using an autolevel. The average position of each species along the vernal pool inundation gradient was estimated by first identifying the depth at which each species reached maximum abundance in each pool, and then averaging this value across all pools in which it was observed. Additional details of the sampling design, site locations and depth calculations are provided in Emery et al. (2012).

Using these survey data, we tested how species performance in the experiment related to depth distributions in the field. First, we developed an index of the inundation tolerance of each species in the experiment by comparing final biomass of plants grown without neighbors in both the shallow and deep treatments. For each species, we calculated the index by dividing average biomass of plants in the deep treatment by average biomass of plants in the shallow treatment. We then tested for correlations between this index and species average depth in the pool in field surveys, as well as for correlations with traits measured in the experiment.

Results

Final target biomass in the experiment ranged over two orders of magnitude, from the diminutive *Downingia bicornuta* to the robust *Pogogyne douglasii* in the shallow treatment. Overall we found significant effects of species, depth treatment, and neighbor size on final target biomass, as well as a significant interaction between species and treatment (ANCOVA, Fig. 1, Supplementary material
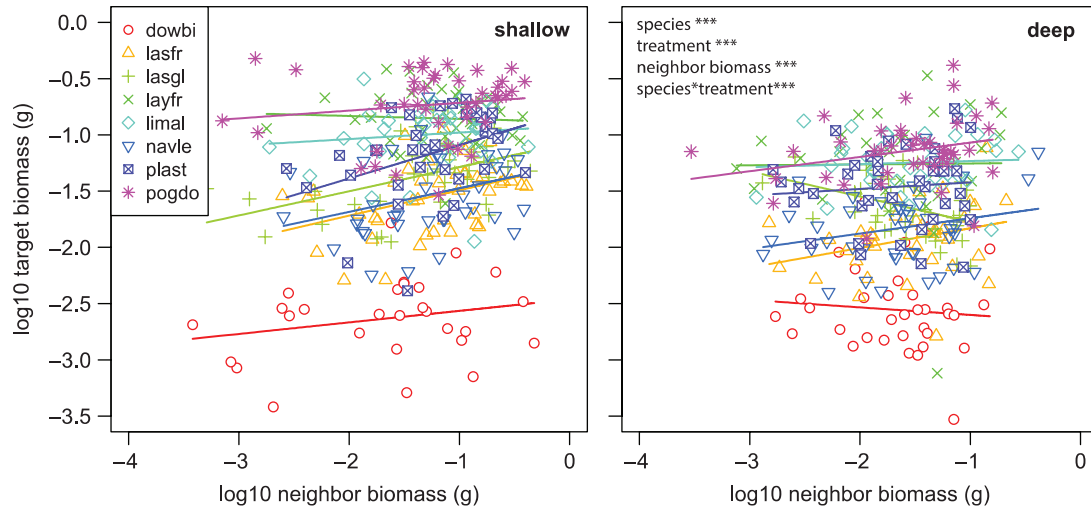
Figure 1. Final target biomass as a function of final neighbor biomass. Data are divided to show differences among depth treatments and among species. Results from type II ANCOVA summarized in panel B (*** = p < 0.001), full results in Supplementary material Appendix 2.

Appendix 1 Table A2). In general neighbor size and target size were positively correlated, though neighbor size effects were more mixed in the deep treatment (Fig. 1).

Analyses of variance of the traits measured at the individual level found significant species differences for the SLA, leaf size and canopy shape, as well as small but significant depth treatment effects for the two leaf traits and a species by treatment interaction for SLA (Supplementary material Appendix 3). Variance partitioning of SLA, leaf size and canopy split the vast majority of the variance roughly evenly between the species and individual level, with very little explained by leaf (Table 2). SLA had the most variance explained by the species level (51%), while canopy shape had the least (44%).

For species average trait values, the first principle component axis captured 39% of the variation among species and primarily reflected variation in SLA, leaf size and canopy shape (Fig. 2), with *Downingia* at one end of the axis with high SLA, small leaves, and a very upright growth pattern with minimal lateral spread. The first axis of the PCA on individual traits (Supplementary material Appendix 4) captured the same traits and slightly less of the total variance (33%). In both PCA analyses, the second axis captured seed size variation but was driven primarily by large seeds in one species, *Limnanthes alba*, and so we focused analyses on the first axis of variation.

For seven of the eight species in the experiment, the best candidate model describing final biomass included a term

capturing trait disparity between the focal individual and the neighbor (Table 3), with goodness of fit (R^2) values ranging from 0.06 to 0.30. *Downingia bicornuta* was the sole exception, where the simplest model with no neighbor effects was the best fit. Among the seven species with trait effects in the best fit model, five were best fit by a trait hierarchy model incorporating individual traits, one species (*Layia fremontii*) was best fit by a model with distances in individual traits, and one species (*Lasthenia fremontii*) was equally well fit by a trait hierarchy in either individual traits or species average traits. It should be noted that among these seven species, in several cases the differences in AICc between the best and second best model were very small, though the second best model was always another model with a trait term. Across all eight species, the

Table 2. Variance partitioning in trait values among nested levels in the experiment. Depth treatment corresponds to inundation post germination. Note that the variance estimate of the lowest level (denoted with a *) includes residual error. Canopy shape is a whole plant trait and is not defined at the leaf level.

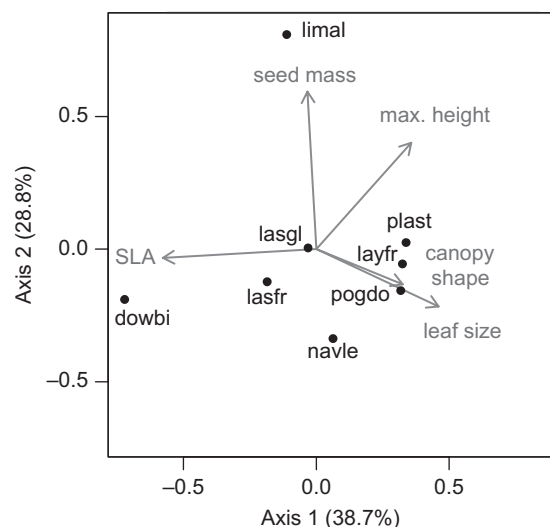| Trait	Depth treatment	Species	Individual	Leaf
SLA	0.007	0.512	0.476	0.005*
Leaf size	0.01	0.476	0.504	0.01*
Canopy shape	0	0.44	0.56*	na

Figure 2. Principle component analysis of species average functional trait values for the eight vernal pool annual plant species in the experiment. Species codes follow Table 1. For PCA of individual plant trait values, see Supplementary material Appendix 4.

Table 3. Summary of model fits for focal plant final biomass for each species. Species codes follow Table 1, with number of focal individuals (N), goodness of fit of the best model (R²), and AICc values for each of six alternative models. Lowest AICc value for each species is given in bold- note that for LASFR two models had equal AICc scores. Models include intercept only, intercept + neighbor biomass, and intercept + biomass + trait difference between the focal and the neighbor. Trait differences were calculated four ways- either using individual trait values for plants or species experiment-wide averages and either using the distance between the focal plant and the neighbor (the absolute value of the difference) or using the difference between the two, preserving the sign of the difference, as consistent with the trait hierarchy hypothesis. The trait used in the models is the axis 1 score of the principle components analysis (either for species or individuals) of the functional traits measured in the experiment.

Species	n	R² best model	AICc					
			Intercept only	Neighbor biomass	Individual trait hierarchy	Individual trait distance	Species ave. trait hierarchy	Species ave. trait distance
DOWBI	62	0.000	**158.4**	160.6	159.9	160.5	161.2	165.6
LASFR	82	0.278	205.8	188.2	**181.2**	185.9	**181.2**	190.0
LASGL	83	0.101	182.4	176.9	**175.7**	176.9	176.7	179.1
LAYFR	76	0.135	207.1	205.3	198.9	**198.2**	206.1	206.0
LIMAL	72	0.063	148.2	149.2	**145.7**	146.7	151.4	148.6
NAVLE	80	0.302	206.0	193.5	**179.5**	191.3	192.1	194.7
PLAST	76	0.185	204.5	194.2	**191.1**	194.2	193.0	193.0
POGDO	82	0.264	207.9	197.58	**184.9**	185.8	193.5	193.1

individual trait hierarchy model had the highest model weight on average (0.48) followed by the individual trait distance model (0.21) and the species average distance model (0.12). The remaining three models had average support value across the species of ≤ 0.07 (Supplementary material Appendix 5). For the six species with the individual trait hierarchy model as the best fit model, the parameter estimates (Supplementary material Appendix 6) indicated that target plants did better when they had higher PC1 axis scores than their neighbor, corresponding to lower SLA, larger leaf size, and canopies with more investment in lateral growth (Fig. 2, Supplementary material Appendix 4).

In assessing inundation tolerance among species in the experiment, most species grew to greater final biomass when grown alone in the shallow treatment with no inundation than in the deep treatment with six weeks of inundation (inundation tolerance index < 1), with *Downingia* being the one species that performed better in the deep treatment (inundation index = 2.14). The inundation index was negatively correlated with species PC1 axis scores (p < 0.028, Fig. 3A), indicating that species with small, high SLA leaves and upright growth forms were more tolerant of inundation than species with contrasting traits, though this correlation was driven largely by the positive response of *Downingia* to inundation. In comparing experimental responses to the field surveys, there was a weak tendency (p < 0.07, Fig. 3B) for species with greater inundation tolerance in the experiment to be found at deeper average depths in vernal pool communities. There was no relationship, however (Fig. 3C) between depth in the pool surveys and the traits measured in the experiment.

Discussion

Trait distance or trait hierarchies?

The primary goal of this study was to experimentally determine whether trait distances ($|T_{focal} - T_{neighbor}|$) or trait hierarchies, measured by trait differences ($T_{focal} - T_{neighbor}$) better predicted the nature of species competitive interactions among vernal pool annual plants. For most species in the

study, the trait hierarchy model was the better fit, indicating that focal species tended to achieve higher final biomass when they had smaller leaves, higher SLA, and more upright growth patterns than their neighbor (Fig. 2, Table 3). This finding is consistent with a number of pairwise plant competition experiments (Gaudet and Keddy 1988, Goldberg and Landa 1991) and more recent studies of forest neighborhood dynamics (Uriarte et al. 2010, Kunstler et al. 2012), but does present a more complex picture of trait dynamics than is typically used in many studies of functional trait and phylogenetic community structure (Kraft et al. 2007). The majority of these studies assume that trait distances between competitors promote coexistence (MacArthur and Levins 1967, Stubbs and Wilson 2004), yet our results suggest that traits instead often capture a competitive hierarchy where coexistence is more likely among species with similar trait values, as this minimizes differences in competitive ability (Mayfield and Levine 2010). This complicates the interpretation of phenotypic and phylogenetic patterns in communities, as competition can drive phenotypic clustering or phenotypic dispersion depending on the context. The majority of species in our study supported the trait hierarchy model, which (all else being equal) should result in phenotypic clustering of competitors, while results from one species (*Layia fremontii*) yielded support for the trait distance model, which should result in phenotypic dispersion. While we did not investigate phenotypic patterns in the field in this study, our results suggest the same traits within a community can drive both niche and fitness differences (sensu Chesson 2000, HilleRisLambers et al. 2012) and therefore produce contrasting phenotypic patterns within the community. Needless to say, our results add to the growing sense of caution that should be taken when interpreting phenotypic (or phylogenetic) patterns in communities in the absence of other information (Mayfield and Levine 2010, Adler et al. 2013).

Incorporating intraspecific variation into trait-based analyses

The second goal of the study was to quantify the magnitude of intraspecific variation in functional traits and to test whether analyses of species interactions that included
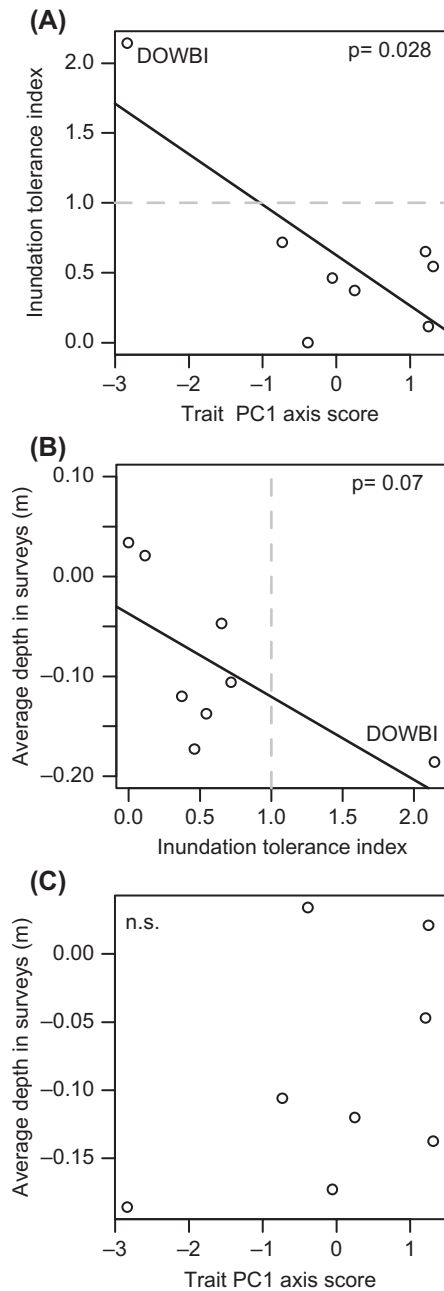
Figure 3. Correlations among experimental results, trait measurements, and plant distributions in field surveys. Inundation tolerance index is calculated as the final biomass ratio of plants grown alone in the deep treatment to plants grown alone in the shallow treatment. Dashed line marks an index value of 1 where there is no difference between treatments. See Fig. 2 for trait PCA. Average depth from field surveys is measured from upper edge of pool. p-values are from linear regression.

individual variation differed from analyses using species trait averages. To this end, we sampled traits on every individual plant surviving to harvest in our experiment, or over 680 individuals. Our results were clear in this respect – the two models of final focal plant biomass including individual trait values had much stronger support (sum of average Akaike weights = 0.69) than the two models using species averages (sum of average Akaike weights = 0.17, Supplementary

material Appendix 5). For some species (e.g. *Layia fremontii*), the species average trait value models actually had lower support than trait-free models (Table 3, Supplementary material Appendix 5), suggesting that in some circumstances, the extra effort of sampling intra-specific trait values may be essential in detecting the effect of traits on species interactions. These experimental findings are consistent with several recent observational studies that report stronger evidence for niche structure when intra-specific trait variation is incorporated (Cornwell and Ackerly 2009, Jung et al. 2010, Paine et al. 2011).

Intraspecific variation can be attributed to both genetic variation and to plastic responses to the environment conditions in which plants are growing. It is well known that there can be considerable genetic variation within plant species that can influence competitive outcomes (Booth and Grime 2003). In our study, seeds were collected from different maternal lines within each vernal plant species, which undoubtedly contributed some genetic variation in traits to the study. Similarly, plastic responses to biotic (neighbor identity) and abiotic (inundation) conditions contributed to within species variation as well. For example, a small (~1%, Table 2) but significant (Supplementary material Appendix 3) amount of variation in the leaf traits was driven by the depth treatments in the experiment. Likewise, the well known ontogenetic differences in leaves in vernal pool species contributed an additional ~1% of total variation to the leaf trait (Table 2). Approximately 50% of the variation in leaf and canopy shape traits was found at the individual level (Table 2), which incorporates both genetic variation and any differences in traits due to neighbor identity. As most studies have tended to ignore intraspecific variation, it is difficult to precisely assess whether the amount of variation observed in this study is characteristic of other plant communities. However, it is likely that this degree of intraspecific variation relative to interspecific variation is on the high end for plant communities, as the diversity (and therefore the total interspecific trait variation) of our experiment is modest. However, disentangling the individual and interactive contributions of genetic and environmental determinants to intraspecific variation within plant species warrants further inquiry within vernal pool plant communities, specifically, and generally within the functional trait-based approach.

Complex responses

In our experiment, we followed a tradition in neighborhood analyses of first accounting for differences in neighbor size before testing for effects of functional traits (Uriarte et al. 2010). In doing so, we found that neighbor biomass and target biomass were often positively correlated (Fig. 1). This could reflect increased allocation to aboveground biomass in response to light competition, facilitation (perhaps via stress amelioration) or shared responses to abiotic variation across the experiment. Our design does not allow us to completely distinguish between these alternatives. However, after accounting for this positive correlation with neighbor biomass, we still found an important role for trait differences in driving competitive responses in seven of the eight species (Table 3).

Landscape context

Finally, we compared our experimental results to survey data from 43 natural vernal pool plant communities at 19 sites across the California Floristic Province. We found that the ability of plant species to withstand prolonged inundation, as species experience at deeper depths in vernal pools, was correlated with the traits measured in the experiment (Fig. 3A), though this was driven primarily by the response of one species (*Downingia bicornuta*). Tolerance of inundation was only weakly correlated to the average depth of species in pools in the field surveys (Fig. 3B), and traits were not correlated with depth (Fig. 3C). The lack of a strong relationship between inundation tolerance and traits to distribution patterns could arise from the broad spatial scale of the survey data, which spanned multiple pools, sites, and climatic regions across the California Floristic Province, or from the fact that our experiment focused on pairwise interactions, whereas plants occur in multispecies mixtures in the field. The existence of a competitive hierarchy, and the suggestion of tradeoff between inundation tolerance and competitive ability (albeit driven by one species in the experiment), is consistent with the results of a transplant experiment (Emery et al. 2009) conducted with vernal pool species, including several species from the study, at Mather Field. In this field experiment, Emery et al. found that fitness increased for most species when they were planted at shallower positions than they naturally occur along the vernal pool inundation gradient as long as neighboring vegetation was removed. In contrast, fitness declined for individuals that were planted below the population boundaries (i.e. at deeper positions than they naturally occur), regardless of neighbor removal treatment. Furthermore, the fitness responses of each species varied between two years that had very different precipitation patterns. The results of this field experiment are consistent with a tradeoff between competitive ability and inundation tolerance driving plant community organization in vernal pools. Here, we have identified functional traits related to competitive outcomes in these species, thus providing insight into the mechanisms contributing to these patterns.

Conclusions

The results presented here illustrate the value of the functional trait approach to the study of communities, but also some of the challenges inherent in the approach. Care needs to be taken in interpreting phenotypic patterns within communities, as our results highlight a relationship between traits and species interactions that is not widely considered in observational studies in this field of research. Similarly, our strongest inferences came from incorporating intraspecific trait variation into our analyses. While the increased burden of sampling every individual, as we did in this study, is likely to be too high in many studies, our results highlight that there is considerable individual variation within the vernal pool plant community and that incorporating this variation increases the power to detect community assembly process.

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