SYMPOSIUM

Adaptations between Ecotypes and along Environmental Gradients in *Panicum virgatum**

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ABSTRACT: Determining the patterns and mechanisms of natural selection in the wild is of fundamental importance to understanding the differentiation of populations and the evolution of new species. However, it is often unknown the extent to which adaptive genetic variation is distributed among ecotypes between distinct habitats versus along large-scale geographic environmental gradients, such as those that track latitude. Classic studies of selection in the wild in switchgrass, *Panicum virgatum*, tested for adaptation at both of these levels of natural variation. Here we review what these field experiments and modern agronomic field trials have taught us about natural variation and selection at both the ecotype and environmental gradient levels in *P. virgatum*. With recent genome sequencing efforts in *P. virgatum*, it is poised to become an excellent system for understanding the adaptation of grassland species across the eastern half of North America. The identification of genetic loci involved in different types of adaptations will help to understand the evolutionary mechanisms of diversification within *P. virgatum* and provide useful information for the breeding of high-yielding cultivars for different ecoregions.

Keywords: ecotype, cline, local adaptation, reciprocal transplant, switchgrass, population structure.

Introduction

Determining how adaptive genetic variation is distributed temporally and spatially is essential to understanding the evolution of species. Studies of adaptation in the wild typically focus on the change in organismal phenotypes over time in response to natural selection (Grant and Grant 2003; Labbé et al. 2007) or the adaptive divergence between populations and ecotypes from different habitats (Clausen et al. 1958; Hereford 2009; Linnen and Hoekstra 2009; Agren and

Schemke 2012; Nosil 2012). Reciprocal transplant experiments are often used to test the hypothesis of adaptation across geographic space (Linhart and Grant 1996; Leimu and Fischer 2008; Hereford 2009), where the higher fitness of local populations versus immigrant transplants is recognized as strong evidence of local adaptation. The results of dozens of reciprocal transplant experiments suggest that local adaptation is common (Linhart and Grant 1996; Leimu and Fischer 2008; Hereford 2009; Lowry 2012*b*).

Although there is now strong evidence for local adaptation from transplant experiments, how adaptive variation is distributed over space has been a source of controversy for nearly a century (Turesson 1922; Clausen 1951; Kruckeberg 1951; Langlet 1971; Antonovics 1976; Quinn 1978; Stebbins 1980; Grant 1981; Levin 1993). The main source of contention is with regard to whether discernible widespread ecotypes exist within species (reviewed in Lowry 2012*a*). Use of the term "ecotype" is inconsistent in modern literature, but for the purpose of this article we define it as groups of populations with a common set of adaptations to environmental differences between habitats. Many early plant biosystematists, including Turesson, who coined the term ecotype in 1922, found strong evidence for the existence of ecotypes and thought of them as intermediate stages in the formation of new species (Clausen 1951). In opposition to this view were botanists who argued that most important adaptive genetic variation was either restricted to pairwise divergences between populations (Antonovics 1976) or distributed continuously along large-scale environmental gradients (Langlet 1971; Stebbins 1980).

The classic works of Clausen et al. (1940, 1948) and Clausen and Hiesey (1958) are among the best studies demonstrating how adaptive variation is partitioned both among ecotypes and along environmental gradients. Those studies involved many populations collected along an elevation transect of California that were transplanted to multiple field sites along that transect. In addition, there

is a long history of common garden experiments with tree species (i.e., provenance trials) demonstrating that phenotypic variation is highly associated with large-scale environmental gradients (Langlet et al. 1971). These classic experiments are now being used for association mapping and offer the possibility of new insight into the genetics of adaptation along gradients (Sork et al. 2013).

A system that offers great potential for understanding how adaptive genetic variation is partitioned both between ecotypes and along environmental gradients is switchgrass, *Panicum virgatum*. A large perennial allopolyploid C$_4$ grass species (Triplett et al. 2012), *P. virgatum* is a dominant species of the tallgrass prairie, woodland, and riparian areas over a large portion of the eastern half of North America, from central Mexico to southern Canada (McMillan 1964; Zhang et al. 2011*a*, 2011*b*; Triplett et al. 2012). It has typically been thought of as being composed of two major ecotypes, upland and lowland (fig. 1; Porter 1966; Cortese et al. 2010; Morris et al. 2011; Zhang et al. 2011*a*, 2011*b*; Lu et al. 2013).

Since the mid-twentieth century, *P. virgatum* has received considerable research attention due to its prominent distribution in many ecosystems, its utility as a forage crop, and its established role as a bioenergy feedstock (McMillan 1965*a*; Porter 1966; Sanderson et al. 1996; Schmer et al. 2008; Varvel et al. 2008; Tobias 2009; Parrish et al. 2011; Youngs and Somerville 2012). As a consequence of broad interest in this species, numerous agronomic field trials and reciprocal transplant experiments have been conducted. These studies have provided insight into divergent adaptations between ecotypes and along environmental gradients across the eastern half of North America (McMillan 1959, 1964, 1965*a*, 1965*b*, 1967, 1969*a*, 1969*b*; Porter 1966; Sanderson et al. 1999*a*, 1999*b*; Casler and Boe 2003; Casler et al. 2004, 2007; Berdahl et al. 2005; Schmer et al. 2008; Wullschleger et al. 2010; Behrman et al. 2013). Much of this research was published decades ago or more recently in crop science and agronomy journals. As a result, the ecology and evolutionary biology community has overlooked much of this important research on adaptive genetic variation in *P. virgatum*.

The primary goal of this article is to bring to light and synthesize the extensive field-based research conducted on *P. virgatum* to better understand how adaptive phenotypic variation is partitioned between ecotypes and along large-scale environmental gradients. To accomplish this, we have divided this work into three major sections. In the first section, we describe the population structure of *P. virgatum* and how phenotypic variation is distributed across the range of the species. In the second section, we review how field experiments conducted with *P. virgatum* contribute to our understanding of adaptation between ecotypes and along environmental gradients. Finally, we suggest a set of

future studies to understand the genetic mechanisms of adaptation in *P. virgatum*.

Population Structure and Phenotypic Variation

There are two major ways that phenotypic variation is partitioned within *Panicum virgatum* (table 1). Upland and lowland ecotypes are phenotypically distinct, even in regions where they occur in close proximity (Porter 1966). Overlaid on top of this ecotypic differentiation is phenotypic variation that is highly correlated with large-scale environmental gradients, especially those that are correlated with latitude. A third major component of variation within *P. virgatum* is ploidy. Classic studies reported a large polyploidy series in *P. virgatum*, with a range of $2n = 18, 36, 54, 72, 90, 108$ (Church 1940; Burton 1942; Nielsen 1944; McMillan and Weiler 1959). However, recent studies have found that the vast majority of *P. virgatum* individuals occur as either tetraploids ($2n = 36$) or octoploids ($2n = 72$; Brunken and Estes 1975; Hopkins et al. 1996; Narasimhamoorthy et al. 2008; Costich et al. 2010; Triplett et al. 2012).

Divergence between Upland and Lowland Ecotypes

The most recognizable division of population structure within *P. virgatum* is the divergence of upland and lowland ecotypes (table 1). Numerous studies have documented the striking phenotypic and physiological differences between these two ecotypes (Porter 1966; Gunter et al. 1996; Sanderson et al. 1996; Wullschleger et al. 1996; Stroup et al. 2003; Casler et al. 2004; Cassida et al. 2005*a*, 2005*b*; Barney et al. 2009; El-Nashaar et al. 2009; Yang et al. 2009; Cortese et al. 2010). Upland plants typically have greener leaves, thinner shoots, and more shoots per plant than the lowland ecotype (Porter 1966; Cortese et al. 2010). The upland ecotype occurs commonly both as tetraploids and octoploids in nature and is primarily found in areas of lower soil moisture than the lowland ecotype in regions where the ecotypes co-occur (Bragg 1964; Porter 1966; Brunken and Estes 1975; Costich et al. 2010). The upland ecotype has long been known to be less resistant to infection by rust (*Uromyces graminicola*) than the lowland ecotype (Cornelius and Johnston 1941). It has often been assumed that the upland ecotype occurs only in northern zones 2–7 of the USDA Plant Hardiness Zone Map (www.planthardiness.ars.usda.gov/PHZMWeb/). Lowland plants typically have bluish waxy leaves, fewer shoots per plant, thicker leaves, and thicker shoots and are taller than upland plants (Cortese et al. 2010; Zhang et al. 2011*a*, 2011*b*). Lowland plants are typically found in wetter riparian areas and are restricted to southern cold hardiness zones 6–10. Until recently, it was thought that the lowland ecotype was uniformly tetraploid (Costich et al. 2010; Trip-
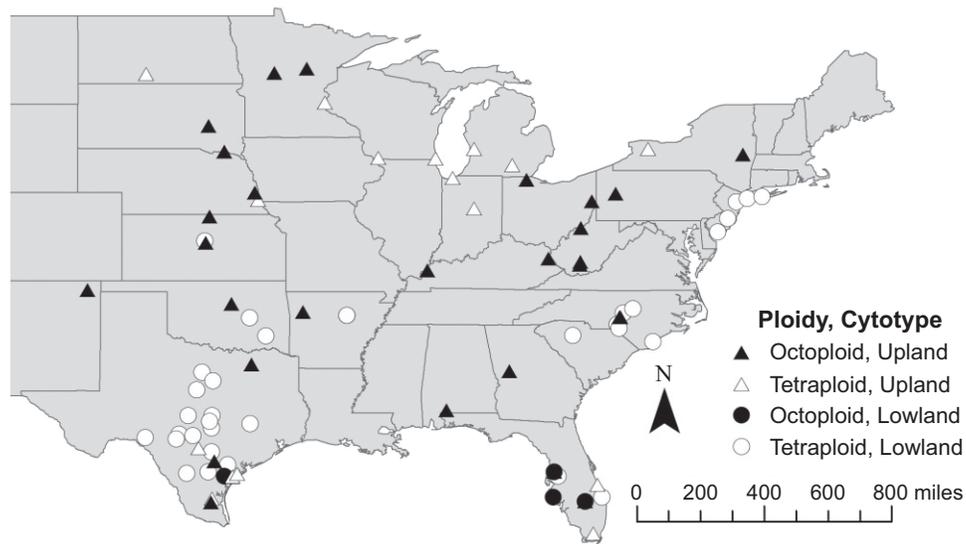
Figure 1: Map of *Panicum virgatum* individuals in which both ploidy and cytotype has been established. Ploidy was determined by flow cytometry. Cytotype was determined by genotyping of *trnL* (UAA) intron chloroplast marker. For details, see table A1, which is deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.rm718 (Lowry et al. 2014).

lett et al. 2012). The two ecotypes are interfertile when they are crossed at the same ploidy level. Failure of crosses between tetraploids and octoploids has been attributed to a postfertilization incompatibility system (Martinez-Reyna and Vogel 2002).

The upland/lowland ecotype division is strongly supported by molecular data. Hultquist et al. (1996) was the first to discover that chloroplast loci are divergent between upland and lowland ecotypes. A decade later, Missaoui et al. (2006) identified a 49-base-pair indel in the chloroplast *trnL* (UAA) intron that can easily distinguish the two ecotypes. From complete chloroplast sequencing, Morris et al. (2011) recently estimated the divergence between the upland and lowland cytotypes to have occurred 0.7–1.0 million years ago. Nuclear markers, including microsatellite loci (Okada et al. 2011; Zalapa et al. 2011; Zhang et al. 2011a, 2011b) and multilocus genotyping by sequencing methods (Morris et al. 2011; Lu et al. 2013), also support the deep division between upland and lowland ecotypes.

Variation Associated with Large-Scale Environmental Gradients

While there is clear morphological differentiation between upland and lowland ecotypes in the areas in which they overlap, there are a number of traits that vary independently of ecotype. The most obvious of these traits are phenological traits (spring emergence, flowering time, and onset of winter dormancy) that vary clinally with latitude (Casler 2012; As-

pinwall et al. 2013). McMillan (1956, 1959, 1964, 1965a, 1967, 1969a, 1969b) conducted the most extensive experiments documenting genetic variation associated with large-scale environmental gradients in *P. virgatum*. Of particular relevance to adaptation, McMillan observed via common garden experiments in Lincoln, Nebraska, and Austin, Texas, that southern populations emerged earlier in the season and entered winter dormancy later in the season. A large portion (69%–83%) of the variation in flowering time was attributed to latitude of origin as well (fig. 2). Across the state of Texas alone, flowering time in the Austin common garden occurred ~13 weeks earlier for northern panhandle populations than for those collected on the southern Texas Gulf Coast (McMillan 1965a). Unfortunately, McMillan did not document the ecotype status of plants in his experiments. Thus, it is difficult to determine how great an influence ecotype has on phenology relative to latitude of origin from his data.

The association of phenology with latitude appears to be driven by the evolutionary divergence of temperature and photoperiodic responses between *P. virgatum* populations (Benedict 1940; McMillan 1959, 1965b; Van Esbroeck et al. 2003, 2004; Casler et al. 2004, 2007; Casler 2012). The degree to which phenology is affected by photoperiod may also vary with latitude of origin. For example, McMillan (1965b) found that *P. virgatum* populations from the northern Great Plains were far more sensitive than those from the southern Gulf Coast to differences in photoperiod between day-length treatments in growth chambers.

Perennial plants evolve responses to photoperiodic cues to maximize growth when conditions are favorable, while entering dormancy in time to avoid unfavorable environmental conditions (Welling et al. 1997; Hoekstra et al. 2001; Li et al. 2003; Bigras and Bertrand 2006; Savage and Cavender-Bares 2013). Phenological differences in the timing of growth and reproduction associated with latitude could also have consequences for physiological differentiation of populations of *P. virgatum*. Physiology is well known to change throughout the development of a plant, and thus mistiming in phenology can have consequences for how plants respond physiologically to major environmental stresses, such as drought and freezing temperatures (Araus et al. 2002; McKay et al. 2003; Fowler and Limin 2004; Bigras and Bertrand 2006; Roux et al. 2006; Lovell et al. 2013; Savage and Cavender-Bares 2013). Recent studies suggest a possible association of leaf-level physiological traits with latitude of origin in *P. virgatum* (Hartman et al. 2012; Aspinwall et al. 2013), but more studies are needed to clarify the strength of these relationships (O'Keefe et al. 2013).

There is also evidence of trait variation in *P. virgatum* being associated with altitude of origin. Quinn (1969) conducted a common garden experiment in Fort Collins, Colorado, with accessions collected from across the eastern half of Colorado. Just like high-latitude populations, high-altitude populations had an earlier flowering phenology and completed growth earlier in the season than lower-elevation populations.

The Evolutionary History and Species Complex of P. virgatum

The strongest division of population structure within *P. virgatum* is between the upland and lowland ecotypes. The differentiation of populations along latitudes and with elevation is also very clear in common garden experiments. Thus, those sources of variation have received considerable research attention. However, there is additional variation within *P. virgatum* that warrants further research because it provides the opportunity to understand the evolutionary history of the species and to better understand adaptation to different habitats.

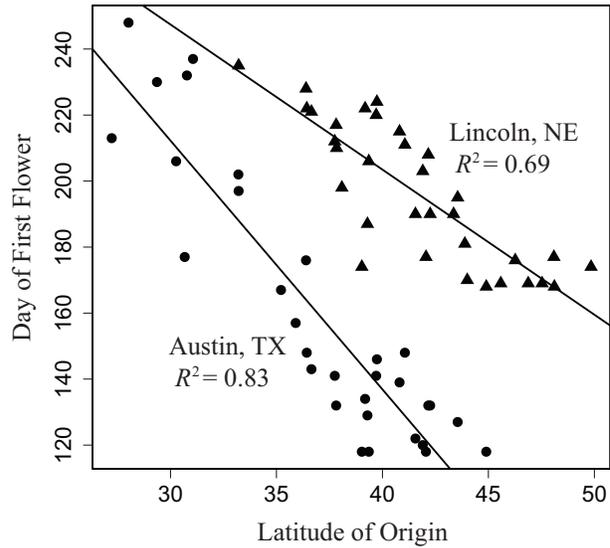The patterns of population structure within *P. virgatum*

Figure 2: Relationship between latitude of origin and date of anthesis (first flower in number of days after Jan. 1) for *Panicum virgatum* accessions growing in McMillan's common gardens in Lincoln, Nebraska, in 1958 (triangles) and Austin, Texas, in 1961 (circles). Data are from McMillan 1959, 1965*a*.

ecotypes may reflect migration during cycles of glaciation. Recent studies have found that the upland ecotype is divided into at least three different regional gene pools (west, north, and east) across the northern part of its range (Zhang et al. 2011*a*, 2011*b*; Lu et al. 2013). Each group may reflect an independent recolonization of northern latitudes by *P. virgatum* from southern refugia following the last Ice Age (McMillan 1959, 1964; Zhang et al. 2011*a*, 2011*b*). Future studies with more populations will be necessary to better clarify the evolutionary history of *P. virgatum* over the course of past migrations with relation to glaciation.

While the division between upland and lowland ecotypes has received the most attention in terms of *P. virgatum* population structure, it is more likely that *P. virgatum* is a species complex composed of multiple ecotypes that are adapted to different habitats. For example, a coastal ecotype of *P. virgatum* occurs in the salt spray zone along the foredunes of barrier islands throughout much of the Atlantic and Gulf Coasts of North America. This

Table 1: Major sources of genetic variation within *Panicum virgatum*

Source	Division	Major phenotypic differences	Fitness consequences
Ecotype	Upland	Thinner shoots and leaves, more shoots per plant	Drought tolerant, higher nitrogen demand
	Lowland	Taller plants, often with bluish leaf color, larger inflorescence	Flooding tolerant, lower nitrogen demand
Latitude	North	Late-season emergence, early flowering, early winter dormancy	Less susceptible to winter kill
	South	Early-season emergence, late flowering, late winter dormancy	Possible tolerance to heat and drought

coastal ecotype occurs as both a hexaploid and a tetraploid ranging from Mexico to Connecticut (Palmer 1975). The coastal ecotype has previously been classified as a distinct species, *Panicum amarum* for the hexaploid and *Panicum amarulum* for the tetraploid (Hitchcock and Chase 1910). However, recent molecular work suggests that the coastal ecotype is no more divergent from *P. virgatum* than upland and lowland ecotypes are from each other (Triplett et al. 2012). Both the tetraploid and hexaploid forms of the coastal ecotype are most closely related to the lowland ecotype across five nuclear genes but have the upland chloroplast cytotype (Triplett et al. 2012). Further, the coastal ecotype has been found to hybridize naturally with the lowland ecotype in Delaware and the panhandle of Florida (Palmer 1975). These findings suggest that a history of migration and gene flow has contributed to the modern diversity of the *P. virgatum* species complex.

Beyond this complexity, recent studies on southern populations of *P. virgatum* have challenged the conventional wisdom of classification of upland and lowland *P. virgatum* ecotypes. Only a few years ago, it was assumed that there was a perfect correspondence between cytotype and upland versus lowland ecotype morphology (Missaoui et al. 2006; Zalapa et al. 2011). Further, plants with lowland morphology and habitat had been thought to be uniformly tetraploid, while more complex ploidy was reserved for upland populations (Costich et al. 2010; Triplett et al. 2012). However, Zhang et al. (2011*a*, 2011*b*) recently found that some populations of *P. virgatum* collected from the deep southeastern United States had the lowland ecotype morphology but were octoploid and/or contained the upland cytotype. In our analyses of populations from Texas (reported for the first time in fig. 1; see also table A1, deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.rm718 [Lowry et al. 2014]), there are similar levels of complexity. In a small area between San Antonio and the Texas Gulf Coast, all four combinations of ploidy (tetraploid/octoploid) and cytotype (lowland/upland) occur. Just inland from the Gulf Coast, there are many tetraploid populations with the upland cytotype. While these populations have a large lowland morphology, they lack the blue waxy leaves that are characteristic of typical lowland populations found throughout much of the interior of Texas. Perhaps these populations are hybrids with the coastal ecotype, or perhaps they are another undescribed ecotype. While populations are easily grouped into upland and lowland ecotypes in the central and northern United States, new biogeographical studies from the southern United States and Mexico will be needed to clarify the more complex patterns of population structure that exist in the southern range of *P. virgatum*. The range of *P. virgatum* extends into southern Mexico (McMillan 1965*a*), yet the population structure and evolutionary his-

tory of that region of the species has never been studied. The bias toward population genetic studies from the northern Great Plains reflects a focus on breeding *P. virgatum* as a forage and bioenergy crop in that region. In general, many more collections need to be classified by ploidy and cytotype to fill in missing gaps in our understanding of population structure within *P. virgatum* and to better reconstruct the patterns of migration and range expansion following the last Ice Age.

Field Experiments on Adaptation between Ecotypes and along Environmental Gradients

Local Adaptation between Ecotypes

While there are clear morphological and physiological differences between upland and lowland ecotypes of *Panicum virgatum*, only one study that we are aware of has explicitly tested for differences in local adaptation between the ecotypes in a region where they co-occur (Porter 1966). Porter focused his research solely on populations from both upland and lowland habitats within one county (McClain) of Oklahoma. In a series of laboratory experiments, Porter (1966) found that *P. virgatum* collected from lowland floodplain sites along the Canadian River were consistently tetraploid (2n = 36) and larger in many morphological traits when compared to octoploid plants (2n = 72) found in upland habitat adjacent to the river.

To test whether upland and lowland populations are locally adapted to their respective habitats, Porter (1966) conducted a field reciprocal transplant experiment between upland and lowland sites. By the third season, all nonlocal plants had died, while indigenous plants survived at both sites. Porter attributed the lack of survival of upland plants at the lowland sites to intolerance to flooding and low levels of nitrogen. Porter's greenhouse experiments supported these claims by showing that the upland plants were reduced in height under flooded conditions, while the lowland ecotype actually increased in height. He hypothesized that the lowland ecotype failed to survive in the upland habitat due to drought susceptibility. Furthermore, Porter showed that upland plants have a greater nitrogen demand and are more dwarfed than lowland plants under low nitrogen. Recent studies have confirmed that there are major differences in nutrient demand between the two ecotypes (Stroup et al. 2003; Yang et al. 2009; Wulschleger et al. 2010). Further, Warner et al. (1987) surveyed physiology from the same populations studied by Porter (1966) and found the upland ecotype to have more chloroplasts and a higher photosynthetic rate than the lowland ecotype.

Porter's results are important because they demonstrate that the upland and lowland ecotypes have evolved habitat

adaptations that are independent of large-scale environmental gradients. That being said, there appears to be intrinsic genetic differences between the upland and lowland ecotypes that have led to differences in the geographic ranges of the ecotypes with respect to major environmental gradients (Casler et al. 2004, 2007; Missaoui et al. 2006; Zalapa et al. 2011; Zhang et al. 2011*a*, 2011*b*).

Reciprocal transplant experiments have yet to be conducted with the coastal ecotype of *P. virgatum* but would help clarify whether this ecotype is also adapted to its indigenous habitat. The evolution of coastal ecotypes is very common in plant species (Turesson 1922; Clausen 1951; Lowry 2012*b*). Reciprocal transplant experiments have frequently found that coastal ecotypes are strongly locally adapted through evolution of salt tolerance and differences in life-history from interior populations (Nagy and Rice 1997; Foster et al. 2007; Lowry et al. 2008; Verhoeven et al. 2008).

Adaptation along Environmental Gradients

Classic common garden experiments and recent agronomic field trials (Nielsen 1947; McMillan 1965*a*; Hopkins et al. 1995*a*, 1995*b*; Sanderson et al. 1999*b*; Casler et al. 2004, 2007; Wullschleger et al. 2010) have demonstrated that the variation associated with the latitudinal gradient in *P. virgatum* is adaptive.

Many studies have established that adaptation in the northern range of *P. virgatum* involves phenology for a short growing season and tolerance to cold winter temperatures (Nielsen 1947; Hope and McElroy 1990; Moser and Vogel 1995; Casler et al. 2004, 2007; Berdahl et al. 2005; Casler and Smart 2013). Nielsen (1947) observed near complete survival of northern lines but near complete mortality of southern lines due to winter injury in a common garden in Madison, Wisconsin. Since then, agronomists have established that moving a *P. virgatum* accession north more than 500 km from its location of origin exposes it to potential winter injury and consequent mortality (Moser and Vogel 1995; Berdahl et al. 2005). Casler et al. (2004) found a 9%–17% reduction in yield for every hardiness-zone *P. virgatum* accessions that were moved north of their origin. McMillan (1965*a*) even observed winter injury and death as far south as Austin, Texas, for an accession transplanted from central Mexico. The mechanisms underlying adaptations to cold winter conditions have received little attention in *P. virgatum*. Phenology likely plays a major role, as southern populations emerge earlier in the season and enter dormancy later (McMillan 1965*a*; Casler et al. 2004, 2007; Aspinwall et al. 2013). Southern populations may also flower too late to produce viable seeds before the first killing frosts of winter in northern locations. In addition, variation in cold acclimation as well as freezing tolerance of rhizomes and emerging shoots could also be important for winter survival (Thomashow 1999, 2010; Clifton-Brown and Lewandowski 2000; Farrell et al. 2006).

A long growing season, high summer temperatures, and aridity have been suggested as the major abiotic factors involved in natural selection of *P. virgatum* adaptation in the southern Great Plains (McMillan 1965*a*, 1969*b*; Casler et al. 2004, 2007). McMillan (1965*a*) found that plants collected from northern states (North Dakota and Minnesota) failed to flower in his Texas common garden. McMillan hypothesized that differences in drought tolerance and root architecture were responsible for the differential survival in Texas. By 1967, only 12% of the clones from outside of Texas were still alive in the Austin common garden, while a majority (57%) of the Texas clones had survived (McMillan 1969*b*). High-elevation accessions were also eliminated by environmental stress in the Austin common garden. Recent field trials have demonstrated that wild accessions and cultivars derived from Texas outperform plants from more northern locations (Sanderson et al. 1999*b*; Aspinwall et al. 2013; Kiniry et al. 2013) and are potentially more drought tolerant (Hartman et al. 2012). Sanderson et al. (1999*b*) found that biomass yield of northern cultivars decreased by an average of 0.72 Mg ha^{-1} for each degree of latitude north of the common garden in which they were grown in Texas (Casler et al. 2004). Determining the mechanisms by which morphological, phenological, and physiological differences among populations contribute to these locally adaptive differences in fitness in the field should be a major focus of future research in *P. virgatum*.

Future Studies on Genetic Mechanisms of Adaptation

The next major step in *Panicum virgatum* adaptation research will be to understand the genetic mechanisms that underlie adaptive differentiation of the upland, lowland, and coastal ecotypes as well as more widespread adaptations associated with latitude. Adaptive latitudinal clines in flowering time across the Great Plains have been studied at the molecular level in detail in sunflowers (*Helianthus annuus*), in which variation appears to be modulated by changes in the photoperiod and gibberellin pathways (Blackman et al. 2011). It would be interesting to determine whether the same or different molecular pathways play a role in the flowering time cline of *P. virgatum* across the Great Plains and whether those genes contribute to other phenological adaptations such as the timing of winter dormancy and spring emergence. Notably, a number of the traits that differ between upland and lowland *P. virgatum* have been the subject of extensive genetic dissection, not only in model plant species, such as rice and

Arabidopsis, but also in panicoid crop species, such as maize and sorghum. These traits include flowering time (Lazakis et al. 2011; Hung et al. 2012; Morris et al. 2013), inflorescence architecture (Vollbrecht et al. 2005; Choi et al. 2012), and plant stature (Peng et al. 1999; Multani et al. 2003). The underlying regulatory networks are likely to be conserved in *P. virgatum*, as they are known to be conserved among grasses (Vollbrecht et al. 2005; Choi et al. 2012), or among land plants more generally (Peng et al. 1999; Multani et al. 2003; Lazakis et al. 2011; Hung et al. 2012). As an example, it recently has been shown that overexpression of a maize floral regulator, *Corngrass1*, can prevent flowering in *P. virgatum* (Chuck et al. 2011).

While local adaptation has been well established as being common (Linhart and Grant 1996; Leimu and Fischer 2008; Hereford 2009), the genetic mechanisms underlying local adaptation are still poorly understood in general (Kawecki and Ebert 2004; Anderson et al. 2011; Argen and Schemske 2012; Pannell and Fields 2013). In particular, it is unclear how often genetic trade-offs (i.e., antagonistic pleiotropy) at individual loci underlie the overall pattern of local adaptation (Anderson et al. 2011, 2013; Colautti et al. 2012; Lowry 2012b; Des Marais et al. 2013). Reciprocal transplant experiments that have incorporated hybrids for genetic mapping have found that trade-offs are rare and that most loci have fitness effects in only one habitat (i.e., conditional neutrality), with no detectable effects in the alternative habitat (Gardner and Latta 2006; Verhoeven et al. 2008; Hall et al. 2010; Fournier-Level et al. 2011; Anderson et al. 2013). Thus far, these studies of the genetic architecture of local adaptation have been conducted only between two contrasting habitats and have yet to be applied to adaptation along large-scale environmental gradients such as latitude. Future research in *P. virgatum* provides the opportunity to address the genetics of adaptation at both the ecotype and large environmental gradient scales.

Overall, it is important that future studies in *P. virgatum* be very clear about what sources of variation are most important for different adaptations. Both ecotype status (upland vs. lowland) and latitude have been established as important sources of variation responsible for adaptation in *P. virgatum* (Porter 1966; McMillan 1969b; Sanderson et al. 1999b; Casler et al. 2004, 2007; Kiniry et al. 2013). The effect of ploidy (tetraploid vs. octoploid) on adaptive trait divergence has also been evaluated in many studies but has rarely been found to have a major effect on phenotype or fitness in the field (Nielsen 1947; McMillan and Weiler 1959; Warner et al. 1987; Wullschleger et al. 1996; O'Keefe et al. 2013). Further, ploidy is often confounded with ecotype (Warner et al. 1987; Wullschleger et al. 1996) or latitude of origin (Nielsen 1947; McMillan and Weiler 1959; O'Keefe et al. 2013), making it difficult to determine the effects these three major sources of variation have on adaptation. Future studies need to include many more replicates from across all three potential sources of variation to distinguish their relative importance.

One of the major recent developments that will facilitate genetic research on adaptation in *P. virgatum* is the ongoing genome sequencing effort (Sharma et al. 2012). The Joint Genome Institute (US Department of Energy) released the first draft of the *P. virgatum* genome (Alamo AP13) in 2012. This genome will facilitate high-throughput development of linkage maps that can be used to identify loci that are involved in regional and ecotypic adaptations. Even though *P. virgatum* occurs as a polyploid, homeologous chromosomes pair independently, leading to disomic inheritance in tetraploid crosses (Okada et al. 2010). This diploid genetic behavior may be the result of *P. virgatum* being the product of an allopolyploid hybridization event (Triplett et al. 2012). Thus, quantitative trait locus (QTL) mapping is feasible in this system, at least for tetraploids. In addition, a deeply sequenced transcriptome is available to facilitate studies of gene expression by RNA sequencing (Casler et al. 2011; Palmer et al 2011; Wang et al. 2012). Genetic transformation has been successfully conducted in *P. virgatum* (Xi et al. 2010; Chuck et al. 2011; Fu et al. 2011; Li and Qu 2011), thus allowing for rigorous tests of gene function. Complementary to linkage mapping approaches, genome-wide association studies are increasingly feasible in nonmodel systems and can be effective at dissecting complex multigenic traits in plants (Brachi et al. 2011). In particular, genome-wide scans show promise for identifying single nucleotide polymorphism (SNP) associations with phenotypes in long-lived perennials (e.g., lodgepole pine [Parchman et al. 2012]) and SNP associations reflecting continuous adaptation along latitudinal and other bioclimatic clines (e.g., loblolly pines [Eckert et al. 2010], *Arabidopsis* [Hancock et al. 2011; Lasky et al. 2012], and sticklebacks [Jones et al. 2012]). In *P. virgatum*, association studies will be facilitated by existing genome-wide maps of SNP variation (Morris et al. 2011; Lu et al. 2013).

Future studies could utilize QTL mapping at multiple locations across latitudes to better understand the genetic architecture of local adaptation over large-scale environmental gradients. The creation of advanced-generation mapping populations between northern upland accessions and southern lowland accessions holds potential to identify loci involved in ecotypic divergence and widespread adaptations associated with environmental gradients simultaneously. The identification of loci involved in climatic adaptations will also help to guide breeding programs that aim to create higher yielding cultivars of *P. virgatum* for bioenergy targeted to different ecoregions of North Amer-

ica. In this way, studies of local adaptation in *P. virgatum* represent an ideal partnership of basic and applied science.

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