Grant Number: CN-16-44

Co-Principal Investigator (UC Campus):

Dr Oliver Fiehn; Department: Department of Molecular and Cellular Biology & Genome Center; Institution: UC Davis

Co-Principal Investigator (Mexico):

Dr Ruben Rellan-Alvarez; Department: Unidad de Genomica Avanzada, Langebio; Institution: Cinvestav

Project Title:

The role of phospholipids in maize adaptation to Mexican highlands

Titulo del Proyecto:

El papel de los fosfolipidos en la adaptación del maiz a los valles altos de México

Amount Requested: $25,000.00

Abstract (English):

Maize adaptation to the Mexican highland offers an opportunity to understand how plants evolve and adapt to new environments, a relevant process that can provide important clues to develop improve crops that can produce the same yields we have achieve today under more stressful conditions due to climate change or using less fertilizers. Evolutionary and population genomics have suggested that maize adaptation to the Mexican highlands involved changes in phospholipid metabolism. The importance of phospholipid metabolism in adaptation to cold, low phosphorus soils and flowering time -three important factors that determine plant growth in the highlands- has also been characterized in the model species Arabidopsis. IIn this proposal we will use maize populations developed with Mexican landraces adapted to the Mexican highlands and lowlands and mutants involved in phospholipid pathway grown common garden experiments lowland and highland field sites and growth chambers simulating these conditions. We will then use precise biochemical phenotyping (high performance lipid chromatography coupled to mass spectrometry) to profile phospholipids and glycerolipids and identify possible patterns of adaptation to highlands in phospholipid metabolic pathways including the underlying genetic architecture that controls it.

Abstract (Spanish):

La adaptación del maíz a valles altos de México ofrece una oportunidad para comprender cómo evolucionan el metabolismo de las plantas para adaptarse a nuevos entornos. Diversos estudios de genómica evolutiva y poblaciones han sugerido cambios en el metabolismo de fosfolípidos tuvieron un papel importante en la adaptación de maíz a las tierras altas de México. La importancia del metabolismo de los fosfolípidos en la adaptación a los suelos bajos en fósforo, temperaturas bajas frías y tiempo de floración -tres factores importantes que determinan el crecimiento de las plantas en valles altos- también se ha descrito en la especie modelo Arabidopsis. En esta propuesta vamos a utilizar poblaciones de mapeo de maíz desarrolladas con variedades mexicanas adaptadas a valles altos y bajos y mutantes implicados en el metabolismo de fosfolípidos, crecidas en experimentos de jardín común en valles bajos y altos así como en cámaras de cultivo simulando estas condiciones. Después utilizaremos un fenotipado bioquímico de alta precisión (cromatografía de líquidos de alto rendimiento acoplada a espectrometría de masas) para obtener un perfil de fosfolípidos e identificar posibles patrones de adaptación a valles altos en las vías metabólicas de fosfolípidos incluyendo incluyen la arquitectura genética subyacente que lo controla.

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Project Plan (Uploaded File): Download
Bibliography (Uploaded File): Download

Attachment (Uploaded File): Nothing Uploaded

Budget Summary:

	UC Campus	Mexican Institution	Total
Salary	$0.00	$0.00	$0.00
Employee Benefits	$0.00	$0.00	$0.00
Supplies & Services	$15,000.00	$7,000.00	$22,000.00
Travel	$0.00	$3,000.00	$3,000.00
Other Costs	$0.00	$0.00	$0.00
Total Request	$15,000.00	$10,000.00	$25,000.00

Budget Detail:
UC Campus

Salaries: $0.00

Employee Benefits: $0.00

Supplies & Services: $15,000.00
$15,000 USD High Troughput Polar Lipid Profilling

600 hundred samples will be analyzed at the Genome Center West Coast Metabolomics Center using HPLC-MS methods developed by the Fiehn Laboratory.

Travel: $0.00

Other: $0.00

Mexican Institution

Salaries: $0.00

Employee Benefits: $0.00

Supplies & Services: $7,000.00
$2,000 RT and Q-PCR analysis
To cover expenses to analyze gene expression of genes involved in phospholipid synthesis pathway from samples

$5,000 USD Field charges.
Two sites (Highland (Metepec) and Lowland (Nayarit). Charges will cover shipping (as required), plant growth and management, and harvest. Additional labor/accommodation costs associated with pollination and phenotypic characterisation will be covered separately by Rellán-Álvarez Lab.

Travel: $3,000.00
$3,000USD Student study stays
Subsistence to support 1 Mexican student stay of 2 months in the US ($1,250 student/month). Support will be used to supplement that available through CONACYT student scholorships to cover the stay and travel to Davis.

Other: $0.00

The role of phospholipids in maize adaptation to Mexican highlands

Background

The ancestor of maize, Balsas teosinte (*Zea mays ssp. parviglumis*), is native from low to mid elevation regions of southwest Mexico. A second subspecies, Chalco teosinte (*Zea mays ssp. mexicana*), is distributed in the cooler, drier highlands of central Mexico, over 1800 meters. Maize (*Zea mays ssp. mays*) was domesticated from *parviglumis* about 9,000 years ago in the Balsas valley (Matsuoka et al., 2002). After domestication, maize colonized the Mexican highlands, possibly aided by significant introgression of adaptive variation from *mexicana* (Hufford et al., 2013). In the Mexican highlands, maize was exposed to higher levels of radiation, lower air pressure, temperature and precipitation; furthermore, owing to the volcanic nature of the Mexican highlands, maize had to adapt to Andosol soils, characterized by low bio-availability of phosphorus (Krasilnikov and Carmen Gutiérrez-Castorena, 2013). Therefore, maize colonization of these highland areas represents a great model to study plant local adaptation. While several studies using evolutionary and population genomics approaches have described the patterns of genetic differentation associated with maize adaptation to the Mexican highlands, studies to functionally validate the underlaying metabolic changes involved in these processes are still largely missing.

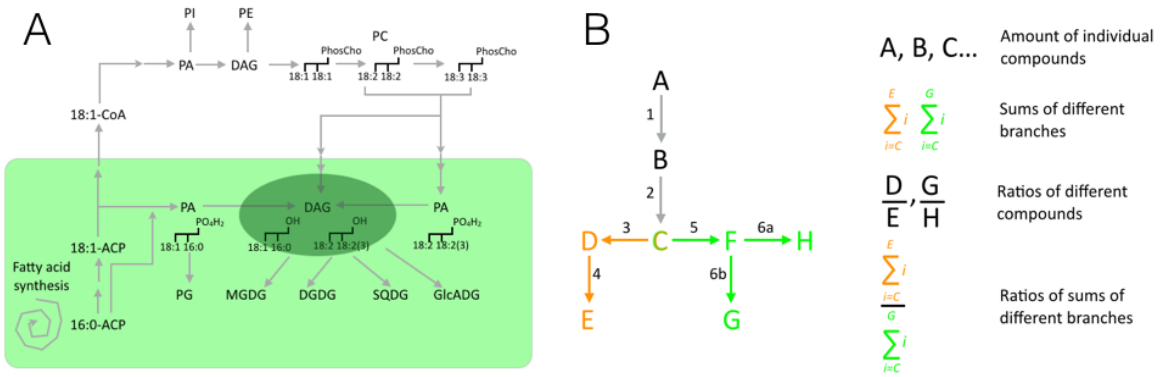
Plants growing in phosphorus limiting soils develop a series of metabolic strategies, including recyling of P containing compounds such as RNA and phospholipids. Phospholipids constitute around 1/3 of the available phosphorus in the plant and are the major component of membranes, specially in chloroplasts (Veneklaas et al., 2012). In addition to their structural role, some of the most abundant phospholipids such as phosphatidylcholine also have interesting signaling functions and are involved in multiple regulatory processes associated with environmental stimuli (Xue et al., 2009). Plants challenged with low temperatures tend to increase the proportion of phospholipids in the composition of membranes with respect to other polar lipids such as galactolipids and sulfolipids (Degenkolbe et al., 2012), probably as a mechanism to modulate membrane fluidity. On the other hand, plants under phosphorus deficiency recycle phospholipids -to free up phosphate- and substitute them with galactolipids and sulfolipids (Veneklaas et al., 2012). The Mexican highland transvolcanic belt that maize colonized is characterized by both lower temperatures and phosphorus bio-availability. Therefore during the process of adaptation to Mexican highlands we hypothesize that maize phospholipid metabolic pathways were under a high selective pressure. Although these pathways are relatively well known (see figureure 1A), recent results suggest that the diversity of polar lipids involved may be greater than we expected (Okazaki et al., 2013). Okazaki and colleagues discovered that Arabidopsis plants challenged with low concentrations of phosphorus were able to synthesize glucuronosyldiacylglycerol using the sulfoquinovosyldiacylglycerol pathway enzymes (Okazaki et al., 2013).

There are several studies that point to an important role of phospholipids in maize highland adaptation. Takuno and colleagues identified genes showing signals of selection between lowland and highland maize populations in Mesoamerica and South America (Takuno et al., 2015). Approximately 90 genes were identified in common between Meso American and South American populations, over a third of which belonged to Gene Ontology groups related to the synthesis of lipids / phospholipids. Interestingly, one of these genes is described as a CONSTANS interacting protein. CONSTANS is a transcription factor that is upstream of the FLOWERING LOCUS T (FT) transcription factor, which controls flowering time by integrating daylength signals.
Using a maize diversity panel, lipid profiles have been shown to be good predictors of flowering time (Riedelsheimer et al., 2012). In *Arabidopsis thaliana*, FT binds to di18:1-PC, a species of phosphatidylcholine (PC) -the most abundant phospholipid in plants- *in vitro* (Nakamura et al., 2014). When the proportion of PC with respect relative to phosphoethanolamine (PE) is genetically reduced () flowering time is reduced, probably because the FT complex with PC favors the union of FT to the promoter

of FT target genes. Additionally, both PC and other intermediate metabolites of PC synthesis are essential for proper embryo development (Lin et al., 2015).

In this project, we will systematically study the role of phospholipid metabolism in maize adaptation to highland conditions. Using high throughout liquid chromatography coupled to mass spectrometry and lipid mass spectra databases to quantify lipids involved phospholipid metabolism. We will use maize genotypes adapted to highland and lowland conditions and a combination of growth chamber experiments simulating highland (low phosphorus and temperature) and lowland (high phosphorus and temperature) and common garden experiments in Mexican highland and lowland locations.
In particular, the maize genotypes that will use are:

- Palomero Toluqueño (landrace adapted to highland conditions) and B73 (US inbred adapted to lowland conditions) maize lines and mutants of genes involved in PC biosynthesis grown in growth chamber with a factorial experiment combining low and high temperature and low and high phosphorus concentrations simulating highland and lowland conditions. In this plant material we will also collect tissue to study the expression of genes involved in the PC pathway.
- A mapping population composed of 100 Palomero Toluqueño (PT, a Mexican highland landrace) x B73 (sequenced US inbred line) Recombinant Inbred Lines grown in the same highland and lowland locations.
- A diversity panel of 30 highland and 30 lowland Mexican maize landraces grown in common gardens experiments in highland (Metepec, 2500 masl) and lowland (Nayarit, 25 m masl) field sites.



figureure 1 A) Biosynthetic pathway of plant glycerolipids. Adapted from Okazaki and cols(Okazaki et al., 2013). Abbreviations: B) Different metabolic variables that can be used in association or linkage mapping studies. Letters represent metabolites and numbers enzymes of an hypothetical pathways.

Hypothesis

We hypothesize that phospholipid metabolism reorganization played a key role in the adaptation of maize to the highlands of Mexico.

Relevance to the UC-Mexus program goals

The current project brings together a young research group in Langebio, México (Rubén Rellán-Álvarez) interested in understanding the biochemical basis of maize local adaptation and a well stablished group (Oliver Fiehn) that has developed methods and databases for lipid analysis in the Genome Center at UC Davis. Rellán-Álvarez has a proven record on the study of how abiotic stresses shape

different plant metabolic pathways and is starting an ambitious research program to understand at the biochemical level how maize has adapted to highland elevations in México. Oliver Fiehn is a world expert in the development and application of metabolomics techniques. His lab has developed analytical methods and data pipelines to identify a great variety of lipids (Kind et al., 2013). This project will enable precise, high throughput biochemical analysis in the UC Davis Genome Center of plants grown in different field locations in México. Rubén Rellán-Álvarez and Oliver Fiehn have experience in carrying out this type of collaborative research. During his PhD, Rubén Rellán-Álvarez visited the lab of Oliver Fiehn several times to perform similar types of work and worked on the logistics of preparing, sending and analyzing the samples in collaboration. Now that Rellán-Álvarez has started his own lab in México this project would provide a fantastic opportunity to establish new collaborations trough common visits and stays of Mexican students that are starting their Masters program in the laboratory of Rellán-Alvarez. In particular, the work of two students, Karla Juarez and Estefany Sánchez are expected to benefit from this collaboration.
Furthermore, is expected that the techniques developed in the Fiehn lab will be transferred to Langebio´s metabolomics lab.

General Aim

The general aim of this proposal is to systematically characterize the changes in phospholipid profiles and expression of genes involved in phospholipid synthesis to shed light on the possible role of phospholipids in Maize highland adaptation.

Specific Aims and relevant methodology

Aim 1. Characterize the profile of phospholipids and other polar lipids, as well as the expression patterns of genes involved in the synthesis of those lipids in B73 and Palomero Toluqueño

Rationale, design and potential problems

In this aim we will get a first characterization of the effect of phosphorus deficiency and cold the phospholipid profiles and the expression of genes involved in PC synthesis in two well characterized genetic backgrounds: B73 and Palomero Toluqueño (a highland adapted landrace). Plants will be grown in a growth chamber simulating highland and lowland weather conditions by regulating the chamber's temperature. The growth chamber's conditions for highlands will be 20°C during the daylight and 10°C during the night time. While for lowlands daylight temperature will be 30°C and 20°C during the night. For both experiments the photoperiod will be 14 hours of light and 10 h of darkness. At each of the temperature conditions half of the plants will be grown under low phosphorus and half under control phosphorus concentrations. So each genotype will be grown under two different phosphorus and temperature conditions. Before this experiment we will optimize lipid extraction procedures to adapt it to maize leaf tissues but once this is optimized we don´t expect too much complications as the rest of the procedures are already performed regularly in both labs. This experiment will set the basis for future experiments using B73xPT F1s to study Allele Specific Expression (ASE) using targeted RNA-Seq in collaboration with the laboratory of Daniel Runcie at the department of Plant Sciences UC Davis. Samples will also be collected to study methylation patterns of phospholipid pathway genes in collaboration with the laboratory of Luis Herrera-Estrella that have recently found interesting methylation pattens of genes involved in lipid recycling upon phosphorus deficiency in *Arabidopsis* plants (Yong-Villalobos et al., 2015).

Methodology

Plants will be grown for a period of 2-3 weeks. Samples will be collected at V3 stage from the youngest full developed leaf. Maize samples will be extracted according to the method of Matyash et al. (Matyash et al., 2008). Phospholipid analysis will be performed at the West Coast Metabolomics Center in UC Davis. Separation and identification of lipids will be accomplished using a method that has been optimized, in the laboratory of Oliver Fiehn, with UPLC-QTOFMS electrospray on modes negative and positive. The method allows the identification of about 500 types of lipids including phospholipids, galactolipids, and sulfolipids. Further identification will be done using the LipidBlast data base (Kind et al., 2013) developed in the laboratory of Oliver Fiehn. Samples will also be collected from the opposite side of the leaf midrib for RT-qPCR analysis of the genes involved in phosphatidylcholine biosynthesis pathway See figureure 1.

Aim 2. Characterize the natural variation and genetic architecture of phospholipid metabolism and identify regions of the genome (QTLs) controlling the re-organization of polar lipids in response to highland conditions

Rationale, design and potential problems

In this aim we will study the natural variation of lipid profiles from a collection of 30 highland and 30 lowland adapted Mexican landraces. This landraces (15 plants each) will be planted in March 2017 in a common garden experiment in both lowland (Nayarit field site) and highland conditions (Metepec field site). Alongside these landraces we will grow a collection of BC1S5 (Backcross with B73 and then selfed during 5 generations) 100 Recombinant Inbred Lines of B73xPT. Samples will be collected at V3-V5 (we expect different rates of development between the different landraces) from 3-5 plants per landrace and RILS in the field, stored in dry ice and transported to the laboratory in Langebio for extraction. Extracted samples will then be shipped to the UC Davis Genome Center for analysis by the laboratory of Oliver Fiehn. Lipid analysis will be performed as described in the previous aim. Phospholipid profiling data from the landraces will give us a very valuable information about genetic highland/lowland genetic variability in the synthesis of these compounds and on the environmental effect of highland and lowland growth conditions. Given the genetic diversity of our panel and the fact that previous data point to an important role of phospholipid metabolism since highland conditions imposed a high selective pressure in these pathways we expect to find great biochemical diversity and even new compounds as recently shown in the model plant *Arabidopsis* (Okazaki et al., 2013).

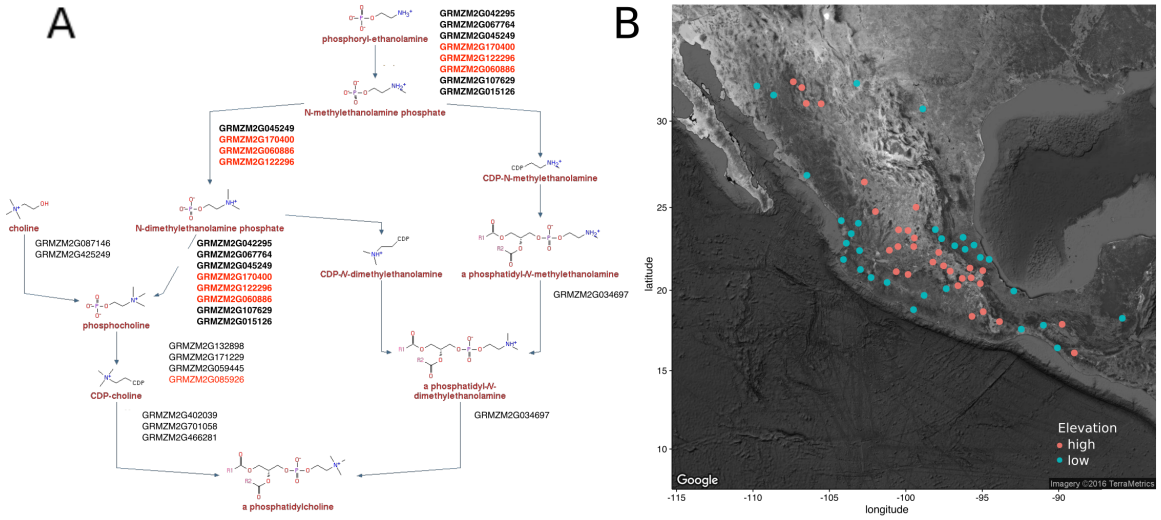
Since, metabolites are the end products of the metabolism they provide a good proxie to the quantify the activities of the enzymes that synthesize them. When combined with tools like linkage or association mapping of populations showing differences to the environmental perturbation being studied, metabolic phenotyping offers a very powerful tool to identify loci and ideally the underlying genes or allelic differences explaining metabolite synthesis and function (Wen et al., 2014). If the pathway of interest is know, metabolite quantification can provide not only information about the amount of different metabolites but also the ratio of different metabolites, pathway branches etc., as indicated in figure 1B. The LC-MS methods developed in the lab of Oliver Fiehn can identify around 400 different species of lipids including those described in figure 1A that are relevant for this project. Using this data we will be able to ask if a certain pathway is enhanced in certain highland or lowland genotypes and by analyzing samples of plants grown in highland and lowland conditions we will be able to tell if certain pathways can be considered adaptive to highland conditions. To do this we will calculate the different set of biochemical phenotypes described in figure 1B and also the ratios between highland and lowland accessions pairs and between highland and lowland conditions.

These biochemical phenotypes obtained from the RILS will be used to run QTL analysis (Broman, 2015). The use of ratios between two metabolite (or sums of metabolites) concentrations as phenotypes in GWAS and QTL analysis has been shown to provide more information than the corresponding metabolite concentrations alone, to identify the allele differences that explain changes in metabolite concentrations (Petersen et al., 2012). The rationale is that ratios are a good proxie to enzymatic

activities and that allelic differences lead to different enzymatic activities. When combined with experiments done in conditions where certain pathways could be considered adaptive, this effect known as *p-gain* becomes very powerful to identify the genetic architecture of

Once QTLs are identified we will use these QTLs to run association mapping on the set of 60 landraces (for which there is already genotypic (GBS) data from the Cimmyt Seeds of Discovery project).

The problems that we expect here are related with the nuances of field sampling experiments. However we have already grown and collected samples from both field sites in collaboration with the laboratory of Ruairidh Sawers at Langebio.



figureure 2 A) Phosphatidylcholine biosynthesis super pathway indicating the different pathways by which PC can be synthesized in maize. Predicted genes are indicated. (In bold those that can perform different steps of the pathway, in red those for which we are already homozygous single insertional mutants). Data was obtained from the Plant Metabolic Network, CornCyc webpage. B) Localization of the 30 highland and 30 lowland maize accessions that will be analyzed in this project. Accessions were selected and ordered from the Cimmyt Seed database as follows: 1) accessions were divided in high elevation (>2000 masl) or low elevation (< 1000 masl) classes; 2) 30 high elevation accessions were randomly chosen with the restriction that they cannot be closer than 50 km from each other; 3) for each a highland line a lowland `pair` was chosen within one degree of latitude band. These lines were selected by Daniel Runcie (UC Davis). As part of another project, they will be test-crossed with B73 the F1s used for RNA-Seq Allele Specific Expression (ASE) in highland and lowland conditions. Combination of lipidomics and transcriptomics data will aid into a better understanding of how this mechanisms are regulated. (See letter of support by Dan Runcie).

Aim 3. Identify the effect of mutations of genes involved in the Phosphatydilcholine biosynthesis pathway

Rationale, design and potential problems

In collaboration with the laboratory of Ruairidh Sawers, the lab of Rubén Rellán-Álvarez is developing different lines carrying insertional mutations in key genes of the Phosphatydilcholine biosynthesis. We have mutants of different alleles homologues to the *Arabidopsis* gene xipotl (Cruz-Ramirez et al., 2004), that encodes an enzyme with phospho-ethanolamine N-methyltransferase activity. In *Arabidopsis*, three different enzymes are needed to complete three consecutive N-methylation steps that are carried out on phospho-bases, phosphoethanolamine, phospho-N-methylethanolamine, and

phospho-N-dimethylethanolamine to produce PC, but in maize, all three steps can be carried out by at least 4 different *xipotl* alleles: GRMZM2G045249, GRMZM2G170400, GRMZM2G122296 and GRMZM2G060886. We have UniformMu or Ds insertional mutant stocks for the last three of them and we are in the process of finding homozygous lines for all of them. We also have a UniformMu insertional mutants of GRMZM2G085926 that encodes an enzyme in the Kennedy pathway that synthesizes PCl *de novo* from choline. Using the same experimental design as in Aim 1 all these mutants will be grown in high/low temperature and phosphorus conditions. Samples will be taken for phospholipid analysis to study the effect of the mutations in phospholipid profiles. Samples will also be collected for RT-qPCR of the genes involved in the PC super pathway. These data will allow us to study if: - we can modify PC concentrations in maize plants and in the future explore the effect of altering this concentrations in flowering time and more - compensation mechanisms between the different branches of the PC pathway exist and what is the importance of each of these pathways in Morphological traits of seedling will also be scored to identify effects in leaf and root development.
In the future this information will be used to design field studies where mutants of these genes will be evaluated in highland and lowland conditions and directly test the effect of the genes in plant fitness in contrasting growing environments. To this end, we have already started making crosses between these insertional mutants and selected RILS of the PTxB73 mapping population with extreme flowering times to test if changes in PC concentrations can indeed modify flowering times in field conditions (Nakamura et al., 2014).

Crosses and genotyping of all the mutants is well underway. Field experiments won't be possible within the timeframe of the UC-Mexus project but data generated in the growth chambers will guide theses experiments in the future.

Summary

In summary we will use precise biochemical phenotyping of phospholipids and other polar lipids to test the hypothesis that changes in phosphoplipid metabolism is an adaptive trait to maize highland adaptation in México. We will carry out a combination of growth chamber and field common garden experiments using maize lines that are adapted to highland and lowland conditions.

Work Group Contributions

RRA group, will carry out growth chamber and field experiments, perform lipid and DNA isolation and make maize crosses and genotyping to generate homozygous mutants of PC synthesis OF group will perform HPLC-MS analysis of samples to generate lipid profile data and train visiting students from RRA lab in data analysis. RRA and OF will work jointly on data analysis of data. RRA group will work closely with the metabolic services at Langebio to implement lipid profile analysis in Langebio.

Potential for Future Funding

Several parts of this project will be done in collaboration with the labs of Ruairidh Sawers and Daniel Runcie (see letters of support), that are already working on maize highland adaptation (not sure if it will be good here to mention the NSF project). Data generated in this project would benefit from data generated by these two labs and may constitute the basis for future grant applications. The lab of Rellán-Álvarez has also submitted an application to the Conacyt Fronteras de la Ciencia (awaiting decision) that would build on the results that could be obtained in this project.

Timeline

Aim 1
Feb - March (2016): Grow B73 and PT in growth chamber under highland and lowland conditions and low and high phosphorus.

March - May (2016): Optimize lipid extraction. UPCL-QTOFMS Lipid profile analysis.

June - July (2016): RT-qPCR analysis and gene methylation patterns from leaf samples taken.

Aim 2
March - April (2017): Sowing of 30 highland and 30 lowland landraces, in Nayarit and Metepec fields respectively. Grow 100 B73xPT RILs, collect V3 leaf samples.

April - May (2017): Lipid extraction and lipid profile analysis.

June - July (2017): QTL identification.

Aim 3
June - August (2016): Generation of insertional mutants on PtdCho synthesis genes. Study mutants for xipotl alleles.

September - October (2016): Find homozygous lines for maize xipotl alleles.

October - November (2016): Grow mutants in growth chamber under Aim 1 conditions. Take V3 leaf samples.

November - December (2016): Lipid extraction. UPCL-QTOFMS Lipid profile analysis. RT-qPCR analysis and gene methylation patterns of leaf samples.

Future Aims
- Crosses and genotyping of PtdCho maize mutants. - Grow mutants in highland and lowland fields.
- Analyze flowering time and root development phenotyping.

Broman KW (2015) R/qtlcharts: interactive graphics for quantitative trait locus mapping. Genetics **199**: 359–361

Cruz-Ramirez A, López-Bucio J, Ramírez-Pimentel G, Zurita-Silva A, Sanchez-Calderon L, Ramírez-Chávez E, González-Ortega E, Herrera-Estrella L (2004) The xipotl mutant of Arabidopsis reveals a critical role for phospholipid metabolism in root system development and epidermal cell integrity. The Plant Cell **16**: 2020–2034

Degenkolbe T, Giavalisco P, Zuther E, Seiwert B, Hincha DK, Willmitzer L (2012) Differential remodeling of the lipidome during cold acclimation in natural accessions of Arabidopsis thaliana. The Plant Journal **72**: 972–982

Hufford MB, Lubinksy P, Pyhäjärvi T, Devengenzo MT, Ellstrand NC, Ross-Ibarra J (2013) The genomic signature of crop-wild introgression in maize. PLoS Genetics **9**: e1003477

Kind T, Liu K-H, Lee DY, Defelice B, Meissen JK, Fiehn O (2013) LipidBlast in silico tandem mass spectrometry database for lipid identification. Nature methods **10**: 755–758

Krasilnikov P, Carmen Gutiérrez-Castorena M del (2013) The soils of Mexico.

Lin Y-C, Liu Y-c, Nakamura Y (2015) The Choline/Ethanolamine Kinase Family in Arabidopsis: Essential Role of CEK4 in Phospholipid Biosynthesis and Embryo Development. The Plant Cell **27**: 1497–1511

Matsuoka Y, Vigouroux Y, Goodman MM, Sanchez G J, Buckler E, Doebley J (2002) A single domestication for maize shown by multilocus microsatellite genotyping. Proceedings of the National Academy of Sciences of the United States of America **99**: 6080–6084

Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D (2008) Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. Journal of lipid research **49**: 1137–1146

Nakamura Y, Andrés F, Kanehara K, Liu Y-c, Dörmann P, Coupland G (2014) Arabidopsis florigen FT binds to diurnally oscillating phospholipids that accelerate flowering. Nature Communications **5**: 3553

Okazaki Y, Otsuki H, Narisawa T, Kobayashi M, Sawai S, Kamide Y, Kusano M, Aoki T, Hirai MY, Saito K (2013) A new class of plant lipid is essential for protection against phosphorus depletion. Nature Communications **4**: 1510

Petersen A-K, Krumsiek J, Wägele B, Theis FJ, Wichmann H-E, Gieger C, Suhre K (2012) On the hypothesis-free testing of metabolite ratios in genome-wide and metabolome-wide association studies. BMC Bioinformatics **13**: 120

Riedelsheimer C, Lisec J, Czedik-Eysenberg A, Sulpice R, Flis A, Grieder C, Altmann T, Stitt M, Willmitzer L, Melchinger AE (2012) Genome-wide association mapping of leaf metabolic profiles for dissecting complex traits in maize. Proceedings of the National Academy of Sciences **109**: 8872–8877

Takuno S, Ralph P, Swarts K, Elshire RJ, Glaubitz JC, Buckler ES, Hufford MB, Ross-Ibarra J (2015) Independent Molecular Basis of Convergent Highland Adaptation in Maize. Genetics genetics.115.178327

Veneklaas EJ, Lambers H, Bragg J, Finnegan PM, Lovelock CE, Plaxton WC, Price CA, Scheible W-R, Shane MW, White PJ et al (2012) Opportunities for improving phosphorus-use efficiency in crop plants. New Phytologist **195**: 306–320

Wen W, Li D, Li X, Gao Y, Li W, Li H, Liu J, Liu H, Chen W, Luo J et al (2014) Metabolome-based genome-wide association study of maize kernel leads to novel biochemical insights.

Nature Communications 5:

Xue H-W, Chen X, Mei Y (2009) Function and regulation of phospholipid signalling in plants. Biochemical Journal **421**: 145–156

Yong-Villalobos L, González-Morales SI, Wrobel K, Gutiérrez-Alanis D, Cervantes-Peréz SA, Hayano-Kanashiro C, Oropeza-Aburto A, Cruz-Ramirez A, Martínez O, Herrera-Estrella L (2015) Methylome analysis reveals an important role for epigenetic changes in the regulation of the Arabidopsis response to phosphate starvation. Proceedings of the National Academy of Sciences **112**: E7293–302

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Fiehn, Oliver	Professor
eRA COMMONS USER NAME OLIVERFIEHN	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Berlin Free University	diploma	1993	Analytical Chemistry
Berlin Technical University	PhD	1997	Analytical Toxicology
Max-Planck Institute Mol. Plant Physiol.	Postdoc	1998-99	Metabolomics

A. Personal Statement

As Director of the NIH West Coast Metabolomics Center at UC Davis I am committed to provide research and services conducting research in metabolomic method developments and biomedical applications. Combined, I oversee the operation of 33 staff using 14 mass spectrometers. I have pioneered developments and applications in metabolomics since 1998 with over 130 publications to date, and am keen to improve these methods with the aim of further standardization and higher content in biochemical information through automatic annotation of metabolites and by using a range of chemical and biochemical database identifiers with every report we generate. For the Metabolomics Society, I have chaired the efforts in standardizing metabolomic reports, and the developments and services in our Center will enable researchers to maximize interpretation of metabolomic findings using a variety of metabolomics, statistical, informatics and genomic approaches.

B. Positions and Honors

Positions and Employment

1994-1997 Research scientist, Technical University of Berlin, Germany
1998-1999 Postdoctoral research scientist, Max-Planck Inst. Molecular Plant Physiology, Potsdam, Germany
1999 Visiting postdoctoral research scientist, Dept. Molecular Biol., University of Wash., Seattle, WA
1999-2004 Research group leader, Max-Planck Inst. Molecular Plant Physiol., Potsdam, Germany
2004-2009 Assoc. Professor, Dept. of Molecular & Cellular Biology, UC Davis Genome Center, CA
2009- Full Professor, Dept. of Molecular & Cellular Biology, UC Davis Genome Center, CA
2012- Director, NIH West Coast Metabolomics Center

Other Experience, Honors and Professional Memberships

2014 Molecular & Cellular Proteomics Lecture Award
2014 Metabolomics Society Lifetime Achievement Award
2012 Phi Lambda Upsilon Rho –Chapter award, Lincoln, NE
2009 Distinguished Scientist Award, CCDG, Chicago
2004-2010, 2013-15 Board of Directors, Metabolomics Society
2008-2010 Treasurer, Metabolomics Society
2006-2011 Editorial Board 'Journal of Biological Chemistry'
2004- Editorial board member 'Metabolomics' and 'Plant Methods'
2010- Editorial board member "Rapid Communication in Mass Spectrometry"
2008, 2009, 2015 Organizer, Metabolomics Society Conference, Boston, Edmonton, San Francisco
2011 Organizer and Chairman, ASMS Asilomar meeting on "Metabolomics"

C. Contribution to Science

Early contributions 1994-1998 were geared at analytical toxicology, specifically in finding unknown organics from complex industrial wastewaters that exerted toxic effects to aquatic organisms. The central theme of this work, identification of unknowns and untargeted analyses, founded the corner stone of postdoctoral and group leader work in Molecular Plant Physiology at the Max-Planck Institute in Germany, then called metabolite

profiling. As group leader in Germany, and then as Professor at UC Davis, this work led to the now flourishing field of metabolomics and its application in biology and medicine.

1. **Development of metabolomics as a technology in biological studies.** The Fiehn laboratory has pioneered metabolomics since 1998. The major idea was to go beyond hypothesis-driven science towards discovery science, broadening the horizon for pleiotropic biological effects and discerning gene functions for orphan genes, novel biochemical pathways and defining metabolic phenotypes, or metabotypes, as central theme of the function of the molecular machinery of cells and organism. We had first used untargeted metabolomics in a seminal publication on plant leaves (Fiehn et al, 2000), opening the door to large scale comparisons of mutant phenotypes by gas chromatography/mass spectrometry. We then added liquid chromatography/mass spectrometry, specifically for hydrophilic metabolites using HILIC techniques and identified many unpublished and novel metabolites discovered in plant phloem (Tolstikov et al, 2003). Realizing the need to link different types of –omics data, we validated protocols to use a single sample for profiling RNA and proteins as well as metabolites, using specific inhibitors and optimized buffer / sample ratios (Weckwerth et al, 2004). More recently, we have focused on workflows for identifying the hundreds of unknown metabolic signals that are typically found in samples (Kumari et al, 2011) of either mammalian or plant or microbial origins. This work remains important to stretch metabolomics to i ts true potential of 'discovery' type work, while robustness and quantifyability becomes an ever more important topic in epidemiological studies.

Fiehn O, Kopka J, Dörmann P, Altmann T, Trethewey RN, Willmitzer L (2000) Metabolite profiling for plant functional genomics. Nature Biotechnology 18: 1157-1161

Tolstikov VV, Lommen A, Nakanishi K, Tanaka N, **Fiehn O** (2003) Monolithic silica-based capillary reversed-phase liquid chromatography/electrospray mass spectrometry for plant metabolomics. Analytical Chemistry 75: 6737-6740

Weckwerth W, Wenzel K, **Fiehn O** (2004) Process for the integrated extraction, identification and quantification of metabolites, proteins and RNA to reveal their co-regulation in biochemical networks. Proteomics 4: 78-83

Kumari S, Stevens D, Kind T, Denkert C, **Fiehn O** (2011) Applying in-silico retention index and mass spectra matching for identification of unknown metabolites in accurate mass GC-TOF mass spectrometry. Analytical Chemistry 83: 5895-5902

2. **Large scale data processing and databases to study metabolism.** Analytical Chemistry was, and partly still is, dominated by reports on single methods and advances in separation. While this view was valid when only a few target molecules were analyzed, metabolomics quickly encountered the challenge to store, compare and disseminate large data sets in publicly available databases that are compliant to data standards developed by community efforts. The Fiehn laboratory takes an active lead in this process and continues to develop and implement novel methods, libraries and databases to improve the status in the field. Given the enormous complexity of metabolism, the Fiehn laboratory now extends libraries of authentic standards to virtual mass spectral predictions, for example for over 200,000 lipids in 29 lipid classes (Kind et al, 2013). Secondly, the laboratory has amassed a 'Rosetta Stone' to translate names and chemical identifiers between over 250 different genomic and chemical databases, termed the Chemical Translation Service (Wohlgemuth et al, 2010). Ultimately, biological interpretations of metabolomic signatures are aided by visualizing all identified metabolites in network graphs via biochemical substrate/product relationships and adding compounds without enzymatic assignments by chemical structure similarities, or by mass spectral similarities (for unknown compounds) (Barupal et al, 2012).

Kind T, Liu K-H, Lee DY, DeFelice B, Meissen JK, **Fiehn O** (2013) LipidBlast in silico tandem mass spectrometry database for lipid identification. Nature Methods 10: 755-758

Wohlgemuth G, Haldiya PK, Willighagen E, Kind T, **Fiehn O** (2010) The Chemical Translation Service—a web-based tool to improve standardization of metabolomic reports. Bioinformatics 26: 2647-2648

Barupal DK, Haldiya PK, Wohlgemuth G, Kind T, Kothari SL, Pinkerton KE, **Fiehn O** (2012) MetaMapp: mapping and visualizing metabolomic data by integrating information from biochemical pathways and chemical and mass spectral similarity. BMC Bioinformatics 13: 99

Kind T, Wohlgemuth G, Lee DY, Lu Y, Palazoglu M, Shahbaz S, **Fiehn O** (2009) FiehnLib: mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. Analytical Chemistry 81: 10038-10048

3. **Discovery of metabolic dysfunctions in human health.** Metabolomics serves two overlapping functions: describing metabolic phenotypes to serve as diagnostic tools in the onset and progression of human diseases, and finding the biochemical mechanisms and causes for diseases by assigning differences in genes or protein functions, as well as the effects of nutrition on the metabolic network and microbiota. For example, we were able to pinpoint a probable differences in diabetic and non-diabetic African-Americans by using metabolomics (Fiehn et al, 2010). In cancer metabolism, we discovere metabolic differences in breast tumors and lung cancers, highlighting the critical roles for lipid metabolism (Hilvo et al, 2011) as well as glutamine dependencies (Budczies et al, 2013). We are further actively investigating diseases of the metabolic syndrome, including diabetes and cardiovascular events, specifically focusing on acylcarnitines and nutritional interventions (Fiehn et al, 2010). We also discovered metabolic differences in cell differentiations, for example between pluripotent and embryonic stem cells, for both primary and lipid metabolism (Meissen et al, 2012).

Hilvo M, Denkert C, Lehtinen L, Müller B, Brockmöller S, Seppänen-Laakso T, Budczies J, Bucher E, Yetukuri L, Castillo S, **Fiehn O**, Oresic M (2011) Novel theranostic opportunities offered by characterization of altered membrane lipid metabolism in breast cancer progression. Cancer research 71: 3236-3245

Budczies J, Brockmöller SF, Müller BM, Barupal DK, Richter-Ehrenstein C, Kleine-Tebbe A, Griffin JL, Orešič M, Dietel M, **Fiehn O,** Denkert C (2013) Comparative metabolomics of estrogen receptor positive and estrogen receptor negative breast cancer: alterations in glutamine and beta-alanine metabolism. Journal of proteomics 94: 279-288

Fiehn O, Garvey WT, Newman JW, Lok KH, Hoppel CL, Adams SH (2010) Plasma metabolomic profiles reflective of glucose homeostasis in non-diabetic and type 2 diabetic obese African-American women. PLoS One 5: e15234

Meissen JK, Yuen BTK, Kind T, Riggs JW, Barupal DK, Knoepfler PS, **Fiehn O** (2012) Induced pluripotent stem cells show metabolomic differences to embryonic stem cells in polyunsaturated phosphatidylcholines and primary metabolism. PloS one 7: e46770

4. **Discovery of metabolic mechanisms in plants, algae and microbes.** Mammalian metabolism describes the interaction of food, the microbiome and mammalian organs in a very complex fashion that makes it very difficult to distinguish cause and effects. For example, we have delineated the relative impact of xeno-metabolites originating from gut microbial metabolism in a controlled nutritional study that improved insulin resistance (Campbell et al, 2014). However, in general, studying metabolism is much easier in systems that have less complex interactions, for example in plants. The Fiehn laboratory has shown that some plants have two metabolically very distinct phloem systems, with the classic system delivering carbon loads but the second, extrafascicular system, responsible for defense and communication (Zhang et al, 2010). We have further discovered very specific enzymes that are responsible for repairing damaged or toxic metabolites, for example repairing the dysfunctional form of hydrated NADPH (Niehaus et al, 2014). On a model species, Chlamydomonas reinhardtii, we could demonstrate that these algae cells have systems that sense the total available nitrogen and counteract metabolic responses way before nitrogen resources are depleted, including up-regulation of novel signaling molecules and a stringent response pathway that was previously only shown for prokaryotes (Lee et al, 2012).

Campbell C, Grapov D, **Fiehn O**, Chandler CJ, Burnett DJ, Souza EC, Casazza GA, Gustafson MB, Keim NL, Newman JW (2014) Improved metabolic health alters host metabolism in parallel with changes in systemic xeno-metabolites of gut origin. PloS one 9: e84260

Zhang B, Tolstikov V, Turnbull C, Hicks LM, **Fiehn O** (2010) Divergent metabolome and proteome suggest functional independence of dual phloem transport systems in cucurbits. Proceedings of the National Academy of Sciences 107: 13532-13537

Niehaus TD, Richardson LGL, Gidda SK, ElBadawi-Sidhu M, Meissen JK, Mullen RT, **Fiehn O**, Hanson AD (2014) Plants utilize a highly conserved system for repair of NADH and NADPH hydrates. Plant physiology 165: 52-61

Lee DY, Park J-J, Barupal DK, **Fiehn O** (2012) System response of metabolic networks in Chlamydomonas reinhardtii to total available ammonium. Molecular & Cellular Proteomics 11: 973-988

D. Research Support - Current

Agilent (PI Fiehn, O. - UC Davis) 01/01/07 to 10/30/2016
Metabolomic libraries and methods by mass spectrometry
Goals: Improve metabolite identification by standardized QTOF and GC-quad MS analyses of reference compounds.

NIH / NIEHS ES020819-01 (PI Hockenbery, D - U Washington/Seattle) 09/01/11 to 08/30/2016
Biomarker discovery for mitochondrial toxicants using metabolic footprinting
Goals: To examine mitochondria dysfunction at sub-chemical thresholds, centered on testing fatty acid overload and metabolite biomarker discovery as a result of BDE-47 exposure in mouse hepatocytes.

P20 NIH HL113452 (PI Hazen, S. Cleveland, Fiehn, O. UC Davis; coPI) 06/01/12 to 05/30/2017
Functional Cardio-Metabolomics
Goals: Untargeted metabolomic analysis by GC-TOFMS and LC-QTOFMS from blood plasma of animal models+human cohorts.

NSF MCB 1153491 (PI Hanson U Florida, Fiehn, O. UC Davis; coPI et al) 04/01/12 to 03/31/2016
Metabolite repair – Uncovering the hidden support system for metabolic networks.

NSF MCB - 1139644 (PI Fiehn, O. - UC Davis) 12/01/11 to 11/30/2016
METABOLOMICS: Integrating cheminformatic resources for investigating photoautotrophic and mixtotrophic metabolism in algae
Goals: To implement novel metabolomic databases and tools for compound identification. To screen 10 algal species under different environmental conditions and map metabolomic databases to algal biomass growth.

NIH U24 DK097154 (PI Fiehn, O - UC Davis) 09/01/12 to 08/30/2017
The West Coast Central Comprehensive Metabolomics Resource Core.
Goals: To provide extensive metabolomic services to biological and medical researchers on the West Coast

NIH U01 DK097430 (PI Subramaniam, S. – UCSD, coPI Fiehn, O) 09/01/12 to 08/30/2017
The Metabolomics Data Center and Workbench (MDCW).
Goals: To provide a central data repository for the NIH regional Metabolomics resource cores.

American Seed Research Foundation (PI Bradford – UC Davis) 04/01/13 to 03/31/2016
Respiratory and Hormonal Metabolism Associated with Seed Germination, Vigor and Quality.
Goals: To investigate seed germplasm with respect to metabolomic changes during germination.

NIH / NIDDK (PI Norris, J - U Denver, CO) 09/01/14 to 08/30/2019
Nutrigenetics & -genomics of Vitamin D and Omega-3 Fatty Acids in Type 1 Diabetes
Goals: To test food biomarkers and metabolic signatures in type 1 diabetes cohort research.

Rubén Rellán Álvarez

- **e-mail**: rrellan@langebio.cinvestav.mx
- **website**:www.rrlab.com
- **twitter**: @rrellanalvarez
- **Github**: github.com/rellan

Last updated: January-24-2016

Career Summary and Education

Current Position:

Assistant Professor
National Laboratory of Genomics and Biodiversity, Cinvestav (2015-Present) Irapuato, Guanajuato. México.

Previous:

- **Postdoc** Department of Plant Biology (2012-2014) Carnegie Institution for Science at Stanford *Advisor*: José Ramón Dinneny
  Research Topic: Development of new methods to visualize root system architecture and gene expression of plants growing in soil.
- **PhD**: Plant Biology, Department of Plant Nutrition (2005-2011) Aula Dei Experimental Station, Zaragoza, Spain *Advisor*: Javier Abadía and Ana Álvarez-Fernández
  Research Topic: Long distance transport of iron and metabolomics of iron deficiency
- **MSc** Plant Biotechnology, Dept. of Biology (2002-2005) Universidad Autónoma de Madrid *Advisor*: Luis Eduardo Hernández in collaboration with Ana Álvarez-Fernández *Research Topic:* Heavy metal and oxidative stress
- **BS** Environmental Sciences (1998-2002) Universidad Autónoma de Madrid

Fellowships and Awards

- ASPB Annual Scientific Meeting Travel Award. Portland, Oregon (2014)
- Marschner Young Scientist Award. International Plant Nutrition Colloquium. Istanbul. Turkey (2013)
- ASPB Western Section Meeting Travel Award. Davis, California (2013)
- Postdoctoral Fellowship for Foreign Researchers (2012) Japanese Society for the Promotion of Science. (Declined)
- Long Term Postdoctoral Fellowship (2011) Federation of European Biochemical Societies. (Declined)
- Doctorate Extraordinary Price, Biology (2011) Autonomous University of Madrid.
- FPI PhD Fellowship (2005–2009). Spanish Ministry of Science.

Grants

- Conacyt Ciencia Básica Young Investigator. Natural Variation of lipid organization upon phosphorus deficiency. (PI, $ 90,000 USD)

Scientometrics Summary

- 20 Publications (2 Reviews)
- 1075 citations
- 9 first authorships
- h-index: 16
- h10: 18

More bibliographical info can be found here:

- Orcid-ID
- Google Scholar Webpage

Selected publications organized by research themes.

Metabolomics of the iron deficiency and resupply response

- Rellán-Álvarez R**, El Jendoubi H, Wohlgemuth G, Fiehn O, Abadía A, Abadía J, Álvarez Fernández A (2011) Metabolite profile changes in xylem sap and leaves of Strategy I plants in response to iron deficiency and iron resupply. Frontiers Plant Science 2: 66 PubMed
- **Rellán-Álvarez R**, Andaluz S, Rodríguez-Celma J, Wohlgemuth G, Zocchi G, Álvarez-Fernández A, Fiehn O, López-Millán AF, Abadía J (2010) Changes in the proteomic and metabolic profiles of Beta vulgaris root tips in response to iron deficiency and resupply. BMC Plant Biol. 10: 120 PubMed

Development of metabolite targeted profiling methods

- **Rellán-Álvarez R**, López-Gomollón S, Abadía J, Álvarez-Fernández A. (2011) Development of a new high-performance liquid chromatography electrospray ionization time-of-flight mass spectrometry method for the determination of low molecular mass organic acids in plant tissue extracts. J Agric Food Chem 59: 6864–6870 PubMed
- **Rellán-Álvarez R**, Hernández LE, Abadía J, Álvarez-Fernández A (2006) Direct and simultaneous determination of reduced and oxidized glutathione and homoglutathione by liquid chromatography-electrospray/mass spectrometry in plant tissue extracts. Anal Biochem. 356: 254-264 PubMed

Root Biology

- **Rellán-Álvarez R**, Lobet G, Dinneny J.R. (2016) Environmental control of Root System Biology Annual Rev Plant Biol. 67
- **Rellán-Álvarez R**, Lobet G, Hildner H, Pradier PL, Sebastian J, Yee MC, Yu G, La Rue T, Trontin C, Schrager A, Haney C, Nieu R, Maloof J, Vogel J, Dinneny JR (2015) GLO-Roots: an imaging platform enabling multidimensional characterization of soil-grown roots systems eLife 4:e07597 PubMed, Github repo

Long Distance Iron Transport and Metal Speciation

- Schüler M, **Rellán-Álvarez R**, Fink-Straube C, Abadía J, Petra Bauer (2012) New functions of nicotianamine in the phloem-based transport of iron to sink organs, in pollen development and in pollen tube growth. Plant Cell 24: 2380-2400 PubMed
- **Rellán-Álvarez R**, Giner-Martínez-Sierra J, Orduna J, Orera I, Rodríguez- Castrillón JA, García-Alonso JI, Abadía J, Álvarez-Fernández A (2010) Identification of a tri-iron(III), tri-citrate complex in the xylem sap of iron-deficient tomato resupplied with iron: new insights into plant iron long-distance transport. Plant Cell Physiol. 51: 91-102 PubMed
- **Rellán-Álvarez R**, Abadía J, Álvarez-Fernández A (2008) Formation of metal- nicotianamine complexes as affected by pH, ligand exchange with citrate and metal exchange. A study by electrospray ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 22: 1553-1562 PubMed

Heavy metal and oxidative stress in plants

- **Rellán-Álvarez R**, Ortega-Villasante C,Álvarez-Fernández A, Del Campo FF, Hernández LE (2006) Stress responses of *Zea mays* to cadmium and mercury. Plant Soil. 249: 41-50
- Ortega-Villasante C, **Rellán-Álvarez R**, Del Campo FF, Carpena-Ruíz RO, Hernández LE (2005) Cellular damage induced by cadmium and mercury in Medicago sativa. J Exp Bot. 56: 2239-2251 PubMed.

Selected conferences talks and invited seminars

Metabolomics of the iron deficiency and resupply response

- XV International Symposium on Iron Nutrition and Interactions in Plants, Budapest, Hungary (2010). **Rellán-Álvarez R**, El Jendoubi H, Wohlgemuth G, Abadía A, Fiehn O, Abadía J, Álvarez- Fernández A. Delving into iron deficiency metabolomics. **Selected Talk**
- XVI International Plant Nutrition Colloquium. Sacramento, California, USA (2009) **Rellán-Álvarez R**, Andaluz S, Álvarez-Fernández A, Fiehn O, López-Millán AF, Abadía J. Changes in the proteomic and metabolic profiles of Beta vulgaris root tips in response to iron deficiency and resupply **Keynote**

Root Imaging

- XVI National Congress of Biochemistry and Plant Molecular Biology, IX Simposium Mexico-USA Queretaro, México. (2015). **Invited Seminar** Towards a root system level understanding of how plants adjust root function and shape and integrate heterogeneous environmental cues. **Selected Talk**
- Rhizosphere 4, Maastricht, The Netherlands. (2015). **Roundtable Organization** Emerging technologies for root systems scale imaging and phenotyping.
- Instituto de Biotecnología, UNAM, Cuernavaca, México. (2015). **Invited Seminar** Multidimensional mapping of root responses to soil environmental cues using a luminescence-based imaging system.
- BASF 2014 Symposium on Unlocking Yield Potential in Soil. Limburgerhof, Germany (2014). **Invited Seminar**

- Annual Scientific Meeting of the American Society of Plant Biology. Portland, USA (2014). **Rellán-Álvarez R**, Muh-Ching Y, Pradier PL, Winfield E, Geng Y, Dinneny J. The Ground Truth: Understanding Root Physiology in Soil Using a Novel Imaging Platform. **Selected Talk**
- PAG XXII Plant Phenotypes Workshop. San Diego, USA (2014). **Rellán-Álvarez R**, Muh-Ching Y, Winfield E, Geng Y, Dinneny J. Growth and Luminescence Observatory of Roots (GLO-Roots) A platform for the Analysis of Root Structure and Physiology in Soil **Invited Seminar**
- XVII International Plant Nutrition Colloquium, Istanbul, Turkey (2013) Rellán-Álvarez R Growth and Luminescence Observatory of Roots (GLO-Roots) A platform for the Analysis of Root Structure and Physiology in Soil. **Invited presentation. (Marschner Young Scientist Award)**
- 30th Annual IPG Symposium on Root Biology, Columbia, Missouri. USA (2013) **Rellán-Álvarez R**, Muh-Ching Y, Geng Y, Dinneny J. Growth and Luminescence Observatory of Roots (GLO-Roots) A platform for the Analysis of Root Structure and Physiology in Soil. **Selected Talk**

Long Distance Iron Transport and Metal Speciation

- 3rd Japan-China Joint Workshop on Plant Nutrition, Kurashiki, Japan. (2011) **Rellán-Álvarez R**, Vázquez S, Álvarez-Fernández A, Abadía J. Iron xylem transport, the long and short of it. **Invited Talk**
- XVIII Reunión de la Sociedad Española de Fisiología Vegetal. XI Congreso Hispano-Luso de Fisiología Vegetal, Zaragoza, Spain (2009). Rellán-Álvarez R, Giner-Martínez-Sierra J, Orduna J, Orera I, Rodríguez-Castrillón JA, García-Alonso JI, Abadía J, Álvarez-Fernández A. Iron is transported as a tri-Fe(III), tri-citrate complex in plant xylem sap. **Selected Talk**

Biographical Sketch
Daniel Runcie

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Department of Plant Sciences
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Davis CA, 95616

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(530) 754-0411

(a) Professional Preparation:

Williams College	Biology	B.A.,	2005
Duke University	Biology	Ph.D.	2012
Duke University	Statistics	M.S.	2012
University of California Davis	NSF Postdoctoral Fellow in Biology		2013-2014

(b) Appointments:

2015-present Assistant Professor in Plant Sciences, University of California Davis
2013-2014 NSF Postdoctoral Fellow in Biology, University of California Davis

(c) Products:

(i) Five most closely related publications:

Burghardt, L., Runcie, D. E., Wilczek, A., Cooper, M., Roe, J., Welch, S. M., Schmitt, J. (2015). Fluctuating warm temperatures decrease the effect of a key floral repressor on flowering time in Arabidopsis thaliana. *New Phytologist*. http://dx.doi.org/10.1111/nph.13799

Donohue, K., Burghardt, L. T., Runcie, D. E., Bradford, K. J., & Schmitt, J. (2014). Applying developmental threshold models to evolutionary ecology. *Trends in Ecology & Evolution*. **http://www.cell.com/trends/ecology-evolution/abstract/S0169-5347(14)00250-X**

Garfield, D. A, Runcie, D. E, Babbitt, C. C., Haygood, R., Nielsen, W. J. and G. A. Wray. Evolvability and Robustness in a Developmental Gene Regulatory Network. PloS Biology. 11(10) e1001696EP–. http://dx.doi.org/10.1371%2Fjournal.pbio.1001696

Runcie, D. E., and Mukherjee, S. 2013. Dissecting High-Dimensional Phenotypes with Bayesian Sparse Factor Analysis of Genetic Covariance Matrices. Genetics, 194, 753-767. http://www.genetics.org/cgi/doi/10.1534/genetics.113.151217

Runcie, D. E., Garfield, D. A. Wygoda, J. A., Mukherjee, S. and G. A. Wray. 2012. Genetics of gene expression responses to temperature stress in a sea urchin gene network. Mol Ecol, 21, 4547-4562. http://dx.doi.org/10.1111/j.1365-294X.2012.05717.x

(ii) Other significant publications

Runcie D. E., Wiedmann, R., Archie, E. A., Altmann, J., Wray, G. A., Alberts, S. C., and J. Tung. 2013. Social environment influences the relationship between genotype and gene expression in wild baboons. Philos T Roy Soc B, 368, 20120345-20120345. http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2012.0345

Runcie, D. E. and M. A. F. Noor. 2009. Sequence signatures of a recent chromosomal rearrangement in Drosophila mojavensis. Genetica. 136 (1) pp. 5-11. http://dx.doi.org/10.1111/j.1365-294X.2012.05717.x

(iii) Synergistic activities

i. Software Development: Bayesian Sparse Factor Analysis of Genetic Covariance Matrices (BSFG) package implemented in MATLAB: http://www.stat.duke.edu/~sayan/bfgr/

ii. Reviewer: **Journals:** The Plant Cell, Evolutionary Ecology, Evolution and Development, Journal of Experimental Zoology Part A, Annals of Applied Statistics, New Phytologist, Nature Communications, European Conference on Computational Biology 2014, BMC Plant Biology, Plant Physiology, Molecular Ecology, Functional Ecology. **Grants:** Netherlands Organization for Scientific Research

iii. Workshop instruction: 3rd Annual Duke Systems Biology Symposium on Epistasis (2008)

iv. Mentoring: Mentored six graduate students in rotation projects. Mentored two undergraduate students in independent research projects, including one in UC Davis-Howard University Ecology and Evolution Graduate Admissions Pathways program.

v. Teaching: PLS205: Design and Analysis of Experiments at UC Davis. Teaching assistant for Cell and Development, General Microbiology, Animal Physiology, Duke University

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COOPERATIVE EXTENSION

UC-Mexus Program

March 6 2016

To whom it may concern:

I hereby express my support and excitement for the proposal submitted to the UC-Mexus Program by Dr. Rubén Rellán-Álvarez (Langebio, México) and Oliver Fiehn (UC Davis) titled: **The role of phospholipids in maize adaptation to Mexican highlands.**

I am studying similar aspects of maize adaptation to Mexican highlands, and have already selected a collection of paired lowland and highland landraces that Drs. Rellán-Álvarez and Fiehn will be analyzing in their project. In my own research, I will be using RNAseq to test for specific loci involved in highland adaptation using the technique of allele-specific expression (ASE) analysis. Specifically, we will create F1 crosses between either highland or lowland maize landraces and the B73 inbred, collect tissue from the F1 plants in highland or lowland environments, and use RNAseq to measure the relative abundance of the landrace vs B73 allele at each gene to test for consistently biased expression associated with elevation.

The project proposed by Drs. Rellán-Álvarez and Fiehn will complement my own studies by measuring lipid abundances in some of the same landraces and F1 crosses. The potential of associating gene expression variation and lipid variation is exciting and will provide new insight into maize stress responses and adaptation.

Sincerely,

Dr. Daniel Runcie
Assistant Professor
Department of Plant Sciences
UC Davis