**Project Title**. IOS Preliminary Proposal: collaborative research:  **“**Uncovering thePareto optimality for nutrient trade-off in Arabidopsis”

**1. Overview: Title:** Uncovering thePareto optimality for nutrient trade-off in Arabidopsis

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 **Abstract:** A challenge for biological systems is the optimization of processes to achieve maximum performance at the lowest cost, which in turn will increase fitness. However, maximizing performance in one task often comes at the expense of becoming worse at other tasks, causing a trade-off. In economics, trade-offs have been studied according to the Pareto principle, which defines optimal solutions for multitasking systems. This Pareto framework has been adapted to characterize biological systems, as the best phenotype for one task might not the best for other tasks, resulting in a trade-off situation. In an agronomically important example of this, plants must adapt their nutrient-foraging strategies to maximize growth under multiple combinations of nutrient limiting conditions. For example, a natural population might optimize the foraging/uptake of a scarce nutrient, at the expense of efficiently foraging for other more abundant nutrients. Our preliminary results show that this is actually the case in the model Arabidopsis: there is a trade-off between the assimilation of the primary nutrients N (nitrogen), P (phosphorous), and K (potassium) for optimal biomass production at the organismal level. In this project, we will characterize the molecular components underlying the trade-off between NUE (Nutrient Use Efficiency) under N-limiting and N-replete conditions, as a function of a changing PK environment (∆PK) in natural populations of Arabidopsis. Specifically, this trade-off is defined as the change in biomass under low vs. high N, as a function of PK concentration (∆PK). We will use the Pareto-front framework to capture a “Pareto optimality” for this trade-off (e.g. high biomass under low vs. high N), by exploring a range of successful solutions in natural populations. [Dear Colleagues, Dennis thinks the pareto notion while sexy sounding is misapplied here. Pareto optimality would say something like if we change this condition then someone will lose, so we can’t change it. But here we have only one goal: a single plant’s biomass. A much closer economic notion is efficient frontier which states that we maximize profit at a given level of risk. Here we are maximizing biomass at a given level of N. Pareto might be applicable if we were optimizing biomass and reproductive fitness and … But not here.] We propose to dissect relevant genes underlying this trade-off using genome-wide association studies combined with phenomics, genomics, transcriptomics approaches. Analyzing this genome-wide date within a conceptual economic framework will enable to determine the extent that developmental and environmental trade-offs have relevance to plant productivity. We aim to:

  ***Aim 1. To quantify phenotypic variation in nutrient trade-offs across Arabidopsis ecotypes. We will assay XXXX Arabidopsis Acessions for*** i) NUE quantification using N15-uptake and biomass ii) Shoot and root morphology using landmarked based morphometrics. These assays will be performed on plants exposed to two N-nutrient levels (low vs. high N). NUE (15N/biomass) will be quantified to measure the trade-off in N-biomass, as a function of (∆PK) PK.

 **Aim 2. *To model the “Pareto optimality” of nutrient trade-offs across Arabidopsis ecotypes*** We will use the meaures in Aim 1, to generate Pareto modeling of the nutrient trade-off in natural Arabidopsis accessions and to define “successful” archetypes (e.g. high biomass under low *and* high N, at low PK). ii) We will perform transcriptomic analysis of the “successful” Pareto-efficient genotypes. iii) We will integrate morphometic and transcriptomic data into a multivariate network and identify key TFs regulating such gene regulatory networks (GRNs).

 **Aim 3.** ***To identify the Genome-wide architecture of the “Pareto optimality” of nutrient trade-off*** We will identify the genes underlying the Pareto optimality by i) Genome-wide mapping of the Pareto-efficient genotypes within archetype of low nutrient input. ii) Validation of key genes identified from transcriptomics and GWAS.

**2. Statement on Intellectual Merit: This project exploits the use of the economical Pareto concept to map nutrient-use optimality at a genome-wide level.** This novel approach is addressed to an ecological and developmental challenge of plant biology. We proposed herein to discover: 1) phenotypic variations of natural plant populations associated with nutrient trade-offs 2) capture the “Pareto optimality” model of nutrient use efficiency and identify successful archetypes of Arabidopsis, 3) map the genome-wide architecture of Pareto-optimal NUE genotypes/phenotypes. This novel approach, will allow the identification of key players connecting efficient nutrient assimilation to plant production in natural populations. We envision the long-term advantage of this study as the ability to target gene candidates to engineer plants with optimized NUE yielding energy, environmental and health benefits.

**3. Statement on Broader Impacts:**

1. **Applications to agriculture**: Improvement of NUE in plants, specifically enhancing growth under low N, Low P environments. This will ameliorate pollution of N and P in groundwaters.
2. **Development of novel interdisciplinary concepts**: genome-wide integration of Pareto optimality.
3. **Training in Systems Biology**: Postdocs & students are trained in Systems Biology by co-mentorship between biologists and Math/Computer scientists.
4. **Collaborations:** This project involves international collaborations with Josh Banta (UT Tyler) who will conduct GWAS analysis, and Drs. Ruffel and Krouk (France) for NUE quantification.

**Key words**: Nutrient trade-off, Pareto optimality, genome wide association (GWAS), Nutrient-Use-Efficiency (NUE), Arabidopsis

**Section II Project**

**Background: Trade-offs and Pareto optimality**. The understanding of biological trade-offs is currently a puzzle in developmental and evolutionary biology. Organisms have evolved to optimize their fitness by maximizing performance at a particular task. However, they often do this at the expense of a second tightly-linked task. This creates a trade-off between the performance for the first and the second task, whose directionality will depend on the relevance of the task for fitness of the organisms in a particular environment. The inability of organisms to have high performance at two tasks raises the following questions: What are the molecular mechanisms that determine trade-offs? Can trade-offs be uncoupled or manipulated to increase the performance for traits of economical interest?

 In economics, the problem of trade-offs has been the focus of extensive studies to characterize the possible solutions when populations of individuals are required to perform more than one task (Winter, 2010). In this regard, the conceptual frame-work of “Pareto efficiency” has been developed to indentify the most economical and efficient combinations of solutions for multi-task problems Ehrgott, 2000. An entity with high-performance at task one is highly desirable, despite having low performance at task two. Similarly, an entity with high-performance at task two, is desirable at the expense of task one. Entities with average performance at each of the two tasks can be selected if the combined performance is beneficial. The combinations of high-performance solutions for a set of tasks problem is called the “Pareto front” of optimal solutions, used in economics to identify inefficient solutions to be eliminated from the system (Benson, 2001). In biological systems, developmental and evolutionary trade-offs exist in both plants and animals (Fig. 1A, Vila-Aiub *et al.*, 2009; Barton, 2010; Shoval *et al.*, 2012; Sheftel *et al.*, 2013)]. For instance, species with higher biomass have a slower reproductive cycle and fewer offspring (Dmitriew, 2011). Yet, mostly optimal solutions to multi-task problems have been presumably maintained by natural selection. Thus, it has been recently shown that Pareto-fronts for multi-tasking phenotypes are often found in natural populations, showing the utility of Pareto frameworks to characterize developmental and evolutionary trade-offs Shoval *et al.*, 2012; Sheftel *et al.*, 2013. An example of this is the morphology of the beak in Darwin’s finches whose shape is optimized for a variety of tasks in feeding habits (Fig. 1A) [Shoval 2013]. In this proposal, we aim to characterize the range of optimal solutions for a trade-offs in nutrition use and growth in plants. We will use the Pareto optimality framework and economics principles, to identify the genetic and molecular bases of natural variation in nutrient use efficiency (NUE) in natural populations of Arabidopsis, as a trade-off between N and two related nutrients (P and K). [Dennis thinks our only goal here is biomass, right?]



Figure 1: The Pareto economic principle applied to nutrient trade- off in Arabidopsis [Shoval Ref 2013 here].

**Nutrient trade-off in plants: Application of the Pareto optimality**. A crucial task for plants is the maximization of nutrient uptake from the environment in order to increase their biomass, yield and fitness Good *et al.*, 2004; White & Broadley, 2005. However, nutrient environments are complex and plants often face a variety of nutrient compositions in agricultural and natural soils. Thus, maximization of the uptake of one nutrient might occur at the expense of efficiently foraging for other nutrients (Hermans *et al.*, 2006). To illustrate this case, we tested the effects on biomass in Arabidopsis exposed to a nutritional matrix containing combinations of the main growth-limiting nutrients in nature, provided worldwide as NPK fertilizers: nitrogen (N), phosphorous (P) and potassium (K) (Fig. 1B; Schachtman & Shin, 2007). Our preliminary results obtained in the Col-0 ecotype, show that at low N concentrations, low amounts of PK improve growth and biomass accumulation (Fig. 1B – green arrow). By contrast, these low amounts of PK are detrimental for growth and biomass accumulation at high N (Fig. 1B – red arrow). Therefore, plants perform better under low N in combination with low input of PK nutrients. However, this optimal biomass at low-N, seems to be at the expense of performance at high N concentration, resulting in a nutritional trade-off situation. We hypothesize that this differential performance (e.g. biomass) under high and low N concentrations (as a function of PK), might reflect a plants adaptation to the fluctuating nutrient conditions that plants face in their native environments. Therefore, the magnitude and directionality of the trade-off observed in Col-0 might be different in other natural *Arabidopsis thaliana* accessions.

 The trade-off in nutrient acquisition under combinations of N vs. PK concentrations can be readily explained in terms of a Pareto front, defined by the ∆ biomass under high N (as a function of ∆PK (red line) or under low N availability (green line) (Fig. 1C). In this Pareto space, Col-0 is a natural variant whose trade offs we have experimentally quantified. The collection of natural variants in this space is expected to form a triangle (Shoval *et al.*, 2012), in which the most optimal solutions (archetypes) of the Pareto front are at the corners of the triangle (Fig. 1C – yellow circles). Therefore, the archetypes will represent the most efficient solutions for the individual tasks of growth under high-N (archetype ②) or low N-nutrient input (archetype ③). In this project, we propose to exploit the natural variation in *Arabidopsis thaliana* accessions to characterize the extent of the Pareto front, to select the archetypes for further study to better understand how this trade-off has evolved in natural populations. This will identify which natural accessions have the most optimal solutions for the N-PK nutrient acquisition problem. Most important for agricultural purposes, our nutrient Pareto trade-off will identify Arabidopsis accessions that maximize N-assimilation (biomass) under low N and PK conditions, opening the potential to the dissection of the genetic basis for this, with agronomic benefits for the environment (Good *et al.*, 2004; Hermans *et al.*, 2006).

**The genetic architecture of Pareto trade-offs**. Pareto archetypes represent optimal solutions in natural populations. Some of these optimal solutions might be economically important as they represent high performance solutions to increase productivity of a trait of agricultural relevance – for instance, plant productivity under low nutrient conditions (Good *et al.*, 2004; White & Broadley, 2005; Kant *et al.*, 2010. Thus, an archetype can arbitrarily be selected to quantify its distance to the rest of the data-points or natural variants. This matrix of values then reflects the natural variation of the optimal solutions defined by the selected archetype, and can be considered as a “Pareto efficiency” trait. In Arabidopsis, Genome-Wide Association (GWAS) Mapping (Atwell *et al.*, 2010) has been shown to be a powerful tool to pinpoint the genetic bases of natural variation in complex traits, including ones related to nitrogen responses in roots (Gifford *et al.*, 2013; Rosas *et al.*, 2013. Indentifying the genetic and molecular bases of optimal solutions (archetypes) is ideal towards understanding how to engineer plants with increased performance or yield. Here, we propose to use the Pareto front of N and PK performance defined with Arabidopsis accessions, and identify the accession-archetype that have the most optimal outcome (high ∆biomass at low N, as a function of PK concentration). We will score the rest of the accessions as the differential performance on the Pareto space, relative to the selected archetype. Arabidopsis ecotypes are expected to capture the range of successful solutions in NUE on the Pareto front, reflecting the diversity of nutrient dynamics in natural conditions. Indeed we have already shown a wide variety in NUE in Arabidopsis accessions in our studies (Fig. 2A), as has been also reported previously for a more limited number of accessions (North *et al.*, 2009; Chardon *et al.*, 2010; Ikram *et al.*, 2011). Therefore, using archetype-related performance, we aim at indentifying genes underlying the Nutrient Pareto trade-off and uncover the genetic basis of complex nutrient use efficiency.

 **In summary in this project**, we propose to address the challenging question of the basis of natural variation of environmentally driven trade-offs in nutrient use efficiency. This will be done in an economically and environmentally relevant trade-off: nutrient uptake efficiency and growth. We will combine population genetics with phenomics, genomics, systems biology approaches and economics conceptual framework of Pareto fronts. We aim to quantify the nutrient trade-off in natural Arabidopsis populations as a function of biomass and morphology (Aim 1), use these data to define the Pareto front of nutrient use efficiency and identify optimal archetypes (Aim 2), and identify the genetic basis for these Pareto-archetypes phenotypes using population genetic and genomic approaches (Aim 3). We envision the long-term advantage of this elegant approach as the ability to target gene candidates to engineer plants with optimized NUE.

**Aim 1: “Phenotypic variations of nutrient trade-off in Arabidopsis ecotypes”**. Arabidopsis is a model for studying natural variations of NUE in plants (Fig. 2A,Chardon *et al.*, 2012; Sulpice *et al.*, 2013. For example, previous studies of ecotypes identified N-efficient ecotypes Tsu-0 and Bur-0 with improved biomass production (Chardon *et al.*, 2010). Here, we propose to study the ecological and developmental basis of a multiple nutrient trade-off in Arabidopsis ecotypes at the organismal level. By means of trait quantification and novel morphometrics analysis, we will study the effect of major PK nutrients under low vs. high N conditions, as a trade-off to produce plant biomass (∆PK biomass). In addition, NUE will be monitored as the ratio between biomass and total N-content using an 15N label. Thus, we aim to identify ecotypes of interest with improved potential of NUE at both low and high nitrogen ranges, ideally at low PK (Fig.1B - green and red arrows).

 **Experimental plan:** To generate testable hypotheses, we will assess the effect of the two selected Low and High N concentrations (0.5mM and 40mM, Fig. 1B) using modified MS medium plates containing low versus high P and K. Under this matrix of 4 conditions, plant growth phenotypes of the 96 selected ecotypes will be assessed, including individual phenotypic traits such as root/shoot biomass and leaf and root architectural traits (López-Bucio *et al.*, 2003; Dubrovsky & Forde, 2012; Rosas *et al.*, 2013). In parallel, all monitored developmental traits will be compared to a landmark-based morphometrics analysis recently developed in the lab (Ristova *et al.*, 2013). In addition, we will measure N-uptake and NUE will be screened using radiolabel 15N experiments in collaboration with Sandrine Ruffel and Gabriel Krouk (INRA, France). Briefly, using 15N as a tracer, the rate of nitrate incorporation into total N will be measured as a function of biomass (Fig. 2A).

 **Research question: How have plants adapted to nutrient availability and how are developmental responses rooted in the evolution of the model plant Arabidopsis?** In addition to identifying key ecotypes with improved NUE under low N-input, this phenotypic study aims to identify genetic and ecological variations of development related to nutrient availability and the geographical origin of the accessions. This morphometric analysis may specifically screen the ecological adaptation of the root architecture to indigenous nutrient regimes (López-Bucio *et al.*, 2003). The combination of phenomics (Aim 1) and genomics (Aim 3) approaches will help uncover the genetic basis for the developmental and ecological adaptations to NUE in distinct nutrient environments.

**Aim 2: Pareto optimality of nutrient trade-off in plants**. In this second aim, we will make use of the Pareto-efficiency concept from economics to study developmental trade-offs in nutrient uptake and resource allocation in Arabidopsis natural populations. This concept has been recently used to show that natural species follow triangular distribution patterns when there are trade-offs between traits that explain tasks Shoval *et al.*, 2012; Sheftel *et al.*, 2013. These analyses reveal “archetypes” defined as the most successful solutions for particular tasks which correspond to the vertices of the triangles (Fig. 1A and C – yellow circle①②③). The area of the triangle is populated with natural variants that have combinations of efficiencies to perform the task. In our study, the task is defined as the plant response at Low N and High N (difference in biomass) under changing levels of PK input (∆PK biomass). Currently, we have described the position of the Arabidopsis accession Col-0 in this matrix (Fig. 1C), and we expect to populate this “Pareto space” with the diverse responses of Arabidopsis accessions as variation in NUE (biomass) under low vs high N, as a function of ∆PK. According to predictions in biological systems Shoval *et al.*, 2012, this is expected to produce a triangular distribution, from which we will define the Arabidopsis accessions that are the most representative of the archetypes.

biomass as a function of (Fig. 1C) (Fig. 1B)The ethat,

 **Research questions: How can Pareto-Optimality be integrated into gene networks?** A key objective will be particularly focused on identifying genotypes with a high Pareto efficiency for ∆PK biomass at low N (Fig. 1C – archetype ③), optimizing NUE under low nutrient input system. We propose to perform trancriptomics (RNA seq) on the accessions that occupy extreme archetypes, and compare their gene genome-wide expression profiles. The genome-wide transcriptional data will be integrated into a multivariate network that connects nutrient signals to genes, and genes to phenotypic traits (from Aim1). A highlight of the power of this systems biology approach was enabled by the construction of a multi-network, which was used to identify N-regulatory biomodules in Arabidopsis Gutierrez *et al.*, 2007; Gutiérrez *et al.*, 2008; Krouk *et al.*, 2009; Krouk *et al.*, 2010. Using this system biology approach, the integration of Pareto-efficiency and transcriptomic data, will enable us to identify new gene regulatory networks (GRN) underlying “N-economy” of plant as a system.

**Aim 3 Genome-wide mapping of the Pareto nutritional optimality**. Genome wide association (GWAS) mapping has proven to be a powerful tool to identify genes and networks underlying natural variation Atwell *et al.*, 2010, and evolutionary trade-off in Arabidopsis Todesco *et al.*, 2010. Using the 250K SNP database of polymorphisms in Arabidopsis, we have previously shown that loci controlling natural variation in complex morphometric root traits Ristova *et al.*, 2013 can be identified using GWAS mapping for structured populations Rosas *et al.*, 2013. For instance, we are currently using nitrogen use efficiency traits to identify which genes underlie natural variation in nitrogen allocation in Arabidopsis accessions. To do this, we have measured the amount of nitrogen (15N uptake) in 71 ecotypes and its partition to the plant’s organs (Fig. 2A). For instance, our preliminary analysis of a GWAS for structured populations shows that in this population, there were 6 highly significant hits (FDR p<0.05), one of them mapping to chromosome II and the remaining five mapping to chromosome five (Fig. 2B). Among the candidate genes, ATPR3 is a peptide transporter (Karim *et al.*, 2005) and ARAC2 is a member of the ROP GTPase gene family, known to control multiple signaling pathways in plant growth (Li *et al.*, 2001. In this aim, we propose to use the Pareto-front quantification as a quantitative trait to map the genetic basis of natural variation in NP-K trade-offs. We aim to pinpoint key players connecting nutrient assimilation to plant production (Good *et al.*, 2004). The validations of such targets will correct our network as well as generate new hypotheses, therefore iterating the systems biology cycle of experimentation and computation.

Figure 2 : Genome-wide assocaitaion of nitrogen allocation in Arabidopsis.

 **Experimental Plan:** In collaboration with Josh Banta (UT Tyler), we propose to benefit from the novel Pareto concept to map the efficient phenotypes at a genomic scale. For instance, for accessions closest to archetype with improved NUE under low N (Fig. 1C archetype③), we will measure the distance of each of the Arabidopsis natural variants in relation to this optimal archetype, and use this quantification as a trait for GWAS for structured populations. This is expected to show the genomic basis of natural variation of N-PK trade-off efficiency in Arabidopsis natural populations.

 **Research Question: How can the genetic architecture of Pareto optimality be engineered to improve NUE?** Here for the first time, we propose to test the genome-wide integration of Pareto optimality. By combining an economical conceptual framework with genome-wide data, we will pinpoint key gene targets and networks that will allow focusing more quickly on candidates that improve NUE (Coruzzi et al., 2012???what ref is this??? I don't have pubs in 2012???????). We envision the long-term advantage of this study as the ability to target such candidates to engineer plants with optimized NUE, therefore yielding energy, environmental and health benefits.

**Broader Impacts:** This NSF IOS proposal is the result of a highly successful collaboration between biologists, physiologists and computer scientists. Understanding the system as a whole, through the combination of genomics, bioinformatics and systems biology approaches, has allowed researchers to derive new testable hypotheses in plant nutritional regulation. Our results relate to a real world scenario with practical applications affecting crop yield and NUE with potential benefits for agriculture and the environment.

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