Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales

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ABSTRACT

All available evidence suggests that genetic diversity is important for ecological performance and resilience through the expression and variance of phenotypic traits. Genetic diversity is a multiscalar variable; it includes heterozygosity at the individual scale, genotypic diversity at the population scale, and local adaptation at the regional scale. The three scales of genetic diversity are predominantly studied in isolation to determine conservation status or restoration potential. However, synergisms among the three scales should enhance conservation and restoration assessments. We studied 49 genotypes of *Vallisneria americana*, a common freshwater submersed aquatic macrophyte that has seen catastrophic declines in its estuarine habitats. Two greenhouse experiments examined phenotypic traits and trade-offs and the effects of genetic diversity on the sustainability of *V. americana* populations. Clone size, an exploitative guerilla strategy, and plant height, a conservative phalanx strategy, were negatively associated, as were average turion size with clone size and turion abundance, suggesting that growth strategies trade-off to affect plant fitness. Leaf size and turion size were lower in individuals with lower heterozygosity. Early clonal expansion and flowering frequency were enhanced when genotypic richness was higher. Coefficients of variation revealed that opportunities for selection differed across *V. americana* source beds. We demonstrate that the three scales of genetic diversity work together to determine population performance and evolutionary potential. Thus, integrating the three aspects of genetic diversity is paramount in addressing the impacts of a changing world on the conservation and restoration potential of at-risk populations.

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1. Introduction

Human-induced changes in natural ecosystems have caused unprecedented declines in biodiversity and have reduced the capacity of ecosystems to cope with short- and long-term environmental change (Griffin et al., 2009). Maintaining species diversity for preserving or restoring ecosystem processes is critical (Naeem et al., 2009). All available evidence indicates that it is equally important to preserve genetic diversity within species in terrestrial (e.g., Madritch and Hunter, 2002; Agashe, 2009; Ellers et al., 2011; McArt and Thaler, 2013) and aquatic (e.g., Duffy, 2006; Hughes and Stachowicz, 2009, 2011; Stachowicz et al., 2013) habitats. This topic is becoming increasingly important as populations are subjected to a variety of natural and human-driven environmental stresses that undermine the persistence and evolutionary potential of populations within landscapes. However, genetic diversity is often neglected in biodiversity conservation activities (Laikre et al., 2009).

Three aspects of genetic diversity affect population performance and ecosystem function: 1. Levels of heterozygosity within individuals (Dudash, 1990; Fenster and Dudash, 1994) that can be reduced due to non-random matings and can influence probabilities of survival (Ellstrand and Elam, 1993). 2. Levels of diversity between individuals (e.g., numbers of alleles, genotypes, and phenotypes; Biernacki and Lovett-Doust, 1997; Williams and Orth, 1998) that allow individuals to occupy different niches and promote population diversity (Vellend, 2006). 3. Adaptation of individuals to local environments (e.g., Montalvo and Ellstrand, 2000; Joshi et al., 2001; Hammerli and Reusch, 2002) that affect performance of individuals in local versus foreign environments (Kawecki and Ebert, 2004). The three aspects of genetic diversity act at different spatial scales, from the scale of the individual, to

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the scale of a single population, to the scale of regions that encompass different environments.

Heterozygosity, genotypic diversity, and local adaptation are not mutually exclusive. Rather they may often act together to affect resilience of populations. For example, local adaptation may lead to increased isolation, which can incur an inbreeding cost (Verhoeven et al., 2011) and therefore lower heterozygosity with consequences to individual fitness. Lower individual fitness, in turn, may affect survival and hence genotypic diversity within populations. Therefore, for conservation and restoration of viable populations to be maximally effective, all scales of genetic diversity from individuals to across populations and regions need to be considered (Bischoff et al., 2010) to optimize population growth and survival. Indeed, in a literature survey that focused on 'heterozygosity', 'genotypic diversity', and 'local adaptation' as search terms, we found that 2378 abstracts of papers published in biological journals over the last decade (2003–2013; Proquest Biological Science Journals Database) included at least one of the terms. However, only 15 papers intentionally focused on the intersection of two of the three scales, and none addressed and integrated all three scales to address conservation or restoration issues.

We tested the role of the three scales of genetic diversity in the growth and reproduction of the submersed aquatic macrophyte *Vallisneria americana* (Michx.) (wild celery) in two controlled and well-replicated greenhouse experiments. *V. americana* is a key species in the functioning of freshwater systems for its ability to immobilize nutrients, accumulate metals, and trap sediments (Biernacki and Lovett-Doust, 1997; Benson et al., 2008) and for its provision of important food resources to aquatic fauna (Sponberg and Lodge, 2005). This dioecious plant is capable of both clonal growth and sexual reproduction (Titus and Stephens, 1983; Titus and Hoover, 1991; Korschgen et al., 1997; Lokker et al., 1997) and is used extensively in the restoration of aquatic systems (Korschgen and Green, 1988). However, little is known about the effects of genetic diversity on the restoration of *V. americana* populations or the conservation of the species (Lloyd et al., 2012; Marsden et al., 2013) despite observed wide variation in genetic diversity at local and regional scales within an estuary (Lloyd et al., 2011).

To examine the effects of genetic diversity on *V. americana* population performance and long-term sustainability, we first tested the hypothesis that genotypes invest in different functional strategies (Lovett-Doust, 1981; Sackville Hamilton et al., 1987) with trade-offs between traits that represent plant stature (leaf length and size), biomass accumulation (average turion size, total turion weight), clonal expansion (ramet production, rhizome length, turion abundance), and sexual reproduction (flowering frequency, length of flowering). Reliable estimation of phenotypic trait differences among genotypes is a necessary pre-requisite to test for the effects of heterozygosity, genotypic diversity and local adaptation on population performance. We then tested the hypothesis that heterozygosity affects an individual's response to high and low quality environments. We predicted that individuals with higher levels of heterozygosity would have higher rates of survival and growth than highly homozygous individuals (Chaves et al., 2011), especially in low quality sediments. At the population scale, we tested the hypothesis that genotypic and functional diversity of populations increases population growth and productivity. Higher productivity can result from two sources: chance presence of highly productive genotypes in more diverse populations or from complementarity in resource use by different genotypes (Hughes et al., 2008). At the regional scale, we tested the hypothesis that genotypes are locally adapted to their native environment. We first predicted that resident genotypes and populations would be more productive in their local habitat than foreign genotypes and populations (Kawecki and Ebert, 2004). We further predicted that

opportunities for selection, or phenotypic variation across all genotypes (Crow, 1958), would be lower in benign habitats where phenotypes respond similarly to the environment, forming observable adaptive peaks (Wright, 1931). In contrast, in more spatially or temporally variable environments we expected opportunities for selection to be greater owing to higher variation in the response of phenotypes to variable environmental conditions. Through simultaneously studying the three scales of genetic diversity, we seek to highlight the importance of the three scales in conservation and restoration and increase knowledge for the conservation and restoration of a species that is critically important in estuarine functioning but has seen widespread population declines throughout its range (Kimber et al., 1995a,b; Korschgen et al., 1997; Lloyd et al., 2011).

2. Materials and methods

2.1. Study area and development of plant repository

In June 2007, we harvested up to 30 *V. americana* shoots with attached roots ("samples") from each of 14 natural source beds located within the Chesapeake Bay (Lloyd et al., 2011). When the extent of the population was limited, we sampled fewer than 30 shoots for a total of 403 shoots harvested. Because *V. americana* populations may be composed of one clone or many different genotypes (Lloyd et al., 2011), we sampled every 5–10 meters to determine the extent of clones and to maximize the sampling of different genotypes (Lloyd et al., 2011). Sampled populations varied in extent and cover and differed in environmental conditions (Table S1). We placed each *V. americana* sample into individual 2.4 l containers (diameter: 16 cm; height: 15.5 cm) filled 10 cm deep with steam-sterilized (Slack Associates, Inc. Model 1964) Chesapeake Bay sediments. Steam sterilization killed any propagules (seeds or turions) that could have contaminated the propagation containers if left in the sediments. Microorganisms were reintroduced by planting the *V. americana* field samples. The plants were grown for two years in the Appalachian Laboratory greenhouse to minimize environmental carryover from the field (Hughes and Stachowicz, 2009). As genotypes matured, we recorded the sex of each genotype to estimate the sex ratio of each source bed (Table S1). After senescence, we harvested turions and stored them in wet sterilized sand at 4 °C until planting the following spring.

Using 11 DNA microsatellite loci designed specifically for this species (Burnett et al., 2009), we identified genets from leaf cuttings and assigned *V. americana* samples to genotypes (Lloyd et al., 2011). In spring 2009, the *V. americana* repository of known genotypes consisted of 161 genets (out of 403 shoots harvested in the field) and 8000 turions.

2.2. Monoculture experiment

We planted 49 cloned genotypes of *V. americana* in 516 greenhouse mesocosms (4.7-l; diameter: 20.7 cm, height: 16.5 cm) in March 2009 to quantify traits, determine trade-offs among growth and reproductive strategies, and test for the effects of heterozygosity on individual performance. Genotypes originating from the HWC (17 genotypes), SWP (20 genotypes), and 12 additional source beds (one genotype each) were planted in monoculture in a replicated ($n = 12$ for each SWP and HWC genotype and $n = 6$ for all other genotypes), competition-free, and controlled greenhouse environment. We focused on HWC and SWP genotypes because the HWC and SWP source beds were diverse and yielded multiple genotypes for use in experiments. The source beds were associated with different environmental conditions