The angle of repose: Facilitation and its effects on the Brachypodium distachyon grass complex
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Chapter 5

Synthesis

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Patchy landscapes in the Mediterranean basin can harbor microhabitats that are significantly different than the prevailing local climatic conditions. In this thesis, I found that neighboring perennial plants positively affected several phenotypic traits in spatially associated \textit{B.\textit{hybridum}} in natural populations (Chapter 2). I then explored whether there was a genetic signal that could explain these differences, but we did not find evidence of population level genetic sub-structuring driven by neighboring perennial plants, using microsatellites (Chapter 3). Lastly, in controlled conditions, I found that the increased seed production from \textit{B.\textit{hybridum}} that had originated underneath perennial plant cover was still retained into the $F_2$ generation from our observational study. However, most of the traits did not show differences regardless of origin of location (Chapter 4). Thus, I provide some evidence, that neighboring perennial plants may play a role in ecotypic variation occurring within populations.

In the observational study (Chapter 2), although I did not find a strong negative association of \textit{Brachypodium} to neighboring perennial plants as aridity increases, there were positive effects on traits from \textit{Brachypodium} that had originated underneath canopy cover in contrast to open patches indicating that in this study, microscale effects were stronger than macroscale effects (aridity gradient). Environmental gradients are commonly used in facilitation studies as a proxy for space and time, but the scale studied can lead to ambiguous results, i.e. landscape (Manzaneda et al., 2012; Metz and Tielbörger, 2016) vs regional, one species (Penner et al., 2019) vs a plant community (Metz and Tielbörger, 2016; Miranda et al., 2011), and species of perennial plant investigated (Maestre et al., 2003; Michalet et al., 2015). Responses to neighboring perennial plants would also be dependent upon the distance from the species’ ecological optimum that it occupies, e.g. drought tolerance within the landscape. \textit{Brachypodium} is native to the region (Schippmann, 1991) and is expected to be adapted to the highly variable conditions found in this semi-arid landscape. Thus, \textit{Brachypodium} may prefer the relatively higher temperatures and higher solar radiation conditions found outside perennial plant cover as aridity increases. At the microscale, when combining all ten sites, it was found that several traits (number of seeds, number of spikes and stem height) were all higher when found underneath perennial plant cover. Higher seed output under plant cover is supported in the literature (García-Cervigón et al., 2016; Tirado and Pugnaire, 2003) and increased stem height can infer higher levels of biomass and the presence of competition for light. I also found that the most arid site flowered earlier than the least arid site which is also supported in the literature (Penner et al., 2019). In addition, when examining within the two most extreme populations (least vs most arid site), I found that only in the least arid site, the plants that had originated outside canopy cover flowered more quickly. However, a difference in flowering time of only a few days does not preclude evidence that different ecotypes are present underneath vs outside canopy cover within \textit{B.\textit{hybridum}} at this site. Nevertheless, differences in fitness related traits of field collected \textit{B.\textit{hybridum}} specimens indicates that neighboring perennial plants do play a role, by either directly or indirectly affecting the phenotypic traits of spatially associated \textit{B.\textit{hybridum}} at the microscale.
Population structure, which can be reflected by differences in allele frequencies, can indicate whether or not selection is present and the extent of genetic drift within and between populations (Balloux and Lugon-Moulin, 2002). In Chapter 3, I used microsatellites to investigate more thoroughly the most arid population, where significant trait differences were originally found in the natural population (Chapter 2), and because *Brachypodium* is known to consist of both and spring and winter ecotypes, I explored whether there was any evidence that different ecotypes could be present between seasons. I did not find any evidence that neighboring perennial plants influenced the genetic substructure over 1.5 years at this site. However, although the population exhibits excessive homozygosity, and is relatively stable, I did find higher levels of heterozygosity outside perennial plants in contrast to underneath canopy cover in the last sampling period. This last sampling period would have been the progeny from the plants that had experienced an exceptionally dry fall and spring (when most precipitation occurs in this region) and could represent a small scale selection event for seeds or seedlings with higher levels of heterozygosity outside the canopy cover. Another possibility is that a relatively large immigration event had introduced new rare alleles increasing heterozygosity into the population as well. Although the vectors for seed dispersal are currently unknown, long distance immigration events are evident in the genetic structure in *Brachypodium* populations (Neji et al., 2015a; Shiposha et al., 2016; Vogel et al., 2009) and therefore also possible to be the case here as well.

Because I found that neighboring perennial plants do affect several phenotypic traits of *B. hybridum*, and because the microhabitat occurring underneath perennial plant canopy cover can result in higher levels of soil moisture (Chapter 2), I proceeded to test whether the trait differences found in the natural population, were still present along a water restriction gradient based upon origin of location. I found that *B. hybridum* plants that had originated underneath canopy cover continued to have had higher seed output and heavier seeds which were retained in the $F_2$ generation (Chapter 4). The higher seed output was also found in the observational study (Chapter 2), but the taller stems and increased number of spikes were not found in the $F_2$ generation. A consistent trend was also found in which most traits increased with an increase in soil water capacity including number of seeds, stem height, biomass, number of spikes, iWUE, with the exception of SLA but not differentiation between origin of location. I thus concluded that neighboring perennial plants can drive trait differentiation of spatially associated *B. hybridum* and differences in fitness related traits can be retained between generations.

**Epigenetics and adaptive phenotypic plasticity**

There are several mechanisms in which trait retention can occur between generations. Epigenetic modifications can alter levels of gene expression and proteins (e.g. DNA methylation) and are reversible (Van Der Graaf 2015). Variation in the effects of epigenetics may arise randomly, can promote the persistence of species to environmental stressors (Duncan
et al 2014) and are likely important in plant populations which experience high degrees of inbreeding and have low genetic diversity (Leung et al 2016). Further, epigenetics are known to play an important role in heritable trait differences in plant height and growth rate in *Arabidopsis* (Groszmann et al., 2011) and have also been found to affect seed development and germination in *Brachypodium* (Wolny et al., 2014). Similarly, adaptive phenotypic plasticity, in which having higher levels of phenotypic variability can increase fitness as the environment fluctuates is similar (Sultan, 1995). Either, or, a combination of both, are likely playing a role with the increased level of fitness of *B. hybridum* that was found underneath neighboring perennial plants both in the field and in controlled conditions in this thesis. The question then becomes, will these differences be retained for longer than two generations? Let's explore the scenario, from which this thesis provides evidence for, as to whether these differences are likely to last in the longer term. In this scenario, small populations of *B. hybridum*, located underneath the protective canopy of a perennial plant which attenuates light intensity and temperature, also experiences an influx of usually limited resources (e.g. water availability, nutrients). This results in an increase in competition stimulating phenotypic changes such as an increase in stem height and heavier seeds. Paired with predominantly local seed dispersal, a positive feedback loop ensues in which retaining higher seed output and heavier seeds between generations increases the survival of *B. hybridum* in this microhabitat. However, in the longer term, long distance dispersal rarely occurs, and the area underneath shrubs is generally significantly smaller than the prevailing landscape. In temporally variable habitats, perennial plants will be able to buffer harsh conditions in some years, but not in others. Therefore, it is possible that the increased fitness that we found in *B. hybridum* underneath canopy cover, is likely to exist only in the short term. However, it is very interesting that we encountered a signal as annual grasses tend to have high levels of phenotypic plasticity (Chapter 2), with even higher levels reported for polyploids (Doyle et al., 2008) like that of *B. hybridum*. Therefore, it is possible that the differences in traits that were retained into the F₂ generation in *B. hybridum* may actually be amplified in a species with lower phenotypic plasticity such as the diploid *B. distachyon*. Future research will be needed to ascertain the precise mechanism such as local adaptation, epigenetics, adaptive phenotypic plasticity or a combination, that play a role in the increase in fitness between generations of *B. hybridum* with an origin of location underneath perennial plant cover as found in this study. A more thorough understanding of the interaction between local microhabitat and plant responses is key to elucidating and more accurately predicting a population's capacity to cope with an increase in environmental stressors.

Model plants such as *Brachypodium* are important resources for investigating the effects of plant development, ecology, evolutionary biology and cell biology for temperate cereals. The extensive genomic resources that have been developed in the past decade on *Brachypodium* have resulted in advances in the understanding of the effects of stress tolerance, fungal pathogens and root microbiota among others on cereal production systems. In addition,
novel current research is investigating the evolutionary implications of facilitation and agricultural sustainability. Here, we provide evidence that neighboring perennial plants facilitate several fitness traits of *Brachypodium* in a semi-arid landscape. With the prevalence of wild populations of *Brachypodium* found throughout the Mediterranean Basin, and the use of this species as a model for temperate cereals, future research should focus on not only a deeper understanding of the effects of facilitation on plant communities but to bridge these findings into more sustainable crop production systems and increased food security worldwide.

**Future Research**

In this thesis, I provide evidence that neighboring perennial plants do affect traits of spatially associated *B. hybridum* and that the fitness trait of increased seed output that were present in wild populations, were retained into the F<sub>2</sub> generation in controlled conditions. Future research would benefit from continuing to examine the possible mechanisms that could be fueling these differences at the microscale. This includes the collection of spatial data within wild populations, paired with an SNP marker set, would allow a Genome Wide Association Study (GWAS) which would allow further insight for important traits. In addition, measuring phenotypic plasticity such as exploring the effects of G x E (genotype-environment interaction) or assigning levels of heritability for traits, would lead to further information in regards to the likelihood of ecotypic variation occurring at the microscale.

Demographic studies encompassing the entire lifespan of *Brachypodium* in native populations would lead to a deeper understanding of how annual grasses and the plant community may respond to future environmental stressors. However, studies conducted on demography are greatly hindered by the difficulty in differentiating between *B. hybridum* and *B. distachyon* in the field, which do co-occur in this region frequently. Further investigation into variables such as seed dispersal distance, the vectors involved in seed dispersal (e.g. mammals, birds) and phenology would be very important in determining the likelihood that local adaptation could indeed occur within *Brachypodium* populations. These variables would be extremely important parameters required as inputs into a model investigating whether or not two ecotypic morphs - one with drought tolerance and one with superior competitive abilities - could not only be driven by neighboring perennial plants but could also coexist in one population. Additional variables such as the timing of germination, ontogeny and relative root investment, could give us further insight into the likelihood that different ecotypes (e.g. winter and spring annuals) could be present within one population. This would lead to a deeper understanding of the role that neighboring perennial plants play on spatially associated annual species in patchy, semi-arid landscapes.