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Hidden biodiversity: Spatial mosaics of eelgrass genotypic diversity at the centimeter to meadow scale

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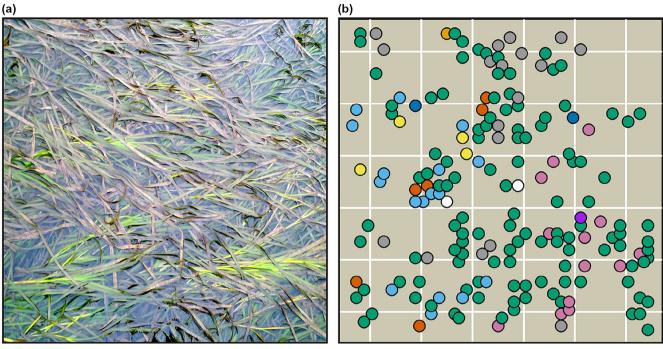
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At first glance, images of homogenous-looking green seagrass meadows (Figure 1a) do not epitomize "biodiversity" in the way that species-rich mosaics of coral reefs or rainforests might. However, intraspecific diversity at the genetic and functional level has been well documented in seagrasses, especially in the eelgrass species Zostera marina (e.g., Hughes et al., 2009). The ecological role of this genetic diversity in eelgrass systems has been highlighted in numerous studies, both in terms of driving local adaptation (e.g., DuBois et al., 2022) and in the importance of genetic diversity for ecosystem functioning (Abbott et al., 2017; DuBois et al., 2021; Hughes & Stachowicz, 2009). Eelgrass is a marine angiosperm that can spread vegetatively as well as reproduce sexually, resulting in individual genets that can range in size from just a single shoot to hundreds of square meters (Reusch et al., 1999). Previous studies have examined the distribution of this genetic variation among versus within populations (Kamel et al., 2012; Olsen et al., 2004; Reynolds et al., 2017), but a picture of eelgrass genetic diversity at the scale at which individual plants interact is missing, largely because of the labor-intensive nature of genotyping the hundreds of shoots that can co-occur in a single square meter.

Through fine-scale, exhaustive genotyping of individual ramets, we discovered unexpected spatial mosaics of diversity that are invisible to the naked eye. First, we genotyped every shoot (n=183) in a single $50~\rm cm \times 50~\rm cm$ area, which revealed 10 intermingling genotypes (Figure 1b; please refer to Kollars & Stachowicz, 2022 for genotyping methods). Although the magnitude of eelgrass genotypic richness itself was not surprising (Hughes & Stachowicz, 2009; Kamel et al., 2012; Reynolds et al., 2017), coordinate-based mapping showed that these genotypes are naturally intertwined at the scale of only a few centimeters. This interdigitating nature of individuals implies complex ecological interactions among genotypes, raising the question of whether these interactions promote or inhibit the maintenance of genotypic diversity within eelgrass populations.

Next, we expanded our sampling to thirty-six $50 \times 50 \text{ cm}^2$ plots within the larger meadow (Figure 2), arrayed in nine blocks and spread along a ~50 m transect parallel to the shore (tidal elevation of approximately -0.1 m mean lower low water). We collected leaf tissue

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Sampling Area: 50 cm x 50 cm

FIGURE 1 Genetic surveys can provide insights into natural history by revealing diversity invisible to the naked eye. (a) An eelgrass meadow in Bodega Harbor, CA seemingly lacks diversity given that only one macrophyte species dominates the seascape, the marine angiosperm *Zostera marina*. (b) Genotyping every individual shoot within a patch (size: $50 \text{ cm} \times 50 \text{ cm}$, n = 183 shoots) revealed the presence of 10 intermingling genotypes. Each circle represents an individual shoot and unique colors represent unique genotypes. The white gridded lines underlying the map show the placement of the sampling grid used to generate the map in Figure 2.

samples by overlaying a grid with 36 evenly spaced points over each plot and removing the inner leaf of the shoot closest to each point (see grid system illustration in Figure 1b). We did not collect a sample at a grid position if there was not a shoot present within ~4 cm of the point (see Kollars & Stachowicz, 2022 for full sampling and genotyping methods). We genotyped a total of 1252 samples that comprised 98 unique genotypes (Figure 2).

This extensive and spatially informed sampling uncovered high variation in local diversity across the meadow. The number of genotypes within a plot ranged from 1 to 10 with a median of 3-4 (or 3.5). In 10 of our 36 plots, one genotype comprised >90% of the sampled individuals, although only three of these plots were full monocultures. We also documented multiple cases in which a single genotype extended across meters of space, the most notable being the "pink"-shaded genotype that occupied an area of nearly 21 m² (right-hand side of the map for the right part of the transect in Figure 2). These richness levels are higher, and the fraction of monocultures lower, compared with previous studies despite sampling only a quarter of the area (Hughes & Stachowicz, 2009). Furthermore, 30% of our plots had more than five genotypes, including one plot that had up to 10 genotypes interdigitated similarly to Figure 1b.

From a distance, the map in Figure 2 shows considerable heterogeneity in local diversity across an area that does not contain any obvious gradient in environmental conditions.

Although our map is still limited by a restricted sampling effort, it provides a snapshot of the distribution and identity of eelgrass genotypes across a meadow. We monitored these plots over 2 years under four clipping treatments and found only modest changes in the diversity and composition of individual plots (Kollars & Stachowicz, 2022). Surveys across a range of northern California populations also showed little change in patterns of genetic structure over a decade (Reynolds et al., 2017). Therefore, we suspect that our snapshot represents what is typical in this bed: some plots are near genetic monocultures, others are highly diverse with interdigitated genotypes, and the reasons for these small-scale spatial mosaics in diversity both within and among patches is entirely unclear. However, variation in the extent of genotypic intermingling does suggest both phalanx and guerrilla growth strategies (Barrett, 2015; Lovett-Doust, 1981) in eelgrass and questions whether spatial mosaics are driven by environmental patchiness (Hutchings, 1988), genetic differentiation, or a combination of the two mechanisms (Ye et al., 2006).

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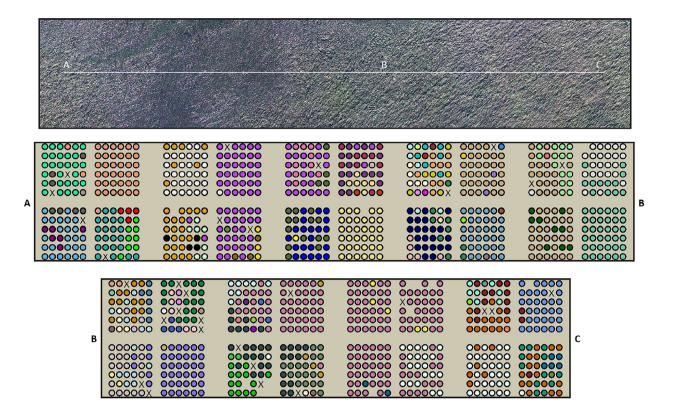


FIGURE 2 More extensive sampling across the meadow showed high variability in the genotypic assembly of neighboring patches. Top image: aerial view of seagrass bed in Bodega Bay, CA from an altitude of 61 m; this section of the meadow is 55 m \times 10 m. The white line overlaying this image shows the sampling transect (\sim 50 m). Letters A to C orient the reader to the placement of the maps of the underlying genetic structure of the meadow along the transect moving from A to B (top map; left hand side of transect) and from B to C (bottom map; right hand side of transect). Scale varies within the genetic map: each plot is 0.5 m \times 0.5 m, the distance between plots within a block is 1 m, and the distance between blocks is 3 m. Samples in each plot were collected along a grid system of 36 points, illustrated by the white lines underlying the map in Figure 1b. Each circle represents an individual shoot and unique colors represent unique genotypes. The absence of a circle in a grid position indicates that there was not a shoot present within \sim 4 cm of the point. An "X" in a grid position indicates that a sample was collected but failed polymerase chain reaction amplification.

This contribution to the natural history of eelgrass raises questions about the maintenance of diversity within partially clonal species in the same way that decades of research has rigorously assessed the mechanisms underlying spatial variation in species diversity. In other words, it opens the door to the study of the community ecology of genotypes (Vellend, 2010; Vellend & Geber, 2005). Eelgrass genotypes are tightly intermingled at very small spatial scales (Figures 1b and 2), which provides ample opportunity for competition and exclusion, but how important are these interactions relative to environmental filtering or stochasticity? Relatedly, are these differences largely the result of priority effects (i.e., the arrival order of colonists determines the composition) and neutral processes, or does environmental heterogeneity result in conditions that favor dominance across a landscape? To what extent are traits of individual taxa (genotypes) driving ecosystem function versus richness and composition per se? Although we cannot yet answer these questions, our previous work allows us to speculate

on the mechanisms that drive fine-scale variation in genotypic assembly across the meadow.

We start by drawing parallels between the drivers of community assembly and those of genotypic assembly within the context of evolution (Vellend, 2010; Vellend & Geber, 2005). Variation in genotypic assembly across a seascape is presumably driven by heterogeneity in the mechanisms underlying that assembly. If we consider an isolated patch of the meadow, the identity and distribution of genotypes within that patch will be determined by patch age as well as which genotypes arrive there (colonization via seeds or vegetative propagation), which ones survive there (random chance or selection/ environmental filtering for tolerant genotypes), and which ones thrive there (intraspecific overlap versus niche differentiation). Spatial variation in these processes could arise from multiple mechanisms including stochastic and deterministic environmental heterogeneity as well as differential disturbance regimes that affect patch age.

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Eelgrass genotypes show considerable genetically based phenotypic variation in traits that influence the outcome of intergenotypic interactions (Abbott & Stachowicz, 2016). However, the extent to which these interactions result in competitive exclusion versus coexistence varies with environmental conditions (Abbott & Stachowicz, 2016; Kollars et al., 2021). For example, in a small-scale field experiment we found that pairs of genotypes with high trait similarity were more likely to coexist (Abbott & Stachowicz, 2016), presumably because certain traits conveyed an advantage over others in particular environments. Under this scenario, the observed variation in patch diversity across the meadow in Figure 2 might be a stochastic function of the identity of the genotypes that initially establish in a particular area. However, in a subsequent experiment, we showed that different genotypes were favored under different environmental conditions (Kollars et al., 2021). This suggests that spatial heterogeneity, or cryptic environmental gradients, could produce monocultures of different genotypes. When we created a temporally variable environment that mimicked field seasonality, coexistence was a more likely outcome (Kollars et al., 2021), and multi-year field experiments showed that high trait diversity increases total eelbiomass due to complementarity (Abbott et al., 2017; Hughes & Stachowicz, 2011). Therefore, it seems plausible that niche differentiation overlain onto a landscape of spatial and temporal environmental heterogeneity contributes to the widespread spatial variation in genotypic diversity we observed (Figure 2), analogous to the mechanisms underlying spatial variation in species diversity patterns. However, this remains largely untested in wild populations.

Our maps revealing the spatial mosaics of genotypic diversity within and among patches in a California eelgrass meadow contribute to a growing effort to map the genotypic variation of partially clonal plants across systems. Variation in genotypic diversity within and among plant populations has long puzzled ecologists (reviewed by Ellstrand & Roose, 1987). With modern advances in genetic tools, we now have fine-scale maps of genotypic diversity across multiple systems including (but not limited to) salt marshes (Richards et al., 2004), fennel pondweed (Hidding et al., 2014), prairie grass (Avolio et al., 2011), and tropical seagrasses (Larkin et al., 2020). With each newly produced map, ecologists can consider how the outcome of recruitment dynamics, intergenotypic interactions, environmental stochasticity, and selection drive genotypic diversity and support the clear parallels in ecological and evolutionary processes described by Vellend (2010). Qualitative synthesis of these studies suggests that, just as in species-rich communities, multiple mechanisms drive genotypic variation

that differ across systems and link to the species' natural history. Tracking spatial genotypic structure (i.e., diversity and composition) over time and/or in response to manipulations will facilitate testing hypotheses for the role of stochasticity, selection, dispersal, and competition in maintaining local genetic diversity.

We conclude by suggesting that genotypic identity, diversity and distribution in the field can be as important to natural history as species properties (Hughes et al., 2008). Given the connection between genetic diversity and ecological performance, fine-scale genotypic variation could be a contributing factor to unexplained variation in field studies in the same way that taxonomic distinction applied at too coarse a scale can obscure important ecological processes and limit our understanding of ecological systems. However, to make incorporation of genetic understanding of natural history into ecological studies feasible, we need to continue to advance techniques to enable the efficient identification of genotypes in systems without observable phenotypic markers.

AUTHOR CONTRIBUTIONS

Nicole M. Kollars, Jessica M. Abbott, and John J. Stachowicz conceived the study. Nicole M. Kollars and Jessica M. Abbott led genotyping efforts. Nicole M. Kollars composed the manuscript with significant input from John J. Stachowicz.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (Kollars, 2022) are available in Zenodo at https://doi.org/10.5281/zenodo.6011003.

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