Title: One hundred years into the study of ecotypes, new advances are being made through large-scale field experiments in perennial plant systems

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ABSTRACT

A hundred years after Turesson first clearly described how local adaptive variation is distributed within species, plant biologists are making major breakthroughs in our understanding of mechanisms underlying adaptation from local populations to the scale of continents. While the genetics of local adaptation has typically been studied in smaller reciprocal transplant experiments, it is now being evaluated with whole genomes in large-scale networks of common garden experiments with perennial switchgrass and poplar trees. These studies support the hypothesis that a complex combination of loci, both with and without adaptive trade-offs, underlie local adaptation and that hybridization and adaptive introgression play a key role in the evolution of these species. Future studies incorporating high-throughput phenotyping, gene expression, and modelling will be used to predict responses of these species to climate change.

Introduction

One hundred years ago, the Swedish botanist Göte Turesson reported a series of observations from common garden experiments in which he found that plants of the same species collected from different habitats had distinctive genetically based differences in morphology and growth form [1,2]. Turesson cogently argued that the differences among populations were due to natural selection, which was a rejection of the Lamarckian paradigm that dominated the literature up to that point [3–7]. To more clearly communicate the phenomena, Turesson coined the term *ecotype* in 1922 to describe distinct groups of plant populations that are locally adapted to particular habitats. These ecotypes were identifiable by having a shared suite of genetically-based traits that could be observed when grown in common garden experiments. A hundred years later, plant biologists continue to build on Turesson's legacy of understanding how natural selection shapes variation within plant species through common garden experiments [8]. In this review, we highlight recent advances in understanding plant adaptations in two perennial plant species through long-term common garden experiments and outline our perspective on where the field is headed next.

Distinguishing genetic from environmentally-based variation in plant species has required a rigorous development of experimental methods in plant evolutionary ecology. Turesson's compelling intellectual arguments in early publications have earned him credit for much of the early work to measure locally adaptive variation within species. However, Langlet [9] correctly pointed out that provenance trials in the field of forestry examining the relationship between ecology and genetics, the study of *genecology*, had long recognized within species adaptive genetic variation, even if they had not clearly articulated those findings in an evolutionary context. Provenance trials consist of planting common gardens at multiple locations along environmental gradients to assess the relationship of fitness to distances from a home environment (Figure 1). In the middle of the 20th-century, Clausen, Keck, and Hiesey combined Turesson's ecotype concept with provenance trials across California [10,11] to establish reciprocal transplant experiments as the gold standard for testing whether ecotypes are locally adapted to their home environments.

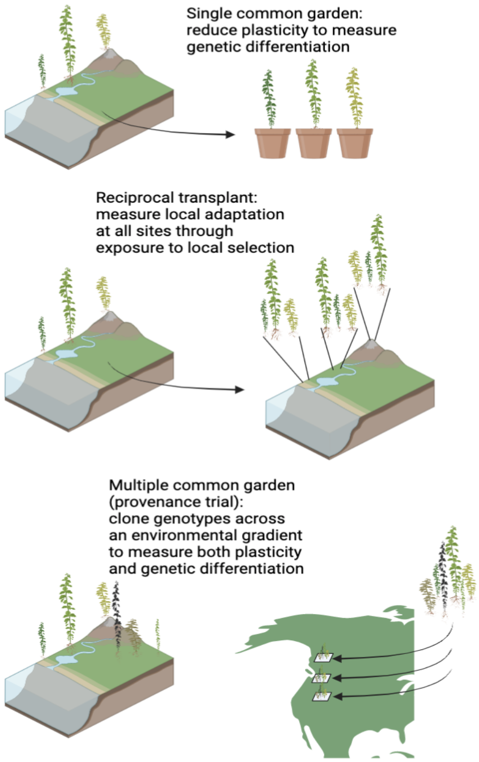


Figure 1: Three types of experiments that test different aspects of local adaptation. Poplar icons represent genotypes.

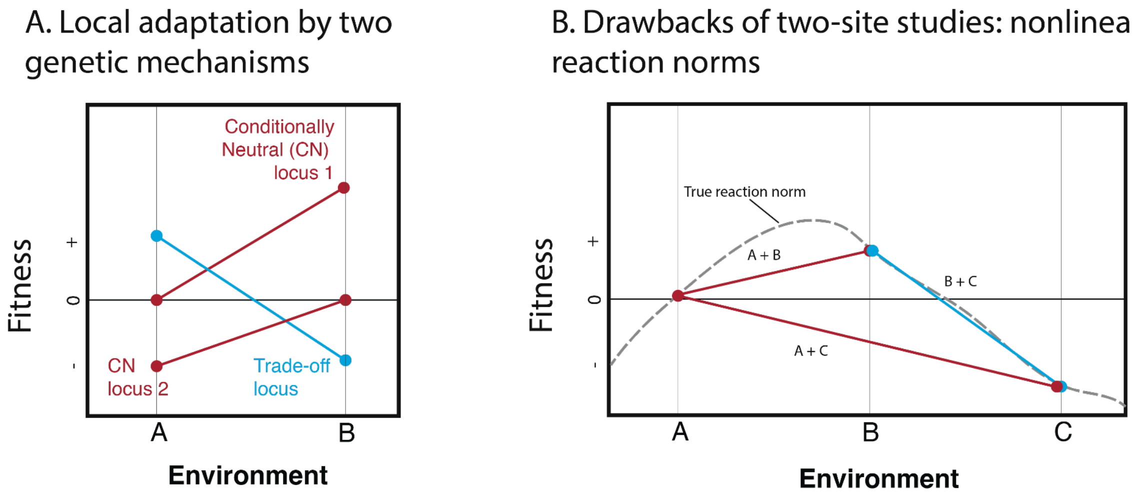
Today, common garden experiments have entered the population genomic era, as whole-genome data sets are becoming increasingly available for accessions planted in geographically widespread common garden experiments. A number of annual and short-lived perennial plant systems, including *Arabidopsis*, *Mimulus*, and *Boechera*, have begun to bring reciprocal transplant common garden field studies into the genomic era [12–14]. Here, we focus on two long-lived perennial systems, switchgrass (*Panicum*) and poplar trees (*Populus*), where larger networks of multiyear common gardens are being used to gain a more comprehensive understanding of local adaptation across geographic space. The results from these two systems illustrate current and emerging approaches to understanding locally adaptive allelic variation is distributed on the scale of continents.

Understanding the role of fitness trade-offs in local adaptation in switchgrass

Switchgrass, *Panicum virgatum*, is a large perennial North American grass species that has a native range from Central America to Southern Canada [15,16]. Across this range, switchgrass exhibits a wide array of genetic variation and adaptive phenotypes. Recent common garden research has leveraged a strategically constructed quantitative trait locus (QTL) mapping population and a large diversity panel to understand the genomics of adaptation from South Texas to South Dakota [17,18]. This recent work builds on historical efforts by forage grass researchers, who have long recognized that there are striking patterns of local adaptation of switchgrass across North America. Classic common garden research by Calvin McMillan in Nebraska and Texas found strong patterns consistent with local adaptation along a latitudinal gradient stretching across the length of the Great Plains [19,20]. In addition to these clinal patterns of adaptive variation, other researchers found evidence that there are several distinct ecotypes of switchgrass that likely originated through geographic isolation into distinct refugia during glaciation. Researchers generally recognized two major switchgrass ecotypes, upland and lowland. The lowland ecotype was generally associated with wetter riparian habitats in the southern United States, while the upland ecotype was thought to primarily occur in more northern regions and typically drier habitats. In the central US where these ecotypes co-occur, Porter [21] conducted a series of experiments to demonstrate strong physiological divergence between the upland and lowland ecotypes. Thus, adaptive genetic variation in switchgrass is distributed both along environmental gradients correlated with latitude and as a mosaic of more discrete ecotype variation [16].

The strength of the switchgrass system in the study of ecological genetics lies in the construction of large-scale, replicated experiments. While McMillan and Porter were able to document morphological differentiation that seemed to be adaptive across the species, modern studies have sought to directly link ecotypic variation to underlying genetic loci. To understand the genetic architecture of local adaptation between northern upland and southern lowland ecotypes, researchers developed genetic mapping populations derived from crosses between upland and lowland germplasm [22,23]. One of these upland × lowland mapping populations was clonally divided through vegetative propagation and planted into 10 field sites spanning 17 degrees of latitude from South Texas to South Dakota and Michigan in 2015 [17]. This experimental design resulted in the exact same set of genotypes being planted across 10 different environmental conditions, allowing for a comprehensive analysis of the genotype, environment, and genotype × environment contributions to trait variation and fitness. This experiment has facilitated QTL analyses across all of these sites to understand how the genetic architecture of trait variation and fitness changes across space [24–27].

Critically, the network of common gardens has allowed researchers to rigorously test a long-held prediction of evolutionary theory: that local adaptation is the result of fitness trade-offs at the individual genetic locus level [28–30]. This theoretical work suggested that the loci involved in adaptation should exhibit a fitness advantage in their home environment but a fitness cost in a foreign environment (Figure 2a), leading to increased phenotypic differentiation among habitats. However, recent studies have indicated that not all loci underlying local adaptation exhibit fitness trade-offs [31]. Most of these studies only used two field sites in a single year, which means that the lack of trade-offs could be the result of failure to measure a sufficient proportion of climate space where trade-offs might manifest (Figure 2b). With the switchgrass common garden network replicated at many sites, it was possible to determine the extent to which there are trade-offs at individual loci. While this work detected a few loci with clear trade-offs across space, there were many more loci that had effects on biomass in one geographic region with non-significant effects in other regions [17]. Thus, some combination of trade-offs and conditional neutrality is responsible for the overall patterns of local adaptation, a pattern that also has been observed previously in two-site reciprocal transplant experiments [31]. Further, using perennial plants for these studies provides the important context of temporal variation in selection pressure, which may be essential to understanding adaptation under climate change.


A. Local adaptation by two genetic mechanisms

B. Drawbacks of two-site studies: nonlinear reaction norms

Figure 2: Detecting the genetic basis of local adaptation. **A.** Two ways that loci can produce local adaptation: single *trade-off* locus increases fitness in one environment, while decreasing it in another (blue reaction norm); two *conditionally neutral* loci can each have effects in only one environment, but combine to form a local advantage (red reaction norms). **B.** Nonlinear reaction norms can result in studies misinterpreting local adaptation patterns. In this scenario, the trade-off for the locus will only be detected if environments B and C are sampled (blue line), owing to an underlying nonlinear reaction norm (dashed gray line).

While QTL mapping populations are powerful tools to understand the impacts of genetic loci on traits and fitness, Genome-Wide Association Studies (GWAS) offer improved precision to identify individual causal genes due to a high number of natural recombination events [12,32,33]. To further elucidate the complement of genes contributing to local adaptation in switchgrass, researchers established a new set of common garden experiments with a diversity panel at 10 field sites (only one site was different from [17]) in North America and 3 sites in Mexico (spanning 24° of latitude) in 2018 and 2019 (Figure 3)[18]. This diversity panel is composed of 732 tetraploid genotypes of switchgrass collected from across eastern North America and clonally propagated at each field site.

As expected, the diversity panel revealed a strong signal of local adaptation, with lowland genotypes generally having higher biomass at southern field sites, while the upland genotypes displayed the opposite pattern [18]. Winter kill in the north has been by far the largest source of mortality in the experiment. Overwinter survival has long been known to be important in switchgrass, but it recently received increased research attention due to it being a primary factor limiting the planting of highly productive southern lowland bioenergy cultivars in the northern United States [34–38]. Many candidate genes underlying adaptive trait variation, including overwinter survival, were revealed through GWAS. Further understanding these fitness-related genes will facilitate future gene editing efforts powered by advances in switchgrass *Agrobacterium*-mediated transformation [39,40].

One of the key results of genome resequencing in the tetraploid diversity panel was that patterns of population genetic structure were discordant with the morphological clustering of plant accessions into ecotypes [18]. Incorporating switchgrass from the eastern USA revealed a third "coastal" ecotype in addition to the previously recognized upland and lowland ecotypes (Figure 4). Population genomic analyses similarly identified three major groups, but these were surprisingly discordant from the morphological ecotypes. This finding contradicts the assumption that ecotypes exist as structure groups, where both structure in trait variation and population genetic structure are inexorably correlated across geographic space [41]. Of particular interest, numerous genotypes with the upland ecotype from the northeastern US clustered with the genetic group containing the new coastal ecotype. Haplotype-level analyses revealed regions of introgression from the mostly upland population into the mostly coastal population, and further that these introgressed regions were enriched for GWAS-detected loci that enhance winter survival [18]. This result suggests that adaptive introgression from the upland population allowed coastal plants to maintain fitness along the northern Atlantic coast.

Figure 3: Representative samples of each recognized switchgrass ecotype. Bars indicate 1 meter. From [18]

In coming years, the network of switchgrass common garden experiments will facilitate a better understanding of how specific abiotic and biotic environmental factors have contributed to adaptive evolution across geographic space. For example, VanWallendael et al. [24] identified two major QTLs for resistance of switchgrass to pathogenic rust fungi. Interestingly, these QTLs both have strong effects in northern sites, but were rarely detected in southern sites, suggesting that genotype x environment interactions play a critical role in shaping biotic interactions across space. In addition to research on pathogens, mutualistic microbes assist with nutrient uptake and may confer additional stress tolerance in leaves [42], and are differentially partitioned across portions of roots [43]. In switchgrass leaves, the fungal microbiome is differentially partitioned across genetic populations, a pattern that may be driven by variation in plant immune response genes [44].

Populus as a model system for ecological, evolutionary, and community genomics

Forestry, more than any other field of plant science, has utilized provenance trials to understand the impact of seed provenance, or origin, on traits important for growth and persistence under varying abiotic and biotic environments [9,45–47]. Indeed, the field of genecology, commonly applied within forestry, largely derives from Turesson's observations of the relationship between heritable trait variation and that of the environment, serving as foundation to the contemporary field of ecological genomics [48]. Thus, understanding the relationship between genotypic or ecotypic variation across environments provides a powerful tool to predicting the health and productivity of reforested regions across space and time [49]. Provenance trials provide invaluable resources which extend our ability to assess adaptation and evaluate populations' capacity for evolution under changing climatic conditions. With the inclusion of new whole genome datasets, extension to new statistical approaches, and the development of new phenotyping platforms the value of provenance trials has only increased.

Populus has become a model genus for studies of local adaptation in forest trees due to its compact genome, rapid growth, ease of vegetative propagation, and extensive natural genomic and phenotypic variation [50,51]. Extensive surveys of range-wide genomic variation in poplar species [52,53], paired with phenotypic measurements from long-term common garden experiments [54–56] have advanced our understanding of the role natural selection has played in shaping adaptive variation within and between Poplar species [57–60]. In addition, due to the propensity of *Populus* species to form natural hybrids and the degree to which hybrids often exhibit heterosis or adaptive introgression for traits valuable in cultivation, the species has become a key model leading advances in breeding and functional genomics [59,61–63]. Finally, poplar is a model system for community genetics - or the study of genetic interactions among species and their abiotic environment [64,65] where heritable trait variation within poplar genotypes have measurable impact to the biotic community impacting ecosystem function [65]. Thus, research using *Populus* capitalizes on its value as a keystone species to understand species' impact across scales of biodiversity, from genes to individuals to populations and communities, to understand the relationship between genetic and environmental variation necessary to predict phenotypic responses across complex environments.

The relatively nascent nature of commercial breeding in forest trees, where many species are only one to three generations from their wild progenitors [66], emphasizes the value that natural hybrid zones provide. Natural hybrid zones circumvent much of the challenge associated with traditional breeding in long-lived species by offering natural, replicated hybrid crosses. Moreover, where ecological differences exist between hybridizing species, zones of contact provide the opportunity to directly evaluate the role natural selection has had on admixture, providing insight into the architecture of adaptation. A unique aspect of *Populus* has been the value of comparative genomics and admixture mapping within naturally replicated contact zones between two species [59,67]. Within the context of climate change, standing genetic variation generated from natural zones of introgression provides a mechanism to facilitate adaptation to novel environments [68–70]. Notably, the contact zone between *Populus trichocarpa* x *balsamifera* indicates expansion of the range of *P. trichocarpa,* which traditionally is characterized by a maritime climate has benefitted from introgression with more continental *P. balsamifera* [59,71]. Thus, a valuable reservoir of adaptive genetic variation may be maintained within zones of introgression with natural selection structuring rangewide genomic and phenotypic variation [55,59,68,71]. Efforts to develop an understanding of the role genome x genome (GxG) interactions may play alongside genotype x environment (GxE) interactions are underway within *P. trichocarpa* x *balsamifera*. Clonally replicated poplar common garden experiments have been established at 18 distinct environments across the United States as part of the PopUp Poplar Network (Figure 5; http://popup-poplars.com). These plantings sourced a latitudinal gradient of native field-collected *P. trichocarpa, P. balsamifera,* and their hybrids spanning six replicated contact zones across the Rocky Mountains, from Washington to Alaska. This model system pairs whole genome sequences, clonally replicated common gardens, and climate modeling to understand how genotypic variation, genomic ancestry, and environmental variation interact to produce phenotypic variation important to climate adaptation and hybrid breeding. This living laboratory provides an unprecedented resource to quantify the role of hybridization to evolution and adaptation, the role of natural selection in shaping quantitative trait variation, and the role interactions between abiotic and biotic factors may have on plant fitness and community and ecosystem function.

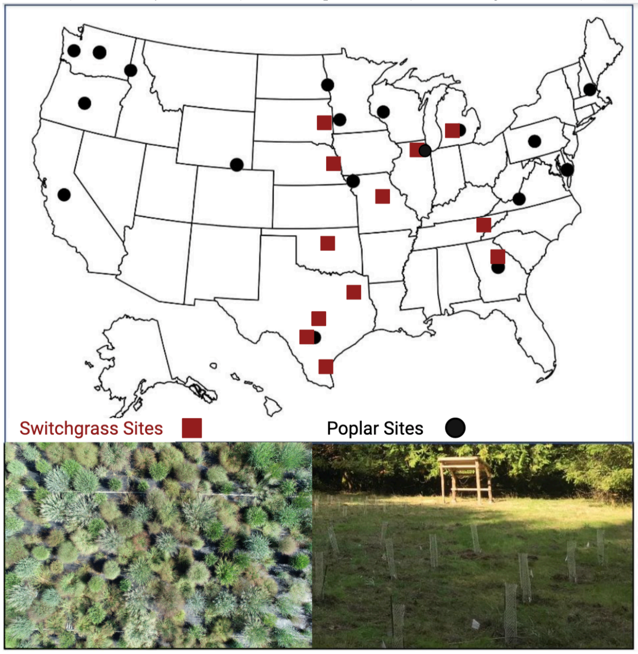


Figure 4: Locations of PopUp Poplar Network sites (black circles) and switchgrass diversity panel planting locations (red squares) across North America. At each poplar location, two replicates of 50 Poplar clones of mixed genomic ancestry between *Populus trichocarpa, P. balsamifera,* and their hybrids have been planted and maintained since 2020. Inset pictures of switchgrass gardens at Kellogg Biological Station (left) and poplar at Evergreen State University (right) (Photo Credit: Robert Goodwin and Dylan Fischer)

Future Directions: Predicting locally adaptive responses to climate change

Current studies of switchgrass are greatly advancing researchers' understanding of how individual loci contribute to the process of evolution at the scale of a continent. One of the most consequential results of this study being conducted in multiple sites and years is that it has enabled the prediction of locus-specific effects on traits and fitness in unmeasured environments. For example, Lowry et al. [17] developed models based on the QTL results from the ten common garden locations to predict allelic effects of loci based on a suite of local environmental conditions. From this modelling effort, it was possible to construct predictive maps across central North America of where individual alleles would be most beneficial to increasing fitness. In the future, these genetic models can be integrated with climate change models to predict how additive allelic effects will shift over time [72]. This future research will complement recent work on trait and fitness predictions for switchgrass at the cultivar level [73].

A major recent advance in predicting plant evolution in response to changed or novel environments leverages spatially-informed genomic datasets with genome-environment associations to predict genotype performance across future environments [74]. These predictions, which assess the degree to which genotype-environment associations are perturbed from locally adapted fitness peaks under climate change, provide an ability to forecast the impact climate change may have on fitness [74–77]. Gougherty et al. [77] recently used genes associated with the flowering-time pathway in *Populus* *balsamifera* to characterize the impact disruptions may have to the maintenance of variation necessary for persistence across the species' range. Broadly, this landscape-level assessment identified regions across the species' distribution that may lack the variation needed to adapt to change and those that may benefit from rescue via migration [77,78]. In combination with advances in machine learning, these new forecasting approaches enhance our ability to predict maladaptation to changed conditions with practical application to restoration under current and future climates [76].

Plant breeding is increasingly incorporating high-throughput phenotyping through unmanned aerial vehicles (UAVs) through drones and through satellite-based remote sensing of hyperspectral data [79,80], and local adaptation studies will follow in the not too distant future. These systems provide models to bridge the genome to phenotype gap for applications both to breeding and evaluation of intraspecific variation essential to restoration efforts [81,82]. Genetic-based trait detection at this spatial and temporal resolution will advance the scale of associations needed to quantify genetic variation for traits important to adaptation. Further, gene expression analyses within hybrid mapping populations and diversity panels will usher in a new era of expression QTL (eQTL) studies to understand how gene regulation contributes to local adaptation. Field eQTL mapping has already recently been conducted in a common garden experiment of a close relative of switchgrass (*Panicum hallii*; [83]). Further, gene expression analyses are now beginning to be incorporated into field reciprocal transplant experiments [13,84,85]. One hundred years after Turesson [2] first clearly articulated how ecotype variation arises from natural selection driving local adaptation, rigorous experimental studies have allowed researchers to more clearly understand the role genetics play in the generation of within-species variation, but the challenge of understanding plants' response to global climate change is a reminder that we still have much to learn.

Funding

This review is based upon work supported in part by the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018409 to DBL, and NSF-RESEARCH-PGR (1856450) to JAH. We received further funding from the U.S. Department of Energy through a grant (DE-SC0017883) to DBL.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to thank the numerous undergraduates, field technicians, graduate students, postdocs, and collaborators who have spent countless hours in the field maintaining and making measurements on our networks of switchgrass common garden experiments and PopUp Poplar Network.

References

1. Turesson G: **The genotypical response of the plant species to the habitat**. *Hereditas* 1922, **3**:211–350.

2. Turesson G: **The species and the variety as ecological units**. *Hereditas* 1922, **3**:100–113.*

This classic paper is where Turesson articulated the conceptual framework for the term ecotype.

3. Bonnier G: **Les plantes de la région alpine et leurs rapports avec le climat**. In *Annales de géographie*. . Persée-Portail des revues scientifiques en SHS; 1895:393–413.

4. Bonnier G: *Nouvelles observations sur les cultures expérimentales à diverses altitudes et cultures par semis*. Librairie générale de l'enseignement; 1920.

5. Hall HM, Clements FE: *The Phylogenetic Method in Taxonomy: The North American Species of Artemisia, Chrysothamnus, and Atriplex*. Carnegie institution of Washington; 1923.

6. Hagen JB: **Experimentalists and naturalists in twentieth-century botany: experimental taxonomy, 1920--1950**. *J Hist Biol* 1984, **17**:249–270.

7. Smocovitis VB: **One hundred years of American botany: a short history of the Botanical Society of America**. *American Journal of Botany* 2006, **93**:942–952.

8. Johnson LC, Galliart MB, Alsdurf JD, Maricle BR, Baer SG, Bello NM, Gibson DJ, Smith AB: **Reciprocal transplant gardens as gold standard to detect local adaptation in grassland species: New opportunities moving into the 21st century**. *J Ecol* 2021, doi:10.1111/1365-2745.13695.

9. Langlet O: **Two Hundred Years Genecology**. *Taxon* 1971, **20**:653–721.*

A classic must read paper for those interested in the debate about how adaptive genetic variation is distributed within species. It covers the long history of tree provenance trials.

10. Clausen J, Keck DD, Hiesey WM, Others: *Experimental studies on the nature of species. III. Environmental responses of climatic races of Achillea*. Carnegie Inst. Wash. Publ.; 1948.

11. Clausen J, Keck DD, Hiesey WM, Others: *Experimental studies on the nature of species. I. Effect of varied environments on western North American plants*. Carnegie Inst. Wash. Publ.; 1940.

12. Exposito-Alonso M, 500 Genomes Field Experiment Team, Burbano HA, Bossdorf O, Nielsen R, Weigel D: **Natural selection on the Arabidopsis thaliana genome in present and future climates**. *Nature* 2019, **573**:126–129.**

In one of the most robust tests of genomic evolution ever conducted in multicellular organisms, researchers measured the genomic impacts of abiotic selection on Arabidopsis genotypes in two divergent climates. They found extensive selection, resulting in a frequency shifts in ~5% of genome-wide variants. These shifts can be paired with climate change predictions, and allow researchers to forecast the evolutionary impacts of imminent climate change.

13. Gould BA, Chen Y, Lowry DB: **Gene regulatory divergence between locally adapted ecotypes in their native habitats**. *Molecular Ecology* 2018, **27**:4174–4188.

14. Lin Y-P, Mitchell-Olds T, Lee C-R: **The ecological, genetic and genomic architecture of local adaptation and population differentiation in *Boechera stricta***. *Proc Biol Sci* 2021, **288**:20202472.

15. Casler MD: **Switchgrass Breeding, Genetics, and Genomics**. In *Switchgrass: A Valuable Biomass Crop for Energy*. Edited by Monti A. Springer London; 2012:29–53.

16. Lowry DB, Behrman KD, Grabowski P, Morris GP, Kiniry JR, Juenger TE: **Adaptations between ecotypes and along environmental gradients in *Panicum virgatum***. *Am Nat* 2014, **183**:682–692.

17. Lowry DB, Lovell JT, Zhang L: **QTL× environment interactions underlie adaptive divergence in switchgrass across a large latitudinal gradient**. *Proceedings of the National Academy of Sciences* 2019,

18. Lovell JT, MacQueen AH, Mamidi S, Bonnette J, Jenkins J, Napier JD, Sreedasyam A, Healey A, Session A, Shu S, et al.: **Genomic mechanisms of climate adaptation in polyploid bioenergy switchgrass**. *Nature* 2021, **590**:438–444.**

The recently published switchgrass genome offers one of the few polyploid perennial plant genomes, as well as an incredibly detailed look at the climatic adaptation of this system. Genome-wide associations made possible by an extensive replicated diversity panel use “multivariate adaptive shrinkage” to share information between planting sites, and improve estimates of genetic associations. This technique, combined with climate associations in the study, offer potential future directions for genomically informed local adaptation studies.

19. McMillan C: **The Role of Ecotypic Variation in the Distribution of the Central Grassland of North America**. *Ecol Monogr* 1959, **29**:286–308.

20. McMillan C: **Ecotypic differentiation within four north American prairie grasses. Ii. Behavioral variation within transplanted community fractions**. *Am J Bot* 1965, **52**:55–65.

21. Porter CL Jr: **An analysis of variation between upland and lowland Switchgrass, *Panicum virgatum* L., in central Oklahoma**. *Ecology* 1966, **47**:980–992.

22. Milano ER, Lowry DB, Juenger TE: **The Genetic Basis of Upland/Lowland Ecotype Divergence in Switchgrass (*Panicum virgatum*)**. *G3* 2016, **6**:3561–3570.

23. Tornqvist C, Taylor M, Jiang Y, Evans J, Robin Buell C, Kaeppler SM, Casler MD: **Quantitative Trait Locus Mapping for Flowering Time in a Lowland × Upland Switchgrass Pseudo-F 2 Population**. *The Plant Genome* 2018, **11**:170093.

24. VanWallendael A, Bonnette J, Juenger TE, Fritschi FB, Fay PA, Mitchell RB, Lloyd-Reilley J, Rouquette FM Jr, Bergstrom GC, Lowry DB: **Geographic variation in the genetic basis of resistance to leaf rust between locally adapted ecotypes of the biofuel crop switchgrass (*Panicum virgatum*)**. *New Phytol* 2020, **227**:1696–1708.

25. Bragg J, Tomasi P, Zhang L, Williams T, Wood D, Lovell JT, Healey A, Schmutz J, Bonnette

JE, Cheng P, et al.: **Environmentally responsive QTL controlling surface wax load in switchgrass**. *Theor Appl Genet* 2020, **133**:3119–3137.

26. Zhang P, Duo T, Wang F, Zhang X, Yang Z, Hu G: **De novo transcriptome in roots of switchgrass (*Panicum virgatum* L.) reveals gene expression dynamic and act network under alkaline salt stress**. *BMC Genomics* 2021, **22**:82.

27. Zhang L, MacQueen A, Bonnette J, Fritschi FB, Lowry DB, Juenger TE: **QTL x environment interactions underlie ionome divergence in switchgrass**. *G3* 2021, doi:10.1093/g3journal/jkab144.

28. Felsenstein J: **The theoretical population genetics of variable selection and migration**. *Annu Rev Genet* 1976, **10**:253–280.

29. Levene H: **Genetic Equilibrium When More Than One Ecological Niche is Available**. *Am Nat* 1953, **87**:331–333.

30. Hedrick PW, Cockerham CC: **Partial inbreeding: Equilibrium heterozygosity and the heterozygosity paradox**. *Evolution* 1986, **40**:856–861.

31. Wadgymar SM, Lowry DB, Gould BA, Byron CN, Mactavish RM, Anderson JT: **Identifying targets and agents of selection: innovative methods to evaluate the processes that contribute to local adaptation**. *Methods Ecol Evol* 2017, **8**:738–749.

32. Lasky JR, Upadhyaya HD, Ramu P, Deshpande S, Hash CT, Bonnette J, Juenger TE, Hyma K, Acharya C, Mitchell SE, et al.: **Genome-environment associations in sorghum landraces predict adaptive traits**. *Sci Adv* 2015, **1**:e1400218.*

33. Bellis E, McLaughlin C, DePamphilis C, Lasky J: **The geography of parasite local adaptation to host communities**. *Ecography* 2021, **44**:1205–1217.

34. Peixoto M de M, Sage RF: **Improved experimental protocols to evaluate cold tolerance thresholds in Miscanthus and switchgrass rhizomes**. *Glob Change Biol Bioenergy* 2016, **8**:257–268.

35. Sage RF, de Melo Peixoto M, Friesen P, Deen B: **C4 bioenergy crops for cool climates, with special emphasis on perennial C4 grasses**. *J Exp Bot* 2015, **66**:4195–4212.

36. Poudel HP, Sanciangco MD, Kaeppler SM, Buell CR, Casler MD: **Quantitative Trait Loci for Freezing Tolerance in a Lowland x Upland Switchgrass Population**. *Front Plant Sci* 2019, **10**:372.

37. Poudel HP, Sanciangco MD, Kaeppler SM, Buell CR, Casler MD: **Genomic Prediction for Winter Survival of Lowland Switchgrass in the Northern USA**. *G3* 2019, **9**:1921–1931.

38. Poudel HP, Lee D, Casler MD: **Selection for winter survivorship in lowland Switchgrass**. *Bioenergy Res* 2020, **13**:109–119.

39. Liu Y, Merrick P, Zhang Z, Ji C, Yang B, Fei S-Z: **Targeted mutagenesis in tetraploid switchgrass (Panicum virgatum L.) using CRISPR/Cas9**. *Plant Biotechnol J* 2018, **16**:381–393.

40. Chen Q, Song G-Q: **Protocol for Agrobacterium-Mediated Transformation and Transgenic Plant Production of Switchgrass**. *Methods Mol Biol* 2019, **1864**:105–115.

41. Lowry DB: **Ecotypes and the controversy over stages in the formation of new species**. *Biol J Linn Soc Lond* 2012, **106**:241–257.

42. Grady KL, Sorensen JW, Stopnisek N, Guittar J, Shade A: **Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops**. *Nat Commun* 2019, **10**:4135.**

43. Singer E, Bonnette J, Kenaley SC, Woyke T, Juenger TE: **Plant compartment and genetic variation drive microbiome composition in switchgrass roots**. *Environ Microbiol Rep* 2019, **11**:185–195.

44. VanWallendael A, Benucci GMN, da Costa PB: **Host genetic control of succession in the switchgrass leaf fungal microbiome**. *bioRxiv* 2021, doi.org/10.1101/2021.03.26.437207

45. Illingworth K: **Variation in the susceptibility of Lodgepole pine provenances to Sirococcus shoot blight**. *Can J For Res* 1973, **3**:585–589.

46. Kremer A, Ronce O, Robledo-Arnuncio JJ, Guillaume F, Bohrer G, Nathan R, Bridle JR, Gomulkiewicz R, Klein EK, Ritland K, et al.: **Long-distance gene flow and adaptation of forest trees to rapid climate change**. *Ecol Lett* 2012, **15**:378–392.

47. Aitken SN, Bemmels JB: **Time to get moving: assisted gene flow of forest trees**. *Evol Appl* 2016, **9**:271–290.

48. Turesson G: **The plant species in relation to habitat and climate**. *Hereditas* 1925, **6**:147–236.

49. Hamilton JA, El Kayal W, Hart AT, Runcie DE, Arango-Velez A, Cooke JEK: **The joint influence of photoperiod and temperature during growth cessation and development of dormancy in white spruce (*Picea glauca*)**. *Tree Physiol* 2016, **36**:1432–1448.

50. Tuskan GA, Gunter LE, Yang ZK, Yin T, Sewell MM, DiFazio SP: **Characterization of microsatellites revealed by genomic sequencing of *Populus trichocarpa***. *Can J For Res* 2004, **34**:85–93.

51. Tuskan GA, Difazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A, et al.: **The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray)**. *Science* 2006, **313**:1596–1604.

52. Keller SR, Olson MS, Silim S, Schroeder W, Tiffin P: **Genomic diversity, population structure, and migration following rapid range expansion in the Balsam Poplar, *Populus balsamifera***. *Molecular Ecology* 2010, **19**:1212–1226.

53. Olson MS, Levsen N, Soolanayakanahally RY, Guy RD, Schroeder WR, Keller SR, Tiffin P: **The adaptive potential of *Populus balsamifera* L. to phenology requirements in a warmer global climate**. *Mol Ecol* 2013, **22**:1214–1230.

54. McKown AD, Guy RD, Klápště J, Geraldes A, Friedmann M, Cronk QCB, El-Kassaby YA, Mansfield SD, Douglas CJ: **Geographical and environmental gradients shape phenotypic trait variation and genetic structure in *Populus trichocarpa***. *New Phytol* 2014, **201**:1263–

1276.

55. McKown AD, Klápště J, Guy RD, Geraldes A, Porth I, Hannemann J, Friedmann M, Muchero W, Tuskan GA, Ehlting J, et al.: **Genome-wide association implicates numerous genes underlying ecological trait variation in natural populations of *Populus trichocarpa***. *New Phytol* 2014, **203**:535–553.

56. Soolanayakanahally RY, Guy RD, Silim SN, Song M: **Timing of photoperiodic competency causes phenological mismatch in balsam poplar (*Populus balsamifera* L.)**. *Plant, Cell & Environment* 2013, **36**:116–127.

57. Evans LM, Slavov GT, Rodgers-Melnick E, Martin J, Ranjan P, Muchero W, Brunner AM, Schackwitz W, Gunter L, Chen J-G, et al.: **Population genomics of *Populus trichocarpa* identifies signatures of selection and adaptive trait associations**. *Nat Genet* 2014, **46**:1089–1096.

58. Holliday JA, Zhou L, Bawa R, Zhang M, Oubida RW: **Evidence for extensive parallelism but divergent genomic architecture of adaptation along altitudinal and latitudinal gradients in *Populus trichocarpa***. *New Phytol* 2016, **209**:1240–1251.

59. Suarez-Gonzalez A, Hefer CA, Lexer C, Cronk QCB, Douglas CJ: **Scale and direction of adaptive introgression between black cottonwood (*Populus trichocarpa*) and balsam poplar (*P. balsamifera)***. *Mol Ecol* 2018, **27**:1667–1680.

60. Suarez-Gonzalez A, Hefer CA, Christe C, Corea O, Lexer C, Cronk QCB, Douglas CJ: **Genomic and functional approaches reveal a case of adaptive introgression from *Populus balsamifera* (balsam poplar) in *P. trichocarpa* (black cottonwood)**. *Mol Ecol* 2016, **25**:2427–2442.

61. Brunner AM, Busov VB, Strauss SH: **Poplar genome sequence: functional genomics in an ecologically dominant plant species**. *Trends in Plant Science* 2004, **9**:49–56.

62. Cronk QCB: **Plant eco-devo: the potential of poplar as a model organism**. *New Phytol* 2005, **166**:39–48.

63. Janes JK, Hamilton JA: **Mixing It Up: The Role of Hybridization in Forest Management and Conservation under Climate Change**. *For Trees Livelihoods* 2017, **8**:237.

64. Whitham TG, Bailey JK, Schweitzer JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf EV, Allan GJ, DiFazio SP, Potts BM, et al.: **A framework for community and ecosystem genetics: from genes to ecosystems**. *Nat Rev Genet* 2006, **7**:510–523.

65. Whitham TG, Allan GJ, Cooper HF, Shuster SM: **Intraspecific Genetic Variation and Species Interactions Contribute to Community Evolution**. 2020, doi:10.1146/annurev-ecolsys-011720-123655.

66. Dungey HS: **Pine hybrids — a review of their use performance and genetics**. *For Ecol Manage* 2001, **148**:243–258.

67. Lindtke D, González-Martínez SC, Macaya-Sanz D, Lexer C: **Admixture mapping of quantitative traits in *Populus* hybrid zones: power and limitations**. *Heredity* 2013, **111**:474–

485.

68. DE Carvalho D, Ingvarsson PK, Joseph J, Suter L, Sedivy C, Macaya-Sanz D, Cottrell J, Heinze B, Schanzer I, Lexer C: **Admixture facilitates adaptation from standing variation in the European aspen (*Populus tremula* L.), a widespread forest tree**. *Mol Ecol* 2010, **19**:1638–1650.

69. Hamilton JA, Lexer C, Aitken SN: **Genomic and phenotypic architecture of a spruce hybrid zone (*Picea sitchensis* × *P. glauca*)**. *Mol Ecol* 2013, **22**:827–841.

70. Hamilton JA, Miller JM: **Adaptive introgression as a resource for management and genetic conservation in a changing climate**. *Conserv Biol* 2016, **30**:33–41.

71. Geraldes A, Farzaneh N, Grassa CJ, McKown AD, Guy RD, Mansfield SD, Douglas CJ, Cronk QCB: **Landscape genomics of *Populus trichocarpa*: the role of hybridization, limited gene flow, and natural selection in shaping patterns of population structure**. *Evolution* 2014, **68**:3260–3280.

72. Waldvogel A-M, Feldmeyer B, Rolshausen G, Exposito-Alonso M, Rellstab C, Kofler R, Mock T, Schmid K, Schmitt I, Bataillon T, et al.: **Evolutionary genomics can improve prediction of species' responses to climate change**. *Evol Lett* 2020, **4**:4–18.

73. Zhang L, Juenger TE, Lowry DB, Behrman KD: **Climatic impact, future biomass production, and local adaptation of four switchgrass cultivars**. *Glob Change Biol Bioenergy* 2019, **11**:956–970.

74. Capblancq T, Fitzpatrick MC, Bay RA, Exposito-Alonso M, Keller SR: **Genomic Prediction of (Mal)Adaptation Across Current and Future Climatic Landscapes**. 2020, doi:10.1146/annurev-ecolsys-020720-042553.*

75. Fitzpatrick MC, Keller SR: **Ecological genomics meets community-level modelling of biodiversity: mapping the genomic landscape of current and future environmental adaptation**. *Ecol Lett* 2015, **18**:1–16.

76. Rellstab C: **Genomics helps to predict maladaptation to climate change**. *Nature Climate Change* 2021, **11**:85–86.

77. Gougherty AV, Keller SR, Fitzpatrick MC: **Maladaptation, migration and extirpation fuel climate change risk in a forest tree species**. *Nature Climate Change* 2021, **11**:166–171.

78. Aitken SN, Whitlock MC: **Assisted Gene Flow to Facilitate Local Adaptation to Climate Change**. *Annual Review of Ecology, Evolution, and Systematics* 2013, **44**:367–388.

79. Feng X, Zhan Y, Wang Q, Yang X, Yu C, Wang H, Tang Z, Jiang D, Peng C, He Y: **Hyperspectral imaging combined with machine learning as a tool to obtain high-throughput plant salt-stress phenotyping**. *Plant J* 2020, **101**:1448–1461.*

Hyperspectral images of plants can provide detailed and high-throughput phenotyping. The resulting images are information-rich, and therefore well-suited to machine learning algorithms used to obtain meaningful phenotypes. This study is a model for use of hyperspectral imaging to detect plant stress in a format that can be used in both controlled and field settings.

80. Thorp KR, Thompson AL, Harders SJ, French AN, Ward RW: **High-Throughput Phenotyping of Crop Water Use Efficiency via Multispectral Drone Imagery and a Daily Soil Water Balance Model**. *Remote Sensing* 2018, **10**:1682.

81. Ludovisi R, Tauro F, Salvati R, Khoury S, Mugnozza Scarascia G, Harfouche A: **UAV-Based Thermal Imaging for High-Throughput Field Phenotyping of Black Poplar Response to Drought**. *Front Plant Sci* 2017, **8**:1681.

82. Sankey JB, Sankey TT, Li J, Ravi S, Wang G, Caster J, Kasprak A: **Quantifying plant-soil-nutrient dynamics in rangelands: Fusion of UAV hyperspectral-LiDAR, UAV multispectral-photogrammetry, and ground-based LiDAR-digital photography in a shrub-encroached desert grassland**. *Remote Sens Environ* 2021, **253**:112223.

83. Lovell JT, Jenkins J, Lowry DB, Mamidi S, Sreedasyam A, Weng X, Barry K, Bonnette J, Campitelli B, Daum C, et al.: **The genomic landscape of molecular responses to natural drought stress in Panicum hallii**. *Nat Commun* 2018, **9**:5213.

84. Kenkel CD, Matz MV: **Gene expression plasticity as a mechanism of coral adaptation to a variable environment**. *Nat Ecol Evol* 2016, **1**:14.

85. Lohman BK, Stutz WE, Bolnick DI: **Gene expression stasis and plasticity following migration into a foreign environment**. *Mol Ecol* 2017, **26**:4657–4670.*

Measuring gene expression in field settings is a methodological challenge, but can offer important insights into natural responses to changes in the local environment. In this study, researchers showed that foreign populations of locally adapted stickleback fish converged on the gene expression profiles of locally adapted populations, but that this plastic response was not fully sufficient to maintain fitness. These results show the importance of disentangling plasticity and genetic differentiation in transplant studies, and offer new tools for local adaptation researchers to understand how populations respond to their environment.