Research Accomplishments

Throughout my research career, I have studied how plants acquire, transport, and use nutrients and how plants adapt in form and function to nutrient deficiencies or toxicities.

During my PhD, I developed different mass spectrometry techniques including one that allowed me to discover the molecular mechanism of long-distance iron transport. I was able to identify for the first time a natural iron complex in a plant tissue and finally characterize the exact form of iron citrate in plant xylem sap. We also showed that the nature of iron citrate complexes is dependent on Fe:Citrate ratios and that since this ratio changes with iron deficiency, the form of iron transport changes under different iron regimes. (Rellán-Álvarez et al., 2010b) .

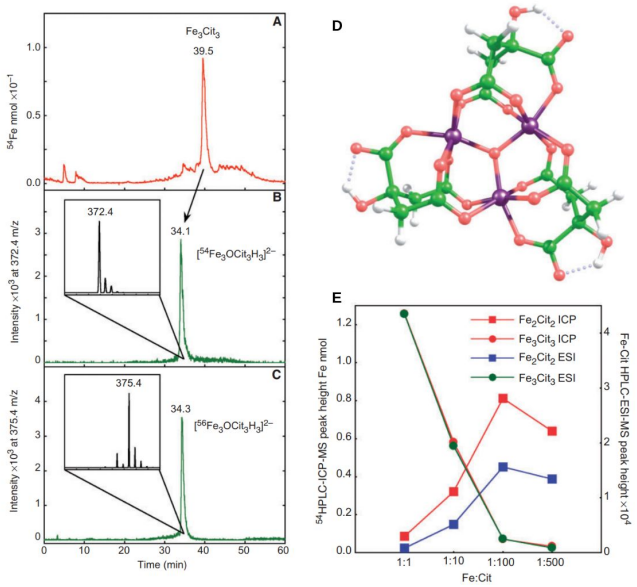

Figure 1. HPLC–ICP-MS (A) and HPLC–ESI-TOFMS (B, C) typical chromatograms of xylem sap samples from Fe-deficient, 12-h Fe-resupplied tomato plants, showing the peak corresponding to the Fe_3Cit_3 complex. Plants were resupplied with 54Fe–*o,o*EDDHA (A, B) or natFe–*o,o*EDDHA (C). HPLC–ESI-TOFMS traces were extracted at m/z values of 372.40 and 375.40 (± 0.05), corresponding to $[54Fe_3OCit_3H_3]^{2-}$ and $[56Fe_3OCit_3H_3]^{2-}$. Isotopic signatures of both molecular ions are shown in insets in (B) and (C). Proposed structure for the Fe_3Cit_3 found in plant xylem as an oxo-bridged tri-iron–citrate complex. Iron, oxygen, carbon and hydrogen atoms are shown in purple, red, green and white, respectively (D). Effect of the Fe:Cit ratio on the Fe_2Cit_2 and Fe_3Cit_3 balance (E).

I showed the dynamic nature of iron citrate complexes and how they change based on the iron:citrate ratios. I also characterized the ligand exchange reactions that occur between different iron ligands such as citrate and nicotianamine (a non -roteinogenic amino acid) and how nicotianamine can interchangeably chelate other micronutrients such as Zn and Cu (Rellán-Álvarez et al., 2008). We showed that citrate and nicotianamine can, up to a certain point, compensate ue for the absence of the other in the role of long-distance iron transport (Schuler et al., 2012).

During my PhD, I used a system biology approach to show that iron deficiency leads to a reduction of available carbon skeletons in the leaves due to reduced photosynthesis that is supplemented n partby organic acids synthesized anaplerotically in roots.(Rellán-Álvarez et al., 2010a; Rellán-Álvarez et al., 2011; Sudre et al., 2013). In total during my PhD and including work from my MSc I published 17 papers, including seven as first author. My PhD work was recognized in the International 2013 Plant Nutrition Colloquium with the Marschner Young Scientist Award.

During my postdoc in the laboratory of José Dinneny, I developed a unique live imaging and image analysis platform named GLO-Roots (Growth and Luminescence Observatory for Roots) that is able to simultaneously track root growth, root system architecture, gene expression in Arabidopsis roots (and other species like Brachypodium, Setaria and Tomato) and soil properties, such as soil moisture, for seedlings growing in a soil-based, substrate that is physiologically close to natural conditions. The system allows seamless integration of different types of data and enables studies aimed at understanding how plant roots

adjust function and shape to adapt to heterogeneous environmental conditions (Rellán-Álvarez et al., 2015). This new imaging system is allowing us to visualize how plant roots respond to localized water application (Sebastian et al., 2016). I am planning to use this system to study how roots respond to different concentrations of nutrients in the soil (Rellán-Álvarez et al., 2016; Mora-Macías et al., 2017).

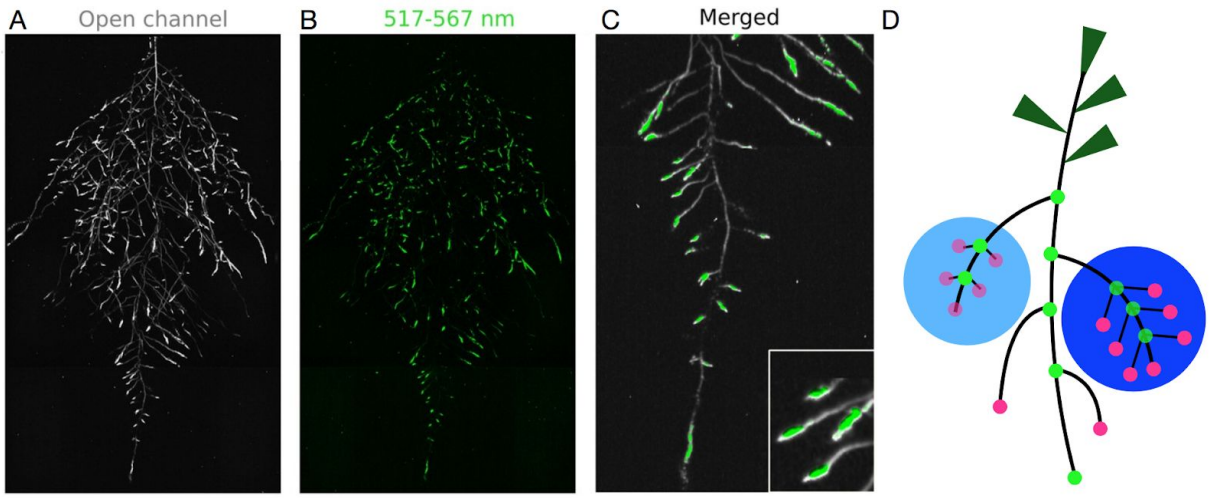

Figure 2. An example of dual color imaging using the GLO-Roots imaging system. A) Open channel showing UBQ10::PpyRE8 expression marking RSA. B) ProZAT12::LUC2 marking ZAT12 expression and C) combination of both channels. D) Example of the type of experimental design that can be carried out with the GLO-Root system to study how root systems integrate information about local environmental conditions at the whole-root level. Different colors indicate different markers and shades of blue indicate different concentrations of a nutrient.

Since I started my own laboratory 2.5 years ago at the National Laboratory of Genetics for Biodiversity in México, I have initiated a new research line focusing on understanding how local adaptation to abiotic stresses shapes metabolic pathways. We are using a combination of biochemical phenotyping, quantitative, and population genetic tools to identify genes involved in controlling metabolic pathways that have been selected for during the process of local adaptation.

To this end, I have secured funding from competitive grants from the Mexican Government research agency Conacyt and collaborative funds between México and the University of California (see future research statement). I am also a collaborator in the NSF PGRP project The genetics of maize adaptation to the highlands, led by Jeffrey Ross-Ibarra.

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Future Research

Functional genomics and metabolomics of plant local adaptation

Since I started my own laboratory 2.5 years ago at the National Laboratory of Genetics for Biodiversity in México, I have initiated a research line focusing on understanding how local adaptation to abiotic stresses shapes metabolic pathways (Fig. 1). We are using a combination of biochemical phenotyping, quantitative, and population genetic tools to identify genes involved in controlling metabolic pathways that have been selected for during the process of local adaptation.

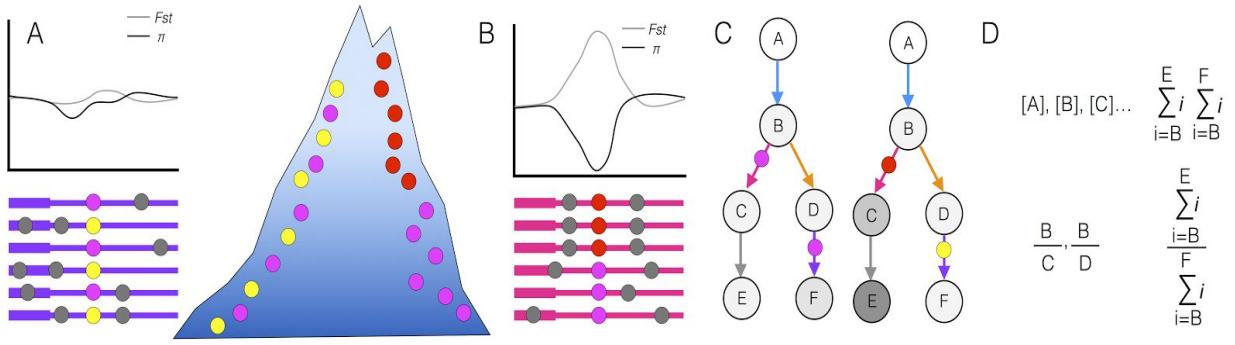

Figure 1. Effect of local adaptation to high elevation and soil conditions (indicated by a gradient of blue) on genetic diversity and differentiation between highland and lowland populations and its consequences on a metabolic pathway under selective pressure. **A)** Example of one gene with variants (magenta and yellow) under no selection at different elevations. Values of population differentiation (*Fst*) and nucleotide diversity (π) are close to 0. **B)** Example of one gene with a genetic variant (red) under selection at high elevations. *Fst* values are high and π values are low in highland conditions. C) Pathway on the left shows lowland conditions where genetic variants have a neutral effect of metabolite concentrations. In the right pathway, the red genetic variants selected for in highland conditions led to the accumulation of C and E, which has a positive effect on adaptation to highland conditions. D) Different types of o data that can be calculated after metabolite concentrations are measured.

Plants are subject to the constraints of the environment in which they live, including temperature, edaphic conditions, and biotic interactions. As plants colonize other geographical areas with different environmental constraints (Fig. 1), their form and metabolic function adapt to the new conditions through changes in the underlying genes. Changes that allow the plant to thrive in local environmental conditions are selected from a variety of sources (e.g., standing variation, new point mutations, interspecific gene flow), but eventually lead to a loss of genetic variation in the genetic regions that were selected for and in genetic differentiation between populations originating under different environmental conditions (Fig. 1A, B).

To analyze how plants adapt to new environmental conditions, I have been studying glycerolipid reorganization during maize adaptation to highland environments. After domestication, maize colonized the highlands of México, where it encountered a whole range of new environmental conditions, including higher UV radiation, different precipitation regimes, colder temperatures and, in some areas, depleted soils with low availability of nutrients, especially phosphorus. Several other independent adaptive events occurred in highland maize as domesticated maize spread across the Americas, making maize highland adaptation an excellent system to study convergent adaptation processes to similar environments. Glycerolipid metabolism was under high selective pressure during maize highland adaptation, because phosphorus deficiency and cold temperatures impose contrasting pressures on different types of glycerolipids. While non-phosphorus containing glycerolipids (such as galactolipids and sulpholipids) are favored under

phosphorus deficiency conditions, phospholipids are favored in cold conditions. We therefore expect to identify genes involved in glycerolipid metabolism that show differentiation between highland and lowland maize lines.

To search for such genes, we are profiling the lipidomes of maize mapping populations, including diversity panels and recombinant inbred lines (RILs) consisting of highland and lowland landraces collected from across the Americas and grown under common garden conditions at high and low altitudes in México. We have identified around 120 glycerolipid species that we then used in different ways (Fig. 1D) as phenotypes for genome-wide association studies (GWAS), Qst-Fst, and quantitative trait locus (QTL) analysis to identify loci involved in regulating glycerolipid metabolism. Using this approach, we identified a QTL at the beginning of chromosome 3 (Fig. 2A, C) that explains the conversion of phosphatidylcholines (PCs, the major phospholipid species) to lyso-phosphatidylcholines (LPCs). RILs with PT alleles at the QTL peak have low concentrations of LPCs and high concentrations of PCs. The opposite is observed for B73 alleles.

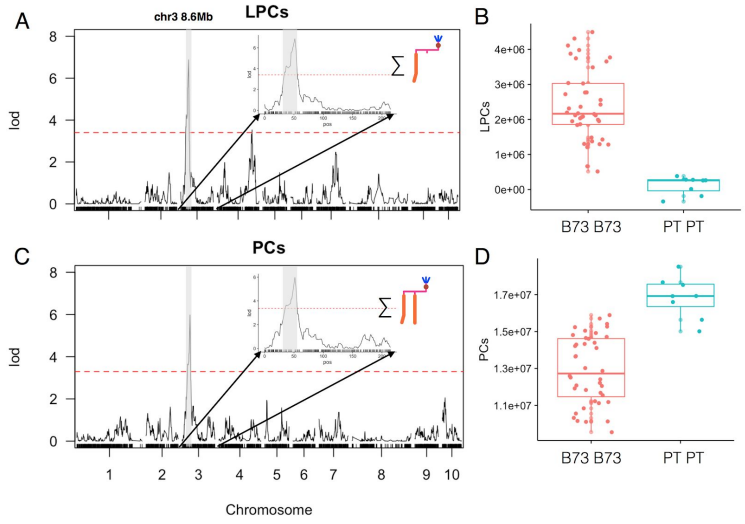

Figure 2. QTL analysis on a B73 x Palomero Toluqueño RIL mapping population using the sum of lyso-phosphatidylcholine (A) and phosphatidylcholine (C) species as the phenotype. LPC (B) and PC (D) concentrations of homozygous RILs at the marker on the chr3 8.6 Mb QTL peak. The blue line indicates the location of the candidate phospholipase A1 gene GRMZM2G353444.

At the QTL peak, we identified a candidate gene with predicted PC to lysoPC activity. In B73, this gene is highly expressed in vegetative leaves and in temperate inbred lines it is upregulated under cold conditions and downregulated in response to heat stress (Waters et al. 2017). We have now sequenced the PT allele and have identified several non-synonymous mutations, including an Ile > Leu mutation in the flap/lid of the protein that could affect the function of the protein. We are currently heterologously expressing the PT and B73 coding sequences (CDSs) in *E. coli* and *A. thaliana* to identify functional differences between the two genes. We have also analyzed a number of datasets to identify possible signals of selection in the highland maize genome. Using 30 whole genome sequences of highland and lowland maize landraces across the Americas (Wang et al. 2017), we have observed that several non-synonymous SNPs in the candidate region, including the Ile > Leu in the flap/lid, were selected for in highland North American maize, but not in South American maize. This mutation is present in the highland variety teosinte mexicana, but is only present in around 20% of the lowland variety teosinte parviglumis. Using public GBS data from 4000 Landrace accessions, we have also observed that another non-synonymous single nucleotide polymorphism (SNP) in the CDS of the gene is much more frequent in highland maize and that the B73 allele is more common in lowland maize (Fig. 3).Together, these data show that the gene was selected for in highland North American maize. We are now analyzing if the B73 allele might be the result of adaptive introgression from highland teosinte mexicana. Efforts in my lab are now concentrated on developing near

introgressed lines (NILs) and mutants (CRISPR-CAS9 T0s have already been generated) of the gene to test if this is the case. This would be one of the first examples where adaptive introgression at the gene level is functionally validated. Interestingly, we have also found found that a couple of genes (GRMZM2G014981 and GRMZM2G481755) that perform the reverse reaction (lyso-PC + fatty acid → PC) of GRMZM2G353444 (PC → lyso-PC + fatty acid) showed high Fst values in between highland and lowland maize populations of South America and México(Takuno et al. 2015). All taken together this data that phospholipid homeostasis and ultimately phosphorus use efficiency were under high selective pressure during maize adaptation to highland conditions.

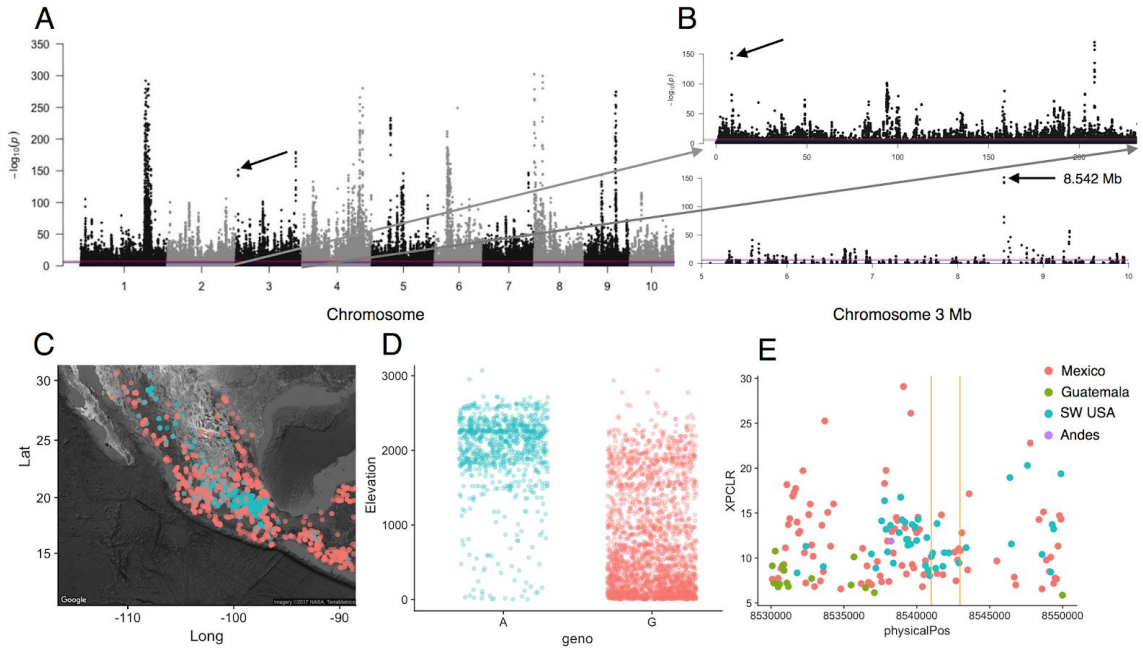

Figure 3. A) Allelic differentiation analysis using PCadapt in a collection of 4000 landraces shows that a G > A mutation located in the CDS of the candidate gene was under selection with altitude. B) Zoom in chromosome 3 and its first 10 Mb The location of the SNP is indicated with an arrow. C) Geographic distribution of the A (blue) and G (alleles) in México showing higher frequency of the A allele in the highlands of the trans volcanic belt. D) Allele frequencies of the A and G alleles of landraces as a function of elevation. E) XP-CLR allele differentiation between highland and lowland material of different populations across America. The higher the value the more allele differentiation (and higher selection) between highland and lowland material. The data were obtained by analyzing the whole-genome sequences of 30 landraces from México, Guatemala, SW USA and the Andes.

Ultimately, we are interested in understanding why this gene was selected in highland conditions and also how glycerolipid metabolism changes in response to different environments, especially environments with extreme temperatures and phosphorus deficiencies. Using this same mapping population, we have also identified other interesting QTLs related to glycerolipid metabolism. In addition, we have collected and are starting to analyze other datasets, including a lipidomics dataset of a highland and lowland landrace diversity panel composed of 60 Mesoamerican and 60 South American lines grown under the same common garden conditions at high and low altitudes as described above. We are using this dataset to calculate Qst-Fst values of glycerolipid contents.

We have also collected data of the F1 offspring of these same 120 landraces crossed with B73 and grown under the same conditions. Together with our collaborator from the highlandadaptation.org project, Daniel Runcie, who will perform RNA-Seq allele-specific expression analysis on the same plants from which we collect tissue for lipidomics, we plan to identify possible patterns of divergence/convergence of glycerolipid metabolism during independent events of maize adaptation to the highlands. We are currently following a

similar strategy using 120 teosinte populations by crossing them with B73 and growing the F1s in highland and lowland sites. We will backcross both landrace and teosinte F1s with B73 and will derive three BC1S5 families for each of the teosinte and landrace donors (Figure 4A).

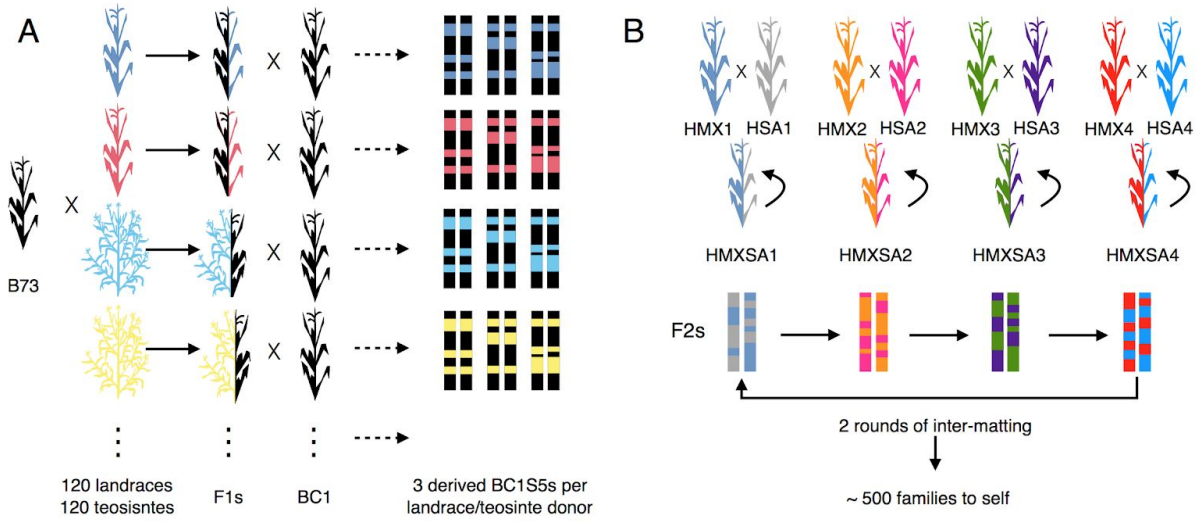

Figure 4. Population building strategy. A) B73 was crossed with 120 landraces from the HiLo diversity panel and 120 teosinte populations from the CIMMYT collection. F1s were sampled for Allele Specific Expression and lipidomic analysis. F1s were then backcrossed with B73 to produce a BC1. From each teosinte and landrace donor BC1, we are planning to generate three BC1S5s. B) Breeding scheme of the Highland MAGIC mapping population we are currently generating. Eight highland (above 2000 masl) landrace parents from across México and South América were selected. Mexican landraces were crossed with South American ones to generate F1s that were then selfed, intermated for two generations and then selfed to produce around 500 families.

We have also started a highland maize magic population consisting of four Mexican highland landraces crossed with four highland South American landraces (Fig. 4B) that should give us greater power to map genes involved in divergent modes of highland adaptation between Mexican and South American maize. We are currently sequencing the eight individual parent plants and have produced the four F1s, which we will examine by both RNA-Seq allele-specific expression and glycerolipid analysis. We have also backcrossed these F1s with B73 and we are producing three BC1S5s per landrace donor. This summer, we started a similar strategy using 120 teosintes donors (Fig. 4A). B73 x teosinte F1s will be grown this winter and next spring in our lowland and highland sites and we will collect tissue for both ASE and lipidomics.

In summary, we use diversity panels and artificial mapping populations encompassing materials from different moments of the evolutionary history of maize to conduct common garden experiments in the geographic areas where these evolutionary processes took place. We then use high-precision molecular phenotyping together with population and quantitative genetics to identify loci under selection and determine their role in local adaptation. Finally, we functionally validate candidate gene functions using heterologous expression and reverse genetics tools.

Understanding how maize adapts to highland conditions is relevant for maize breeding because one of the main adaptations to highland conditions is the ability to withstand early season cold conditions and thereby extend the growing season. The ability to extend the growing season has been linked with increased photosynthetic rates per acre in miscanthus, a relative of maize (Dohleman and Long 2009). Indeed, the

most limiting factor for early maize planting in the US Midwest is soil temperature. We hypothesize that the ability to withstand early season cold conditions is linked with glycerolipid metabolism and in fact In the maize sister genus *Tripsacum* (that is adapted to colder conditions than maize) it has been recently shown that genes involved in phospholipid metabolism have much higher protein evolution rates than maize, pointing to a positive selection in these pathways (Yan et al. 2017).

I feel that my research program would fit well in the Biochemistry Department. We use mapping populations to understand how evolutionary processes involved in adaptation to new environmental and climatic conditions have affected maize glycerolipid metabolism, a fundamental component of the photosynthetic machinery. By tapping into the phenomenal genetic diversity of maize, we seek to understand how one of the most important crops has been able to adapt to new environments and is now cultivated in five continents. In the near future, our ability to apply targeted cisgenesis, allele swapping, and other emerging molecular editing techniques (Rodríguez-Leal et al. 2017), coupled with our knowledge of extant variation, will provide a much more effective and less controversial (Fahlgren et al. 2016) tool than random incorporation of transgenic events.

While I believe this line of research would fit very well within the scope of research conducted at the Biochemistry Department and I would actively seek collaborations with other members of the department, I have enough experience leading projects and have established enough collaborations to pursue an independent line of research, and obtain independent sources of funding that would help diversify the portfolio of research currently being performed at the department. I think my research program is well suited to receive support from various funding agencies, such as DOE, USDA and NSF (especially through the PGRP program), as it focuses on basic evolutionary and plant developmental processes that have direct applications in plant breeding. . I am especially looking forward to being part of the renewal of the PGRP Maize Highland Adaptation grant, where I am currently serving as a collaborator.

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Teaching and Mentoring Statement

One of the reasons I am applying for an Assistant Professor position at North Carolina State University is to have more contact with undergraduate students. Throughout my career, I have worked in research institutes and have had little interaction with undergraduate students. However, I have had the opportunity to mentor 6 undergraduate students during summer internships. This experience made me appreciate the impact educators can have in shaping students' career paths at this early stage of their education.

As a first-generation university student raised in a rural area, I believe higher education is one of the best ways for people to cross societal barriers and to empower students to conduct original work in their professional lives. I was lucky enough to have teachers and professors during my high school and undergraduate studies who inspired me to pursue a career in the plant sciences.

Since my arrival at LANGEBIO in 2015, I have been a member of the Plant Biotechnology and Integrative Biology Graduate Programs. I have served on the committees of 14 students and have mentored or co-mentored 6 Master's and PhD students. I have taught classes to Master's and PhD Students on Plant Development, Plant Biochemistry, and Plant Genetics. I started an informal class in Reproducible Science and, together with a colleague, am developing a semester-long class on this topic, as I consider the skills covered in this course to be fundamental in modern biology. I would be happy to teach classes at the undergraduate and graduate level on Plant Biochemistry and Plant Genetics and also on Reproducible Science. I am also keen to organize a field course on maize adaptation in México for a small group of graduate students.

I encourage my students to learn by doing. For undergraduates, this usually involves collecting phenotypic data, either by direct observation or by mining data from previous studies, and storing these data in a format that can be analyzed with the appropriate tools. Most of our analyses involve identifying genotype–phenotype associations under different environmental conditions.

At the graduate level, I like to cultivate skills that are valuable in professional paths beyond academia, while at the same time emphasizing the importance of critical thinking, which is essential for academic research. Preparing a precise document, presenting and defending one's ideas, working in interdisciplinary groups, and meeting deadlines to achieve a certain objective are a few of the tasks performed in research labs that are easily transferable to non-academic jobs.

Most of the work I have done since I started my own lab involves developing different lines and mapping populations that are then grown in contrasting environments. I place a lot of emphasis on bringing students to the field so they can learn how to "read the plants". I believe that the ability to *read* plant phenotypes and to observe how they are shaped by genetics and the environment is one of the most valuable lessons we can teach students in the Plant Sciences and is an asset that is valuable across different professional paths in academia, industry, or environmental policy.