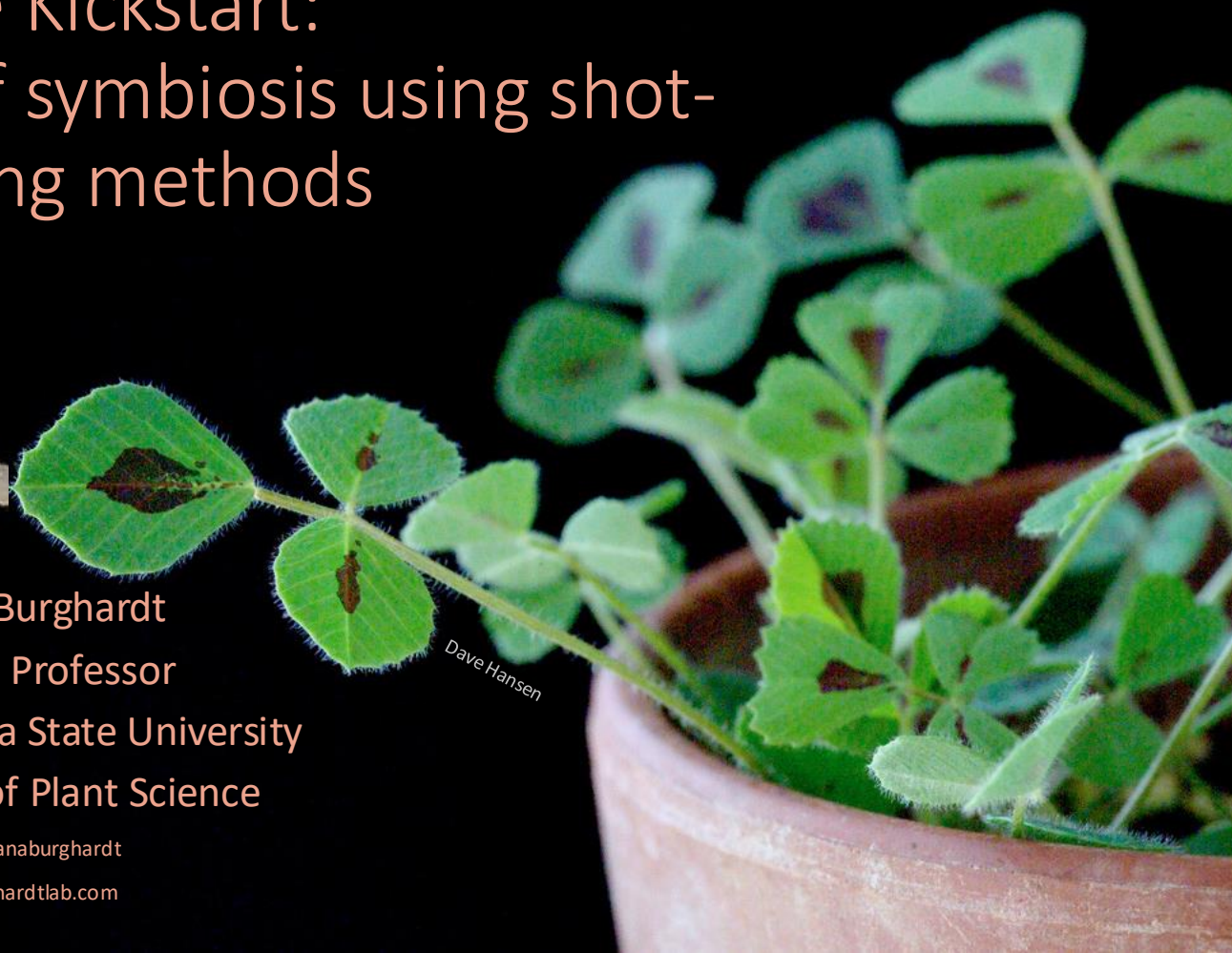
Microbiome Kickstart: Uncovering principles of symbiosis using shot-gun sequencing methods



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The Burghardt Lab: Examining plant-microbe-climate interactions in natural and managed landscapes

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Learning objectives

- Define symbiosis and understand variation in usage
- Understand basic categories of symbiosis and brainstorm examples
- Define holobiont and hologenome concepts
- Articulate how to use shotgun sequencing to measure symbiont fitness at multiple scales of genetic variation-
- Fall in love with legumes and rhizobia

What is Symbiosis?

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from Greek συμβίωσις, *symbíōsis*, "living together", from σύν, *sýn*, "together", and βίωσις, bíōsis, "living")

“any type of a close and long-term biological interaction between two biological organisms of different species, termed symbionts, be it mutualism, commensalism, or parasitism”.- Oxford Dictionary

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"any type of a close and long-term biological interaction between two biological organisms of different species, termed symbionts, be it mutualism, commensalism, or parasitism".- Oxford Dictionary

Broad-

"the living together of unlike organisms" 1879, Heinrich Anton de Bary 1879

Heinrich Anton de Bary was a German surgeon, botanist, microbiologist, and mycologist . He is considered a founding father of plant pathology (phytopathology) as well as the founder of modern mycology. His extensive and careful studies of the life history of fungi and

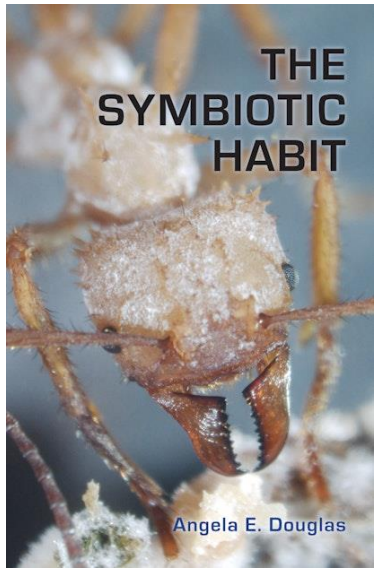
Lynn Margulis was an American evolutionary biologist, and was the primary modern proponent for the significance of symbiosis in evolution. Historian Jan Sapp has said that "Lynn Margulis's name is as synonymous with symbiosis as Charles Darwin's is with evolution." In particular, Marg

Narrow-

" symbiosis as a persistent mutualism"

Angela Douglas is a proponent of this framing.

Used colloquially and by general biologists



A non-exclusive list of axis of symbiosis variation!

- Ecological outcome—mutualism, commensalism, parasitism
- Dependence—obligate vs. facultative
- Transmission—horizontal vs. vertical
- Participants—one-to-one vs. many-to-one
- Location– ecto vs. endo
- Generational—synchronous vs. asymmetric
- Others?

Activity: Think of three of your favorite symbioses and see if you can slot them into the following categories

- Ecological outcome—mutualism, commensalism, parasitism
- Dependence—obligate vs. facultative
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- Participants—one-to-one vs. many-to-one
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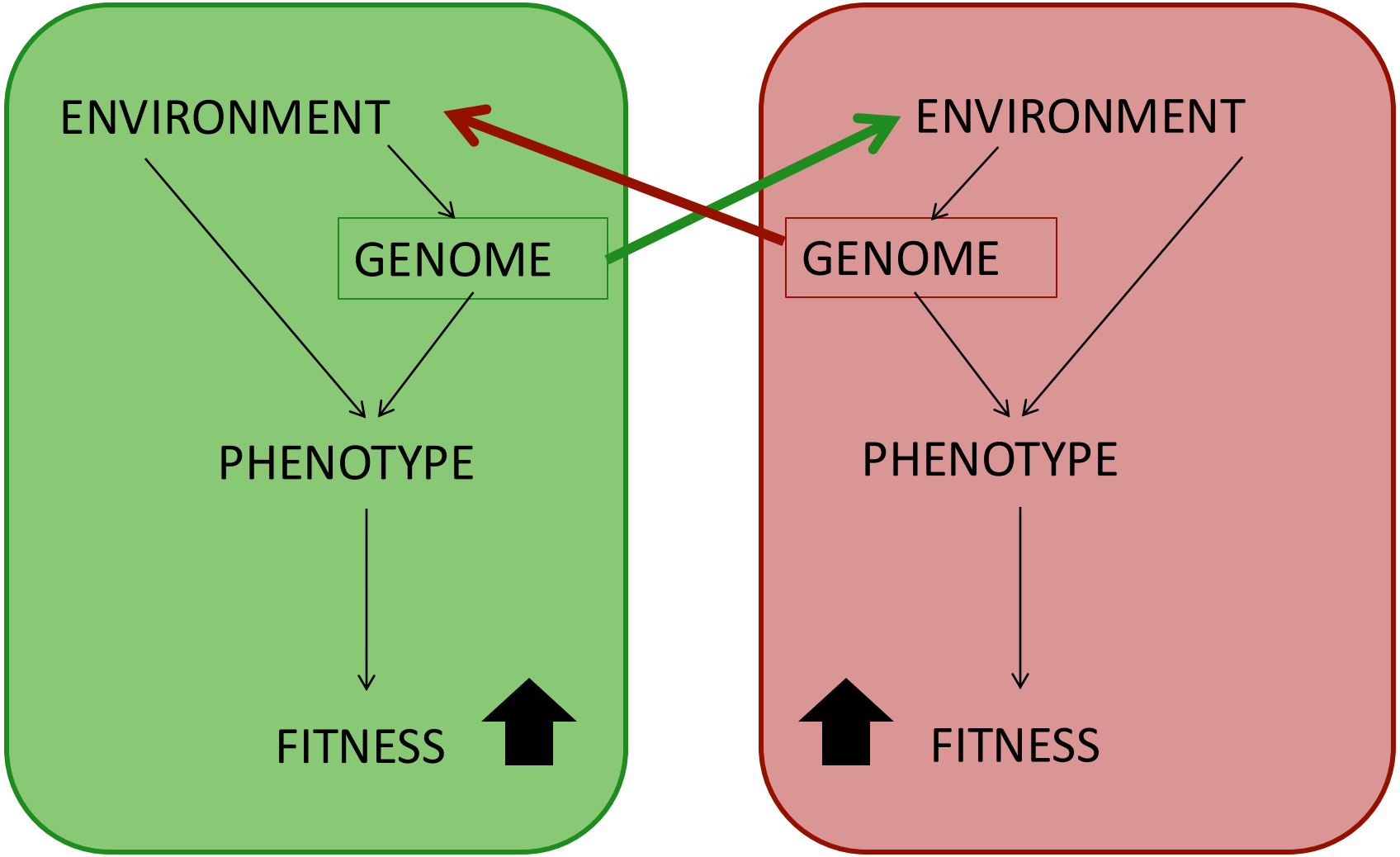
- Others?

Why should microbiome scientists care about the symbiosis concept?

One to one genome interaction model…

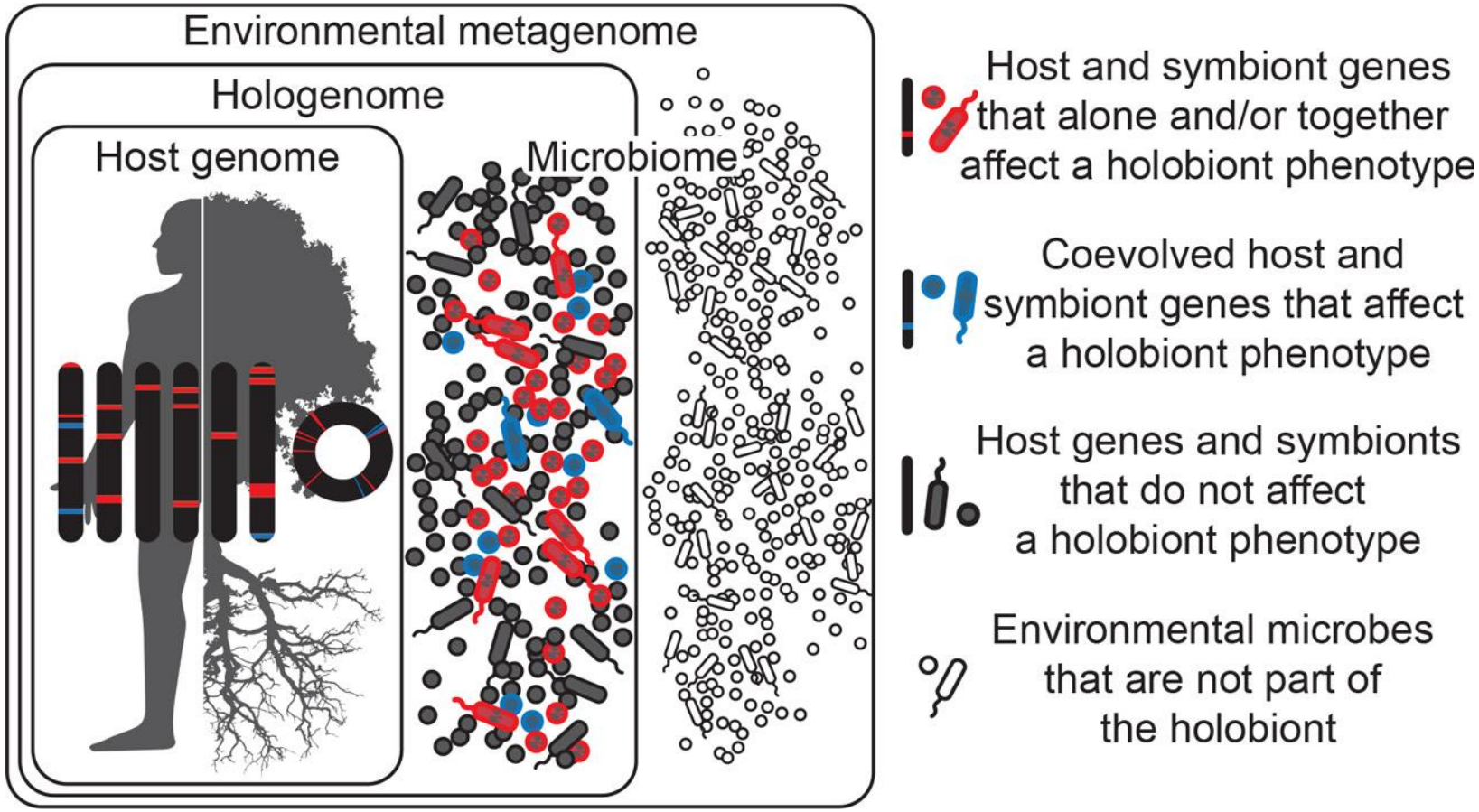


Interacting genomes in symbiosis

- **Holobiont** is a term used to describe an individual host and its microbial community, including viruses and cellular microorganisms
- It is derived from the Greek word holos, which means whole or entire.
- Lynn Margulis was the first to coin the term

- **Hologenome**-encompass the genomes of the host and all of its microbes at any given time point

- **Hologenome evolutionary theory** (still being sorted out…)

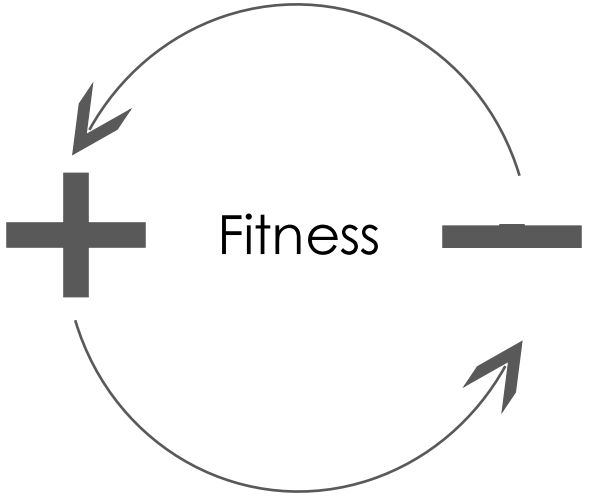


Theis… Bordenstein et. al , 2016

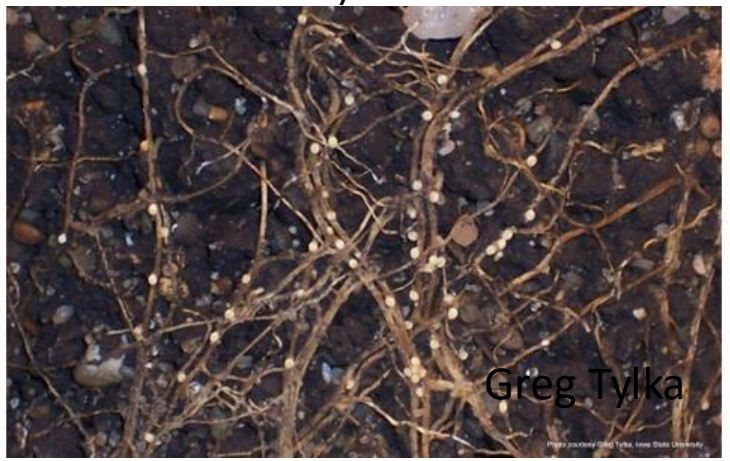
Symbiosis influences host growth & nutrition

Soybean cyst nematode

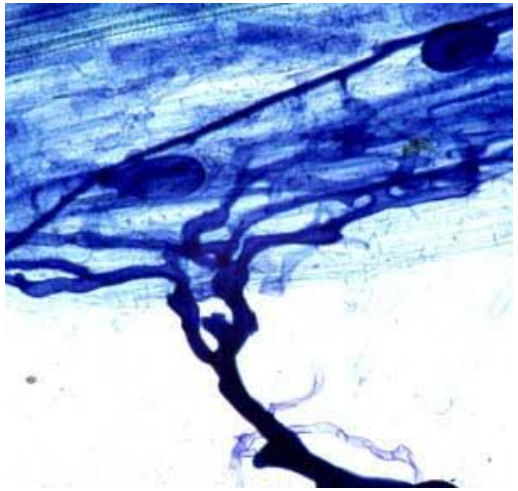
Parasitism



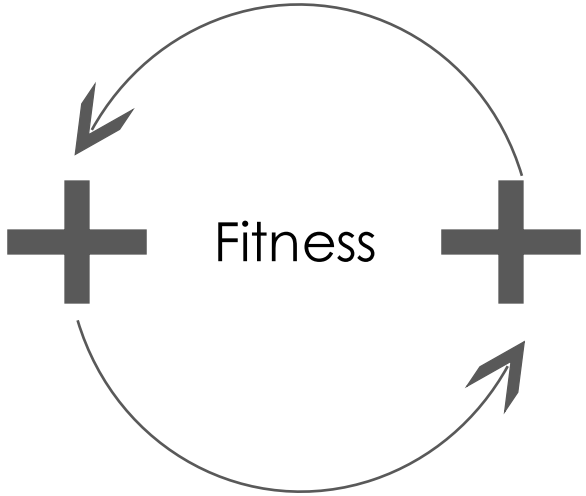
Soybean



Arbuscular mycorrhizal fungi



Mutualism

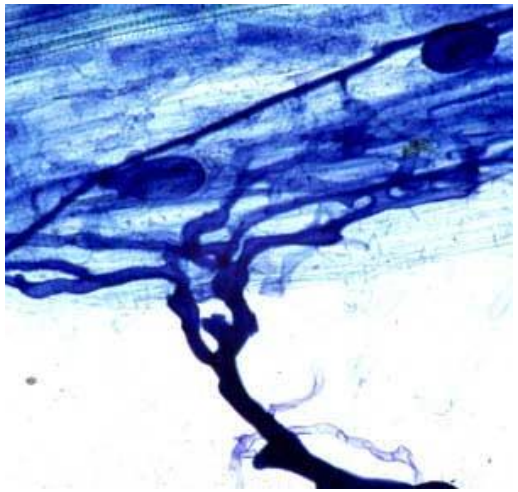


Corn

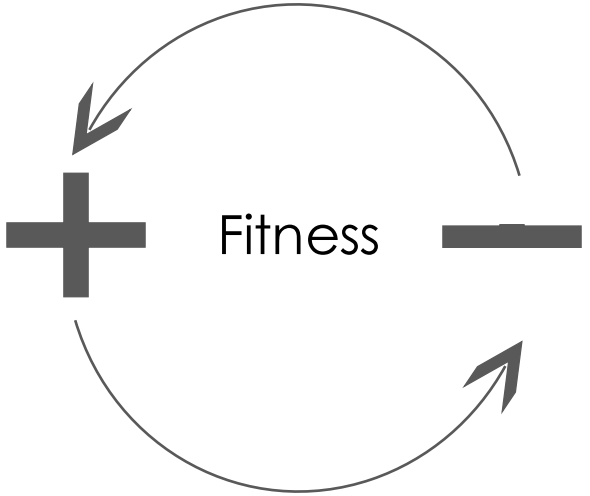
It is rare to measure the relative fitness of microbial symbionts

Soybean cyst nematode

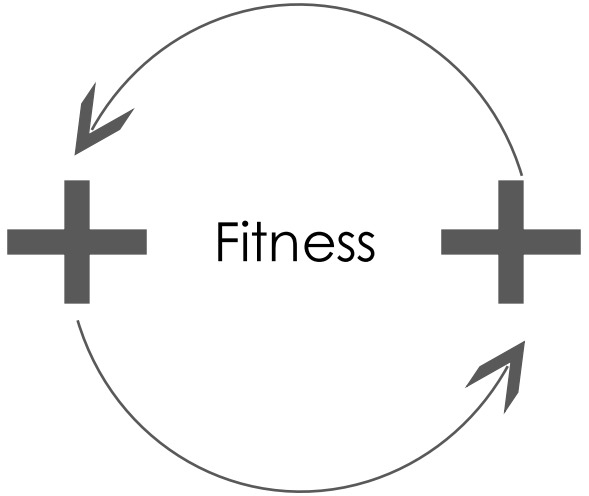
Arbuscular mycorrhizal fungi



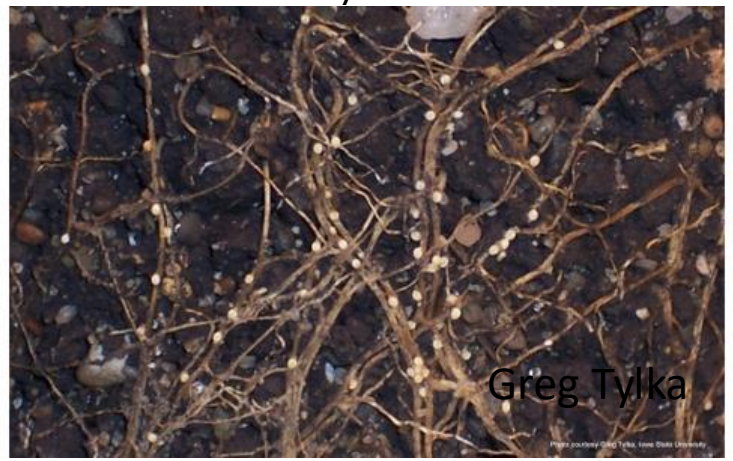
Parasitism



Mutualism

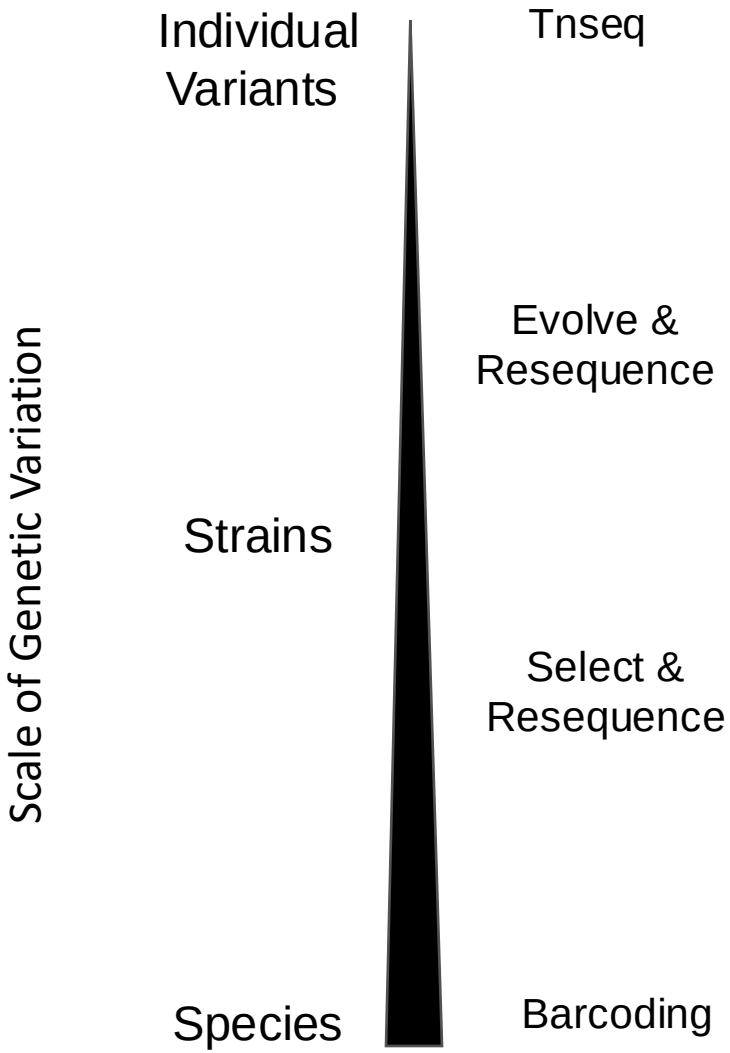


Soybean



Corn

Whole genome shotgun sequencing allows measurement of microbial symbiont fitness at many different levels of genetic variation



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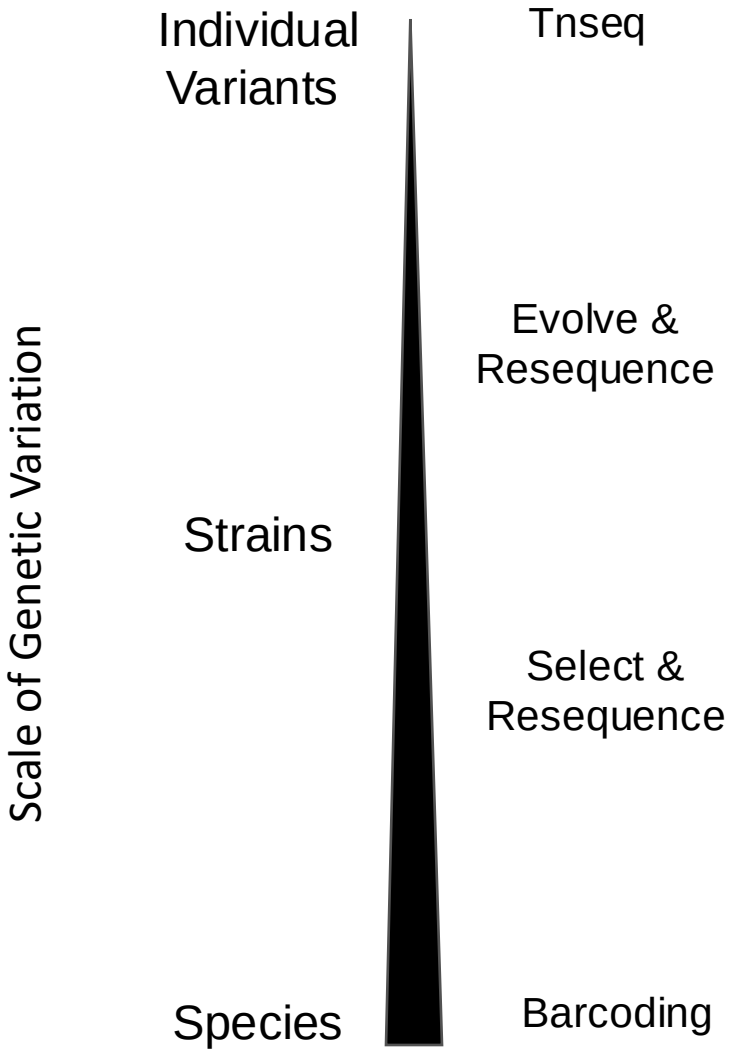


Lifestyle adaptations of *Rhizobium* from rhizosphere to symbiosis

Rachel M. Wheatley[a,1], Brandon L. Ford[a,1], Li Li[b,1], Samuel T. N. Aroney[a], Hayley E. Knights[a], Raphael Ledermann[a], Alison K. East[a], Vinoy K. Ramachandran[a,2], and Philip S. Poole[a,2]

ADAPTATION

Science

Experimental evolution makes microbes more cooperative with their local host genotype

Rebecca T. Batstone[1,2*], Anna M. O'Brien[1,3], Tia L. Harrison[1], Megan E. Frederickson[1,4,5]

Select and resequence reveals relative fitness of bacteria in symbiotic and free-living environments

Liana T. Burghardt[a], Brendan Epstein[a], Joseph Guhlin[a], Matt S. Nelson[b], Margaret R. Taylor[b], Nevin D. Young[a,c], Michael J. Sadowsky[a,b,d], and Peter Tiffin[a,1]

ISME

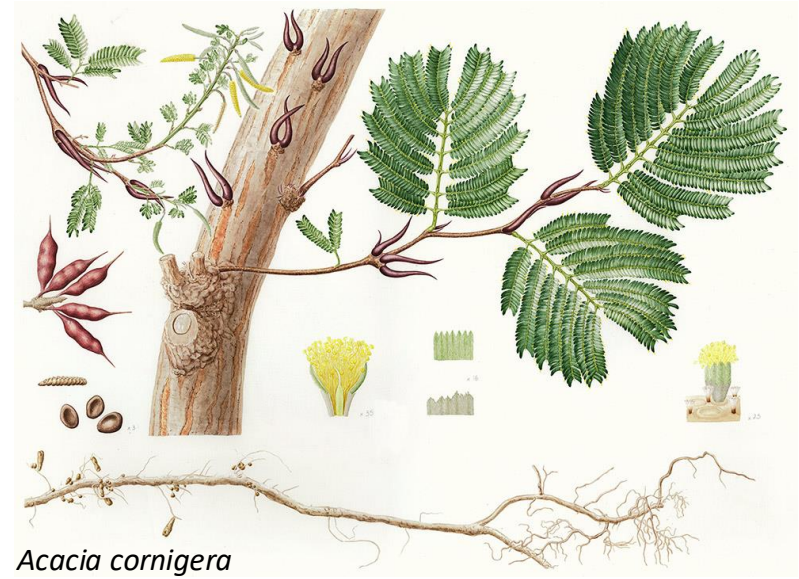
Variation in rhizosphere microbial communities and its association with the symbiotic efficiency of rhizobia in soybean

Qin Han[1] · Qun Ma[1] · Yong Chen[1] · Bing Tian[1] · Lanxi Xu[1] · Yang Bai[2] · Wenfeng Chen[3] · Xia Li[1]

Legumes & Rhizobia

Trifolium pretense


Glycine max


Sarah Roberts

Acacia cornigera

Hippophae rhamnoides L.


Lens culirnaris


(Rasbak)

Pisum sativum


Franz Eugen Köhler

Arachis hypogaea


Lupinus perennis

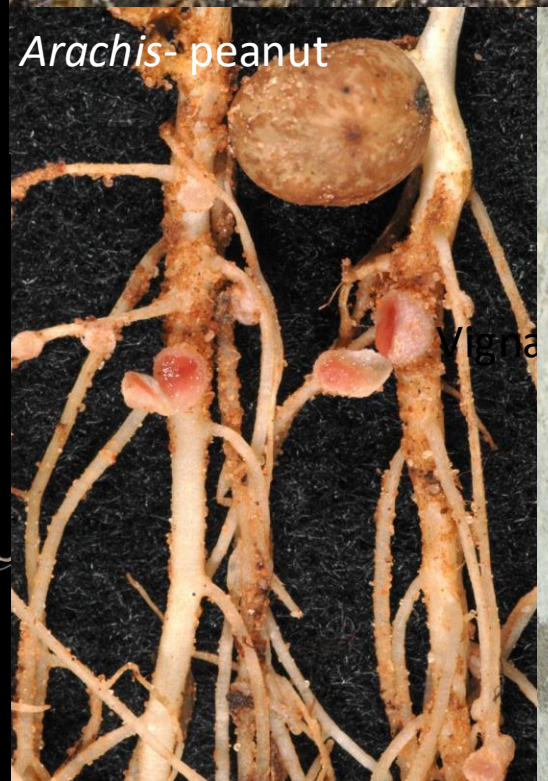

Pisium- Pea


Trifolium- clover


Lotus

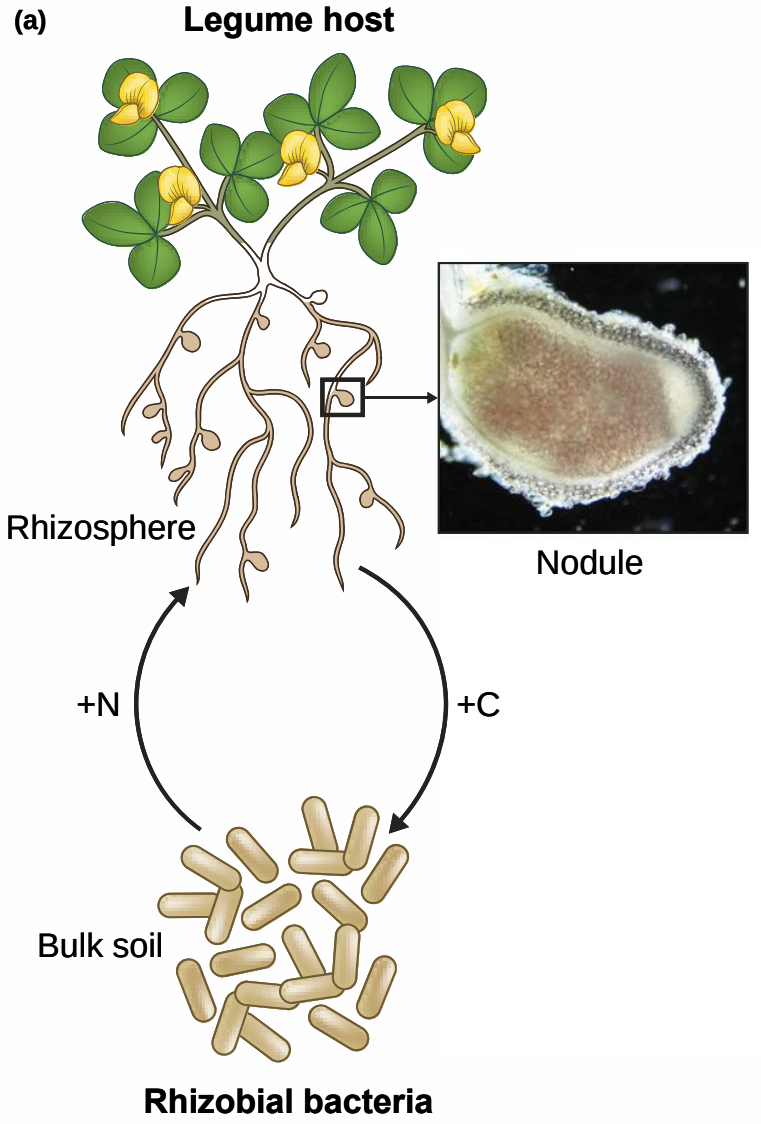

Medicago- alfalfa


Glycine- soybean
wisteria


Arachis- peanut
Vigna

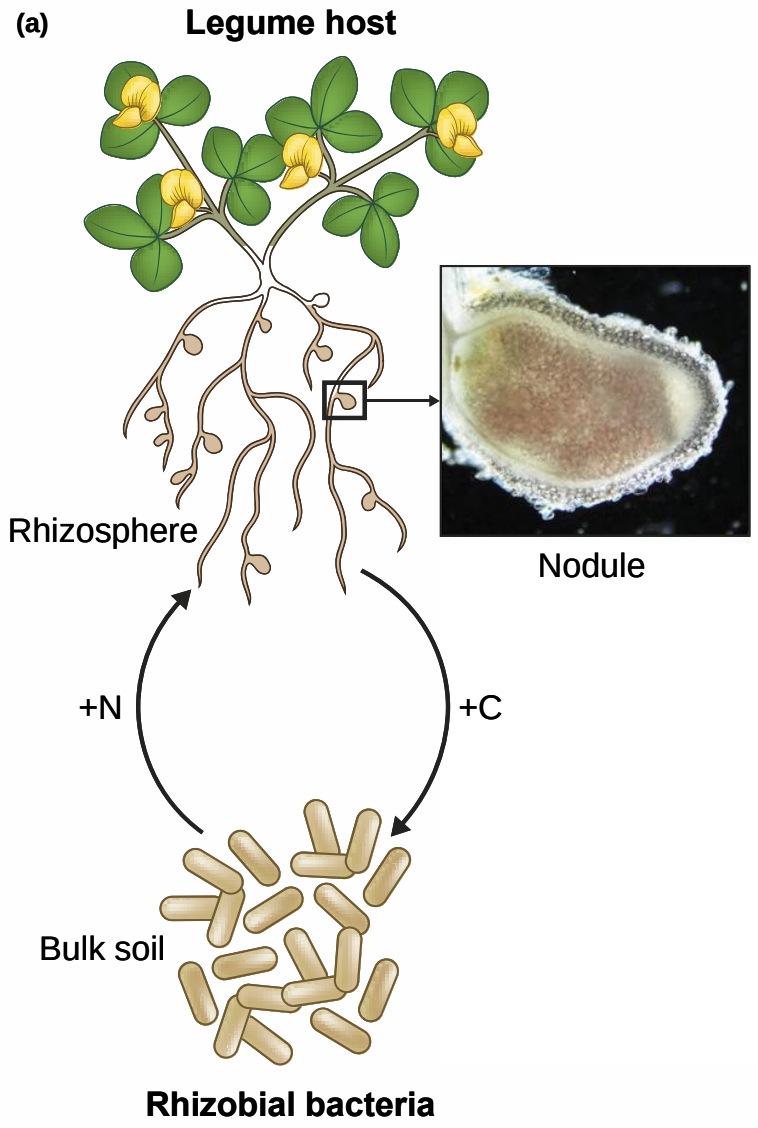

Wisteria

Facultative symbiosis are ubiquitous, important, and poorly understood from the microbial perspective



(Burghardt et al. 2020, *New Phytologist*)

Facultative symbiosis are ubiquitous, important, and poorly understood from the microbial perspective



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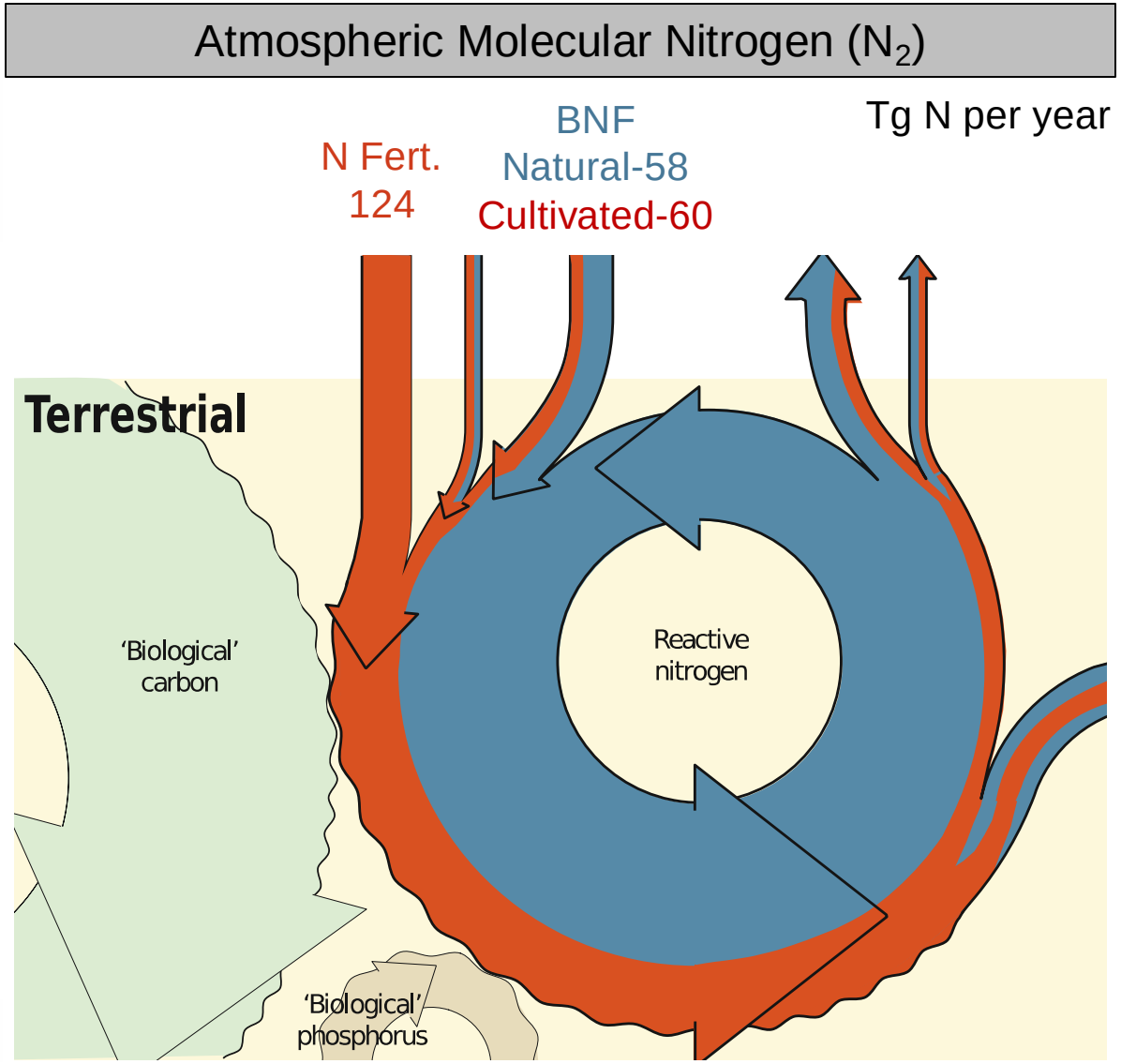

Modified from: Gruber & Galloway 2008 with updated fluxes from IPCC Chapter 6 2013

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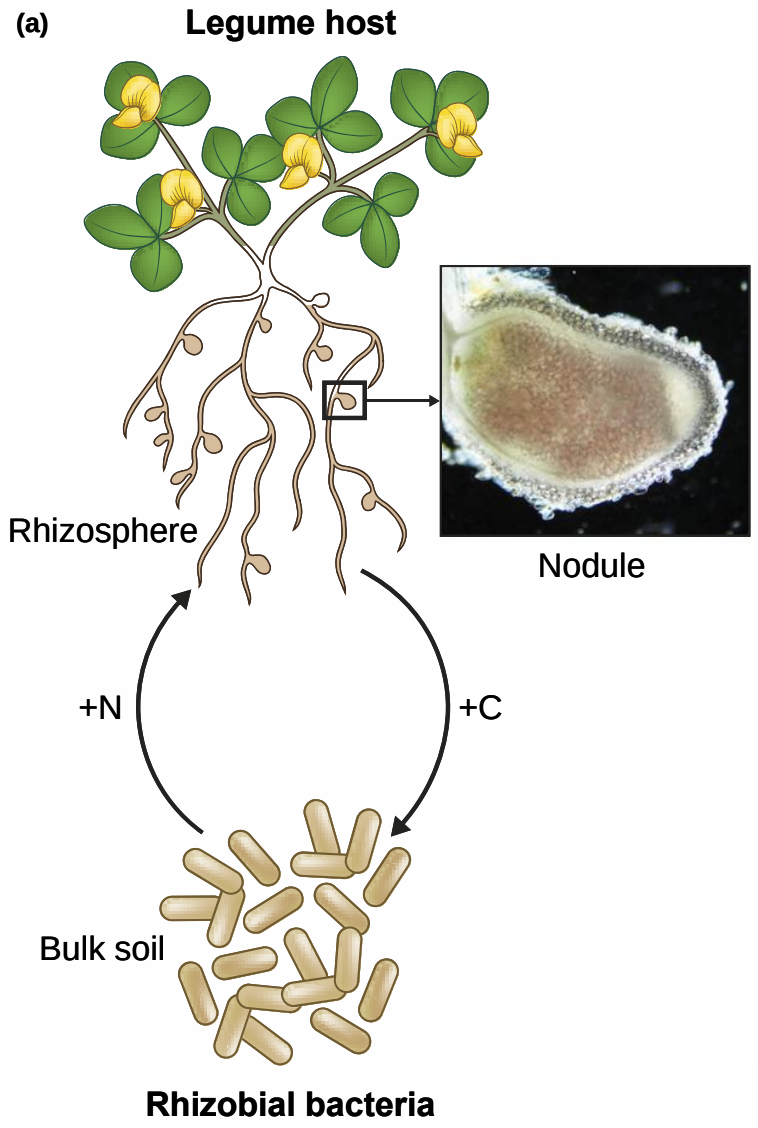

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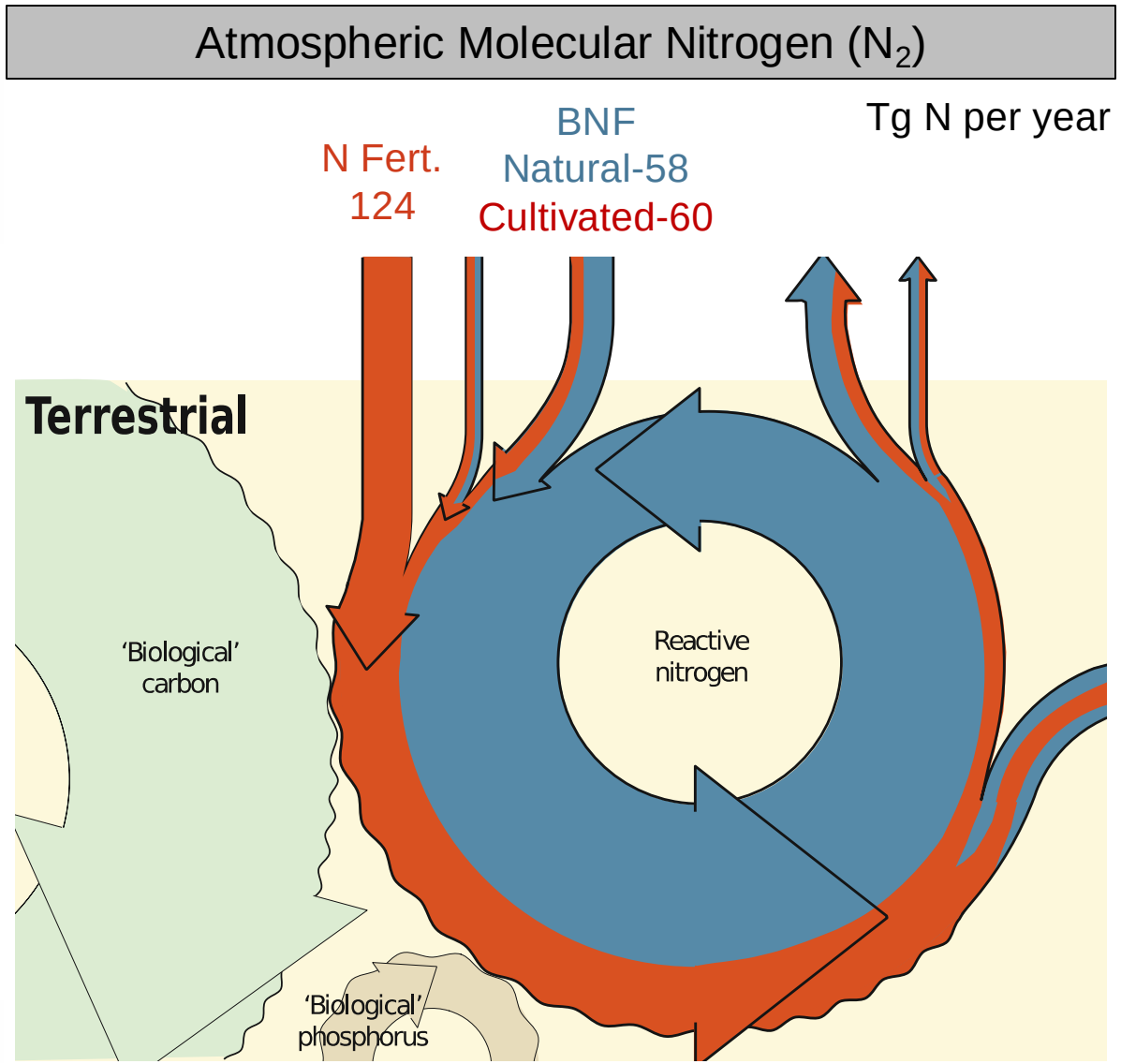


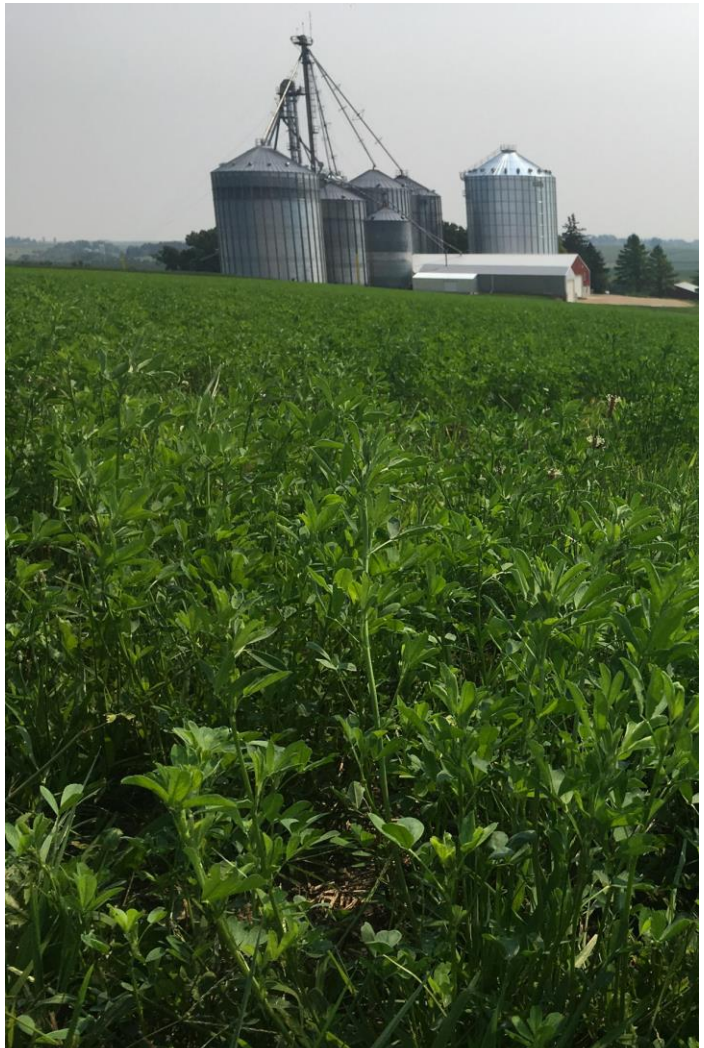
Modified from: Gruber & Galloway 2008 with updated fluxes from IPCC Chapter 6 2013

Barriers to managing Biological Nitrogen Fixation

- Failure to establish
- Competition problem
- Mutualism Breakdown

Legume-Rhizobia System: Medicago-Sinorhizobium

Crop:
M. sativa (alfalfa)

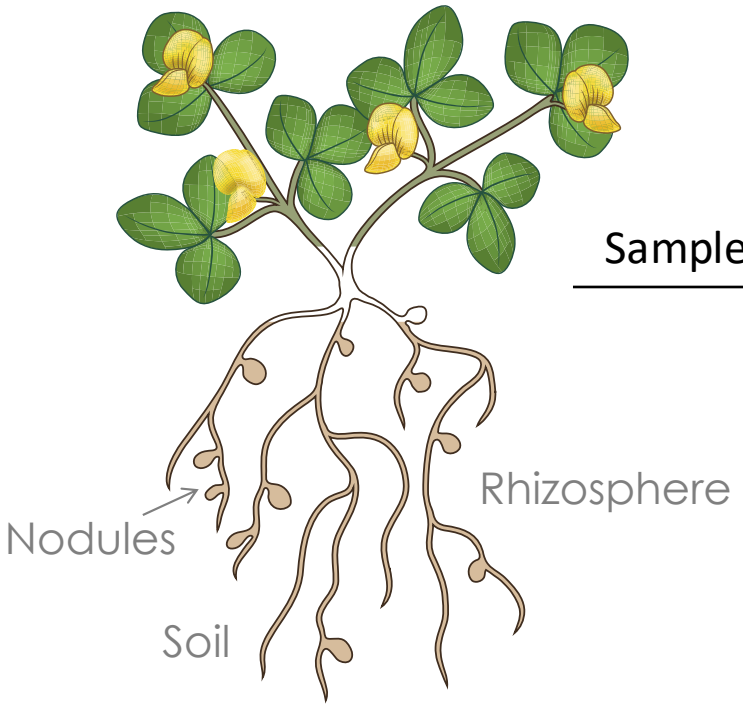


Genetic model:
Medicago truncatula

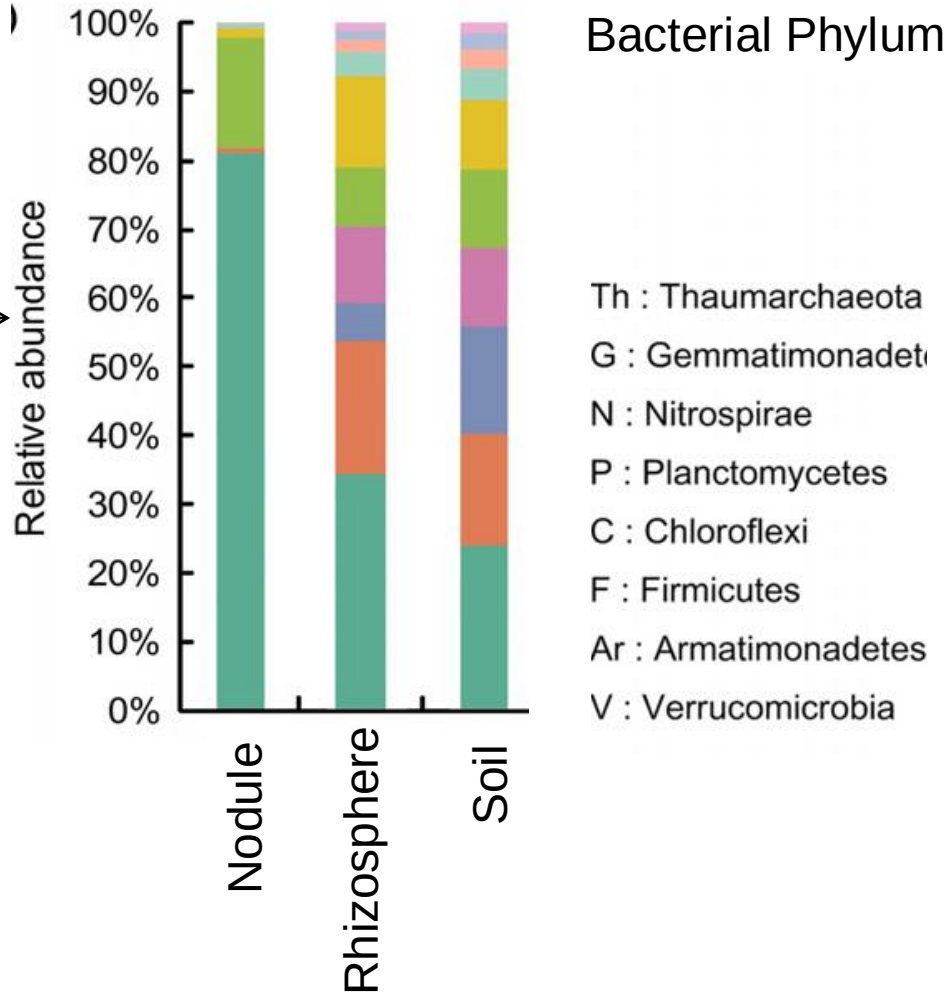
Genetic model:
Sinorhizobium meliloti

Naturalized species:
M. lupulina; M. polymorpha

Medicago nodules are highly selective



Sample



Xiao Xiao et. al. Molecular Ecology 2017

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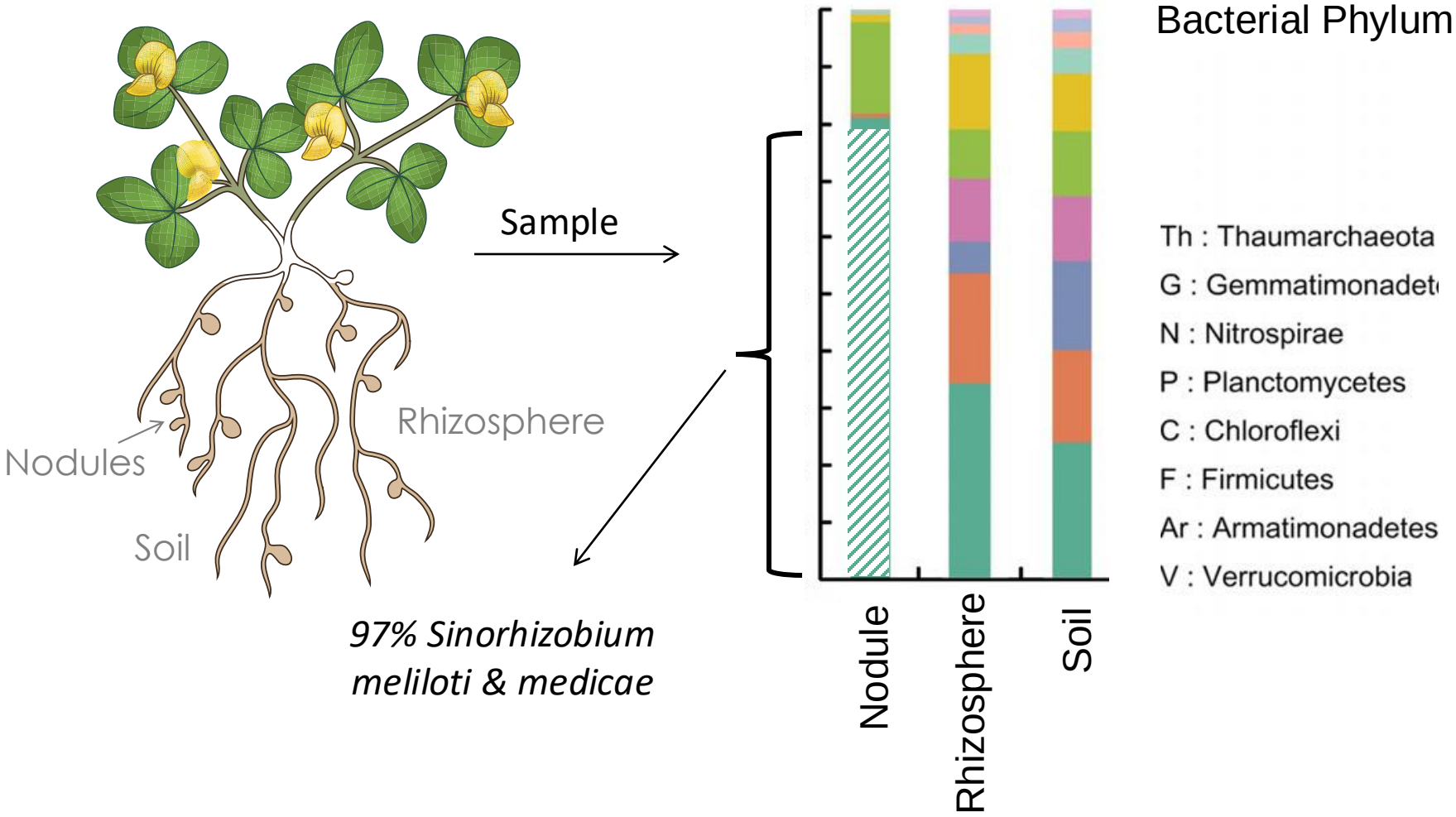
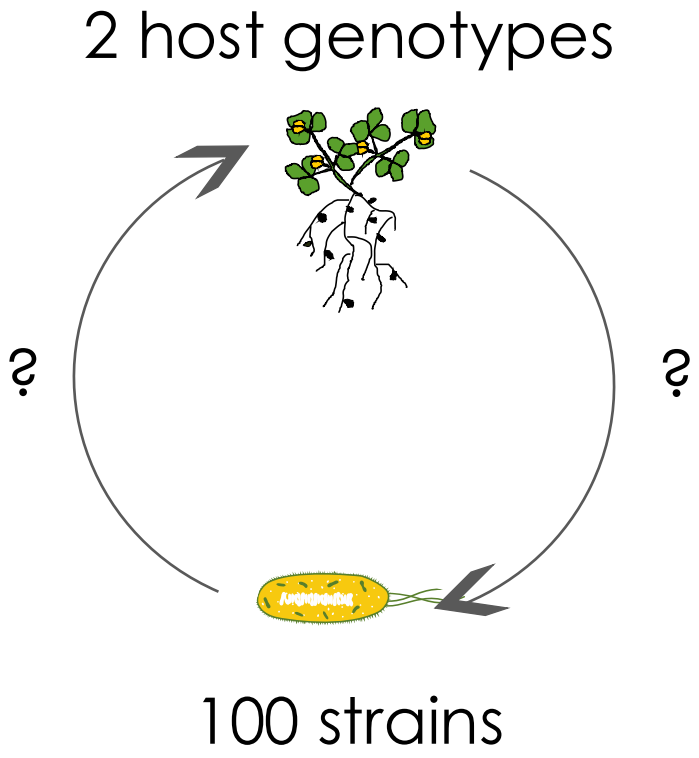


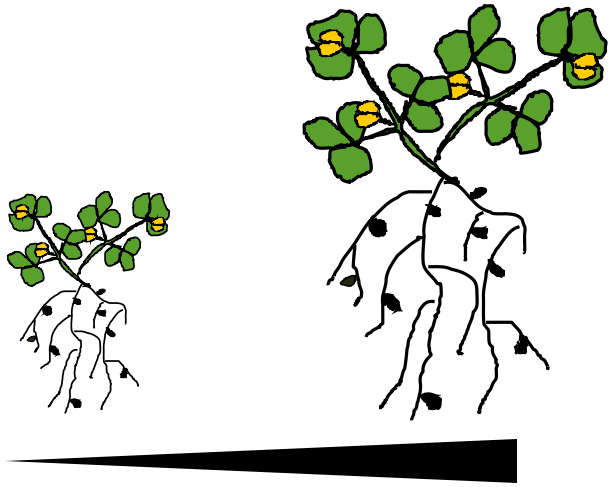
Xiao Xiao et. al. Molecular Ecology 2017

Traditional single-strain experiments to test effect of intra-specific genetic variation on partner fitness



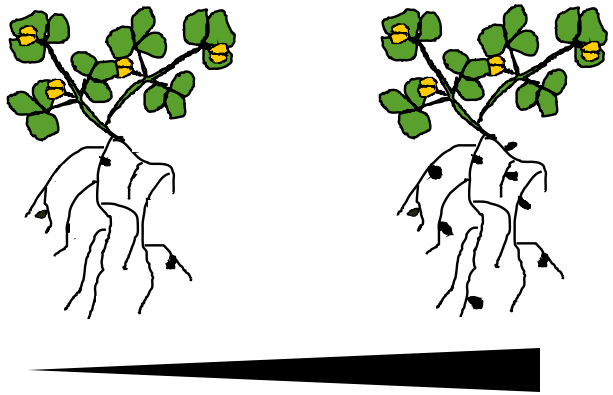
Measure fitness proxies: traits correlated w/ fitness

+ Plant Fitness



Plant biomass

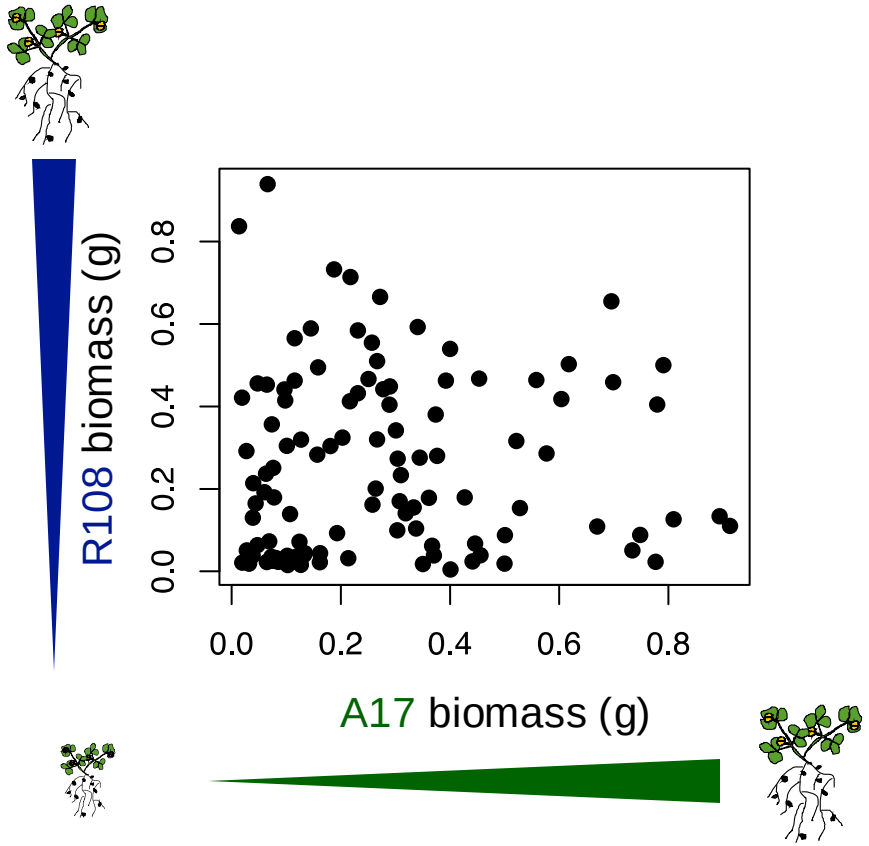
Bacterial Fitness +



Nodule number
Nodule size

Intra-specific strain variation influences plant fitness

Plant Fitness

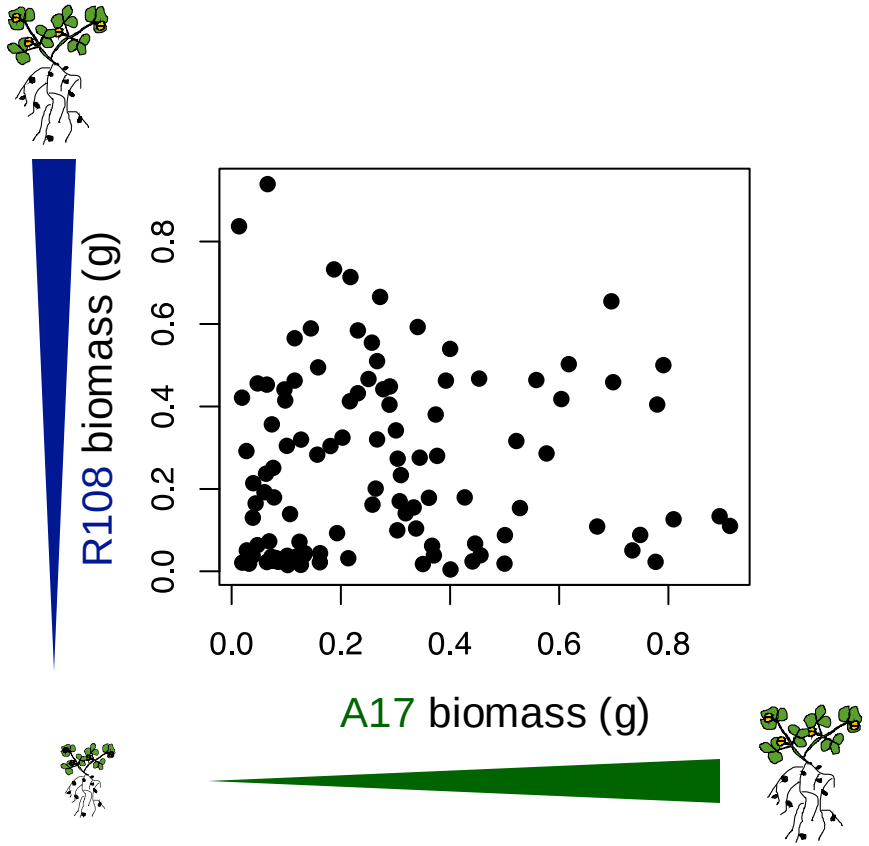


ALL OF THIS FUNCTIONAl VARIATION IS WITHIN and AS

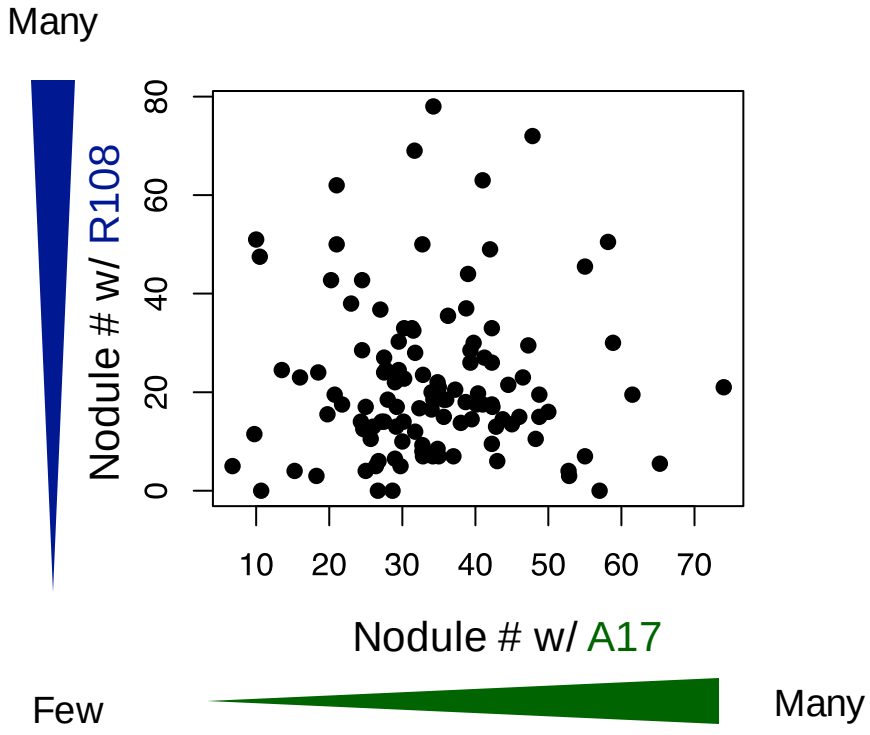
Burghardt et al. 2018, *PNAS*

Intra-specific host variation influences bacterial fitness

Plant Fitness



Bacterial Fitness



Burghardt et al. 2018, *PNAS*

In nature… strain exist alone! So how can we measure symbiont fitness?

Burghardt et al. 2018, *PNAS*

A microbial triathlon: Routes to shaping evolutionary trajectories

Nodules

Rhizosphere

Soil

[Culture]

Selection

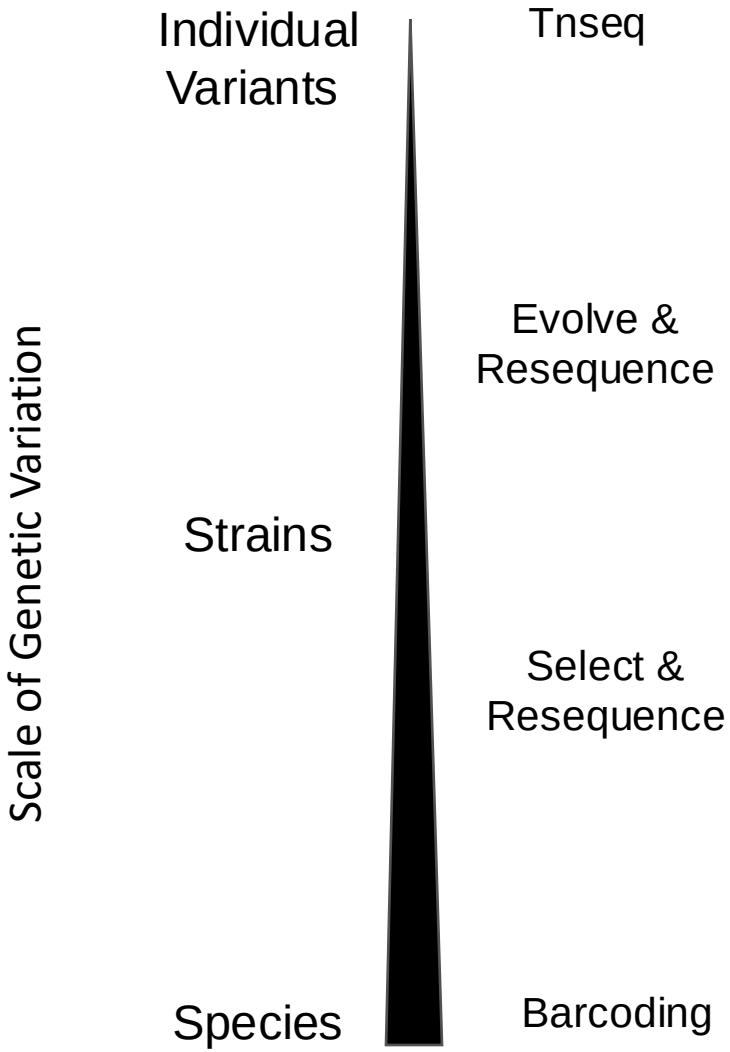
Population size

Frequency of exposure

Drift

Mutation

Whole genome shotgun sequencing allows measurement of microbial symbiont fitness at many different levels of genetic variation

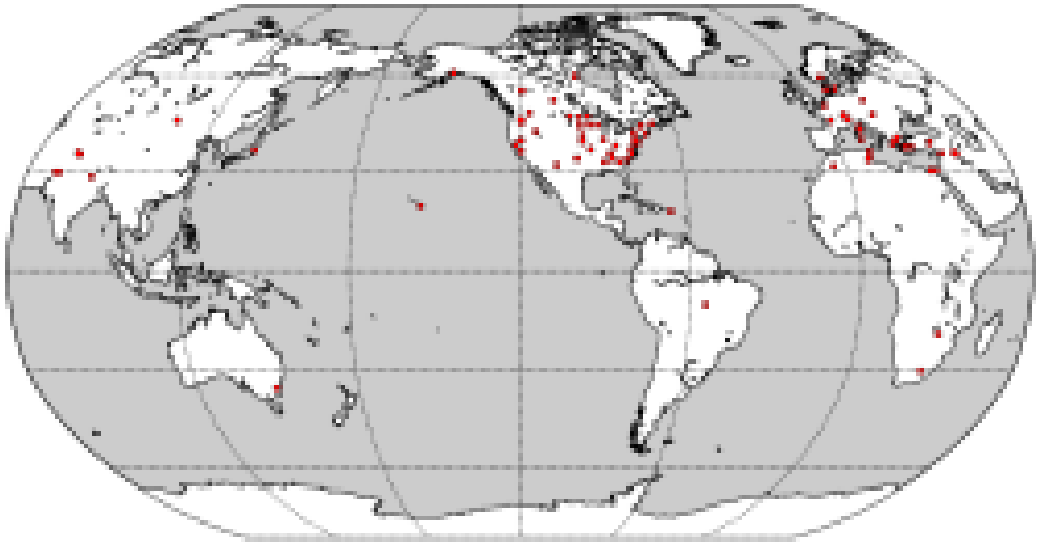


Select and resequence reveals relative fitness of bacteria in symbiotic and free-living environments

Liana T. Burghardt[a], Brendan Epstein[a], Joseph Guhlin[a], Matt S. Nelson[b], Margaret R. Taylor[b], Nevin D. Young[a,c], Michael J. Sadowsky[a,b,d], and Peter Tiffin[a,1]

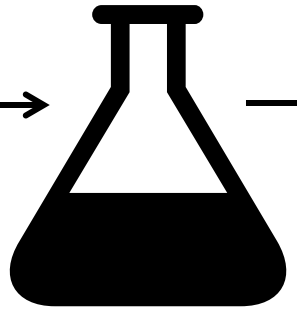
‘Select and Resequence’ Method

1.) Sequence individual strains

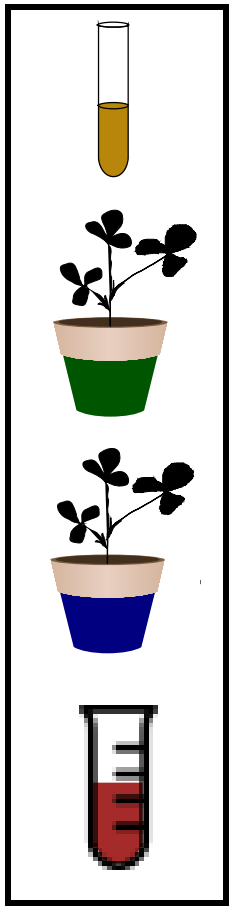


2) Grow strains in culture

3) Mix cultures to form multi-strain inoculum



4) Impose selection



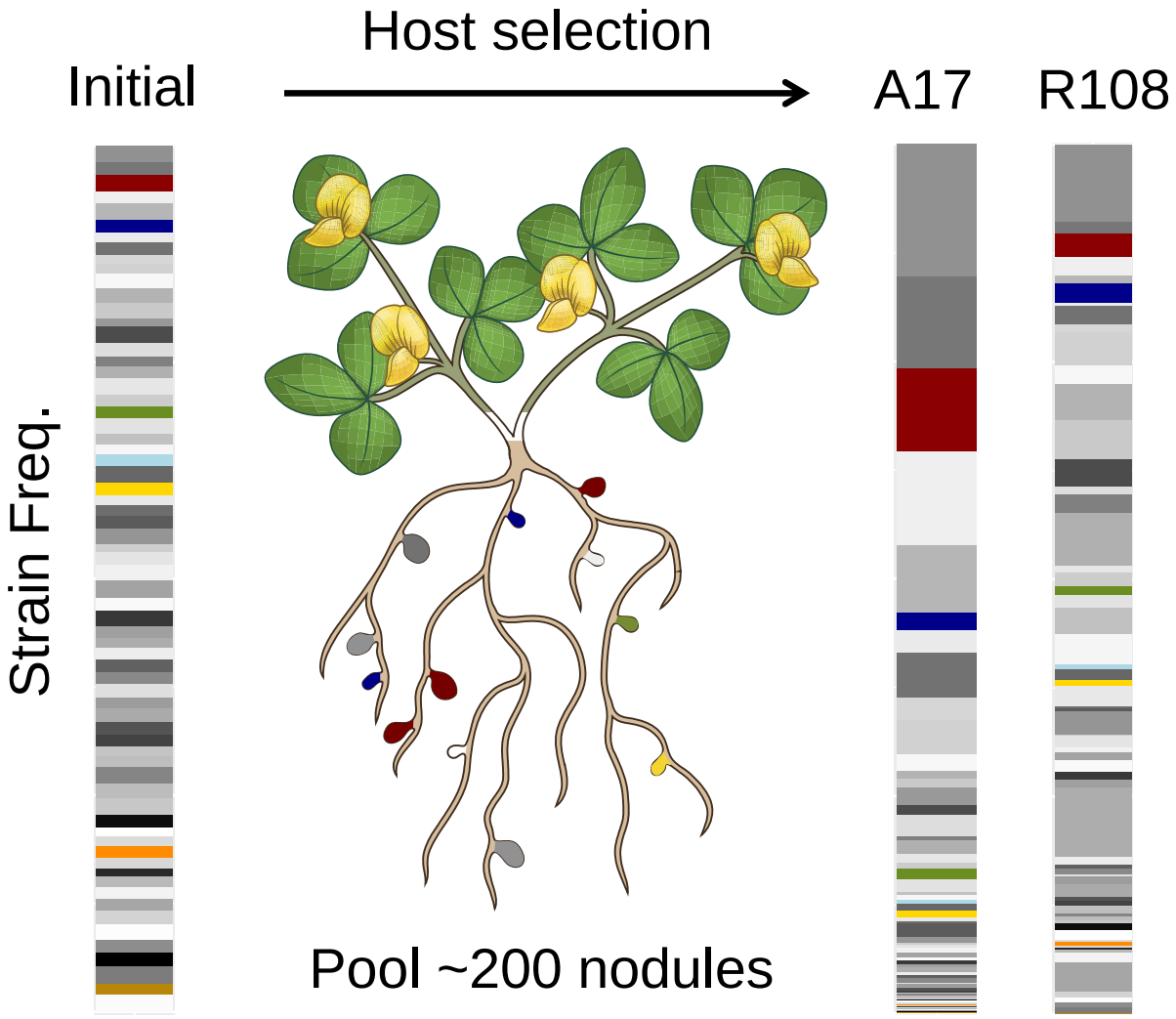
5) Extract DNA

6) WGS pooled sequencing

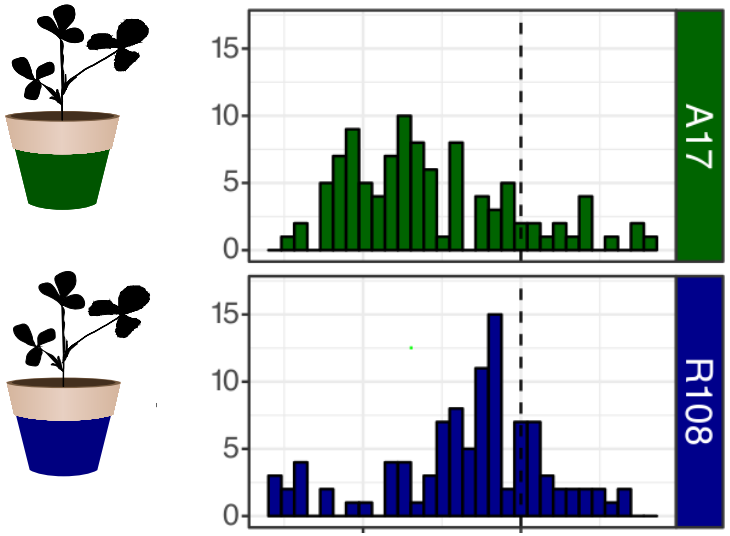
7) Infer strain frequencies using HARP

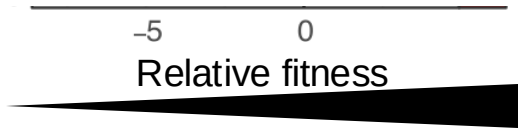
Burghardt et al. 2018, *PNAS*

Medicago genotypes enrich different strains



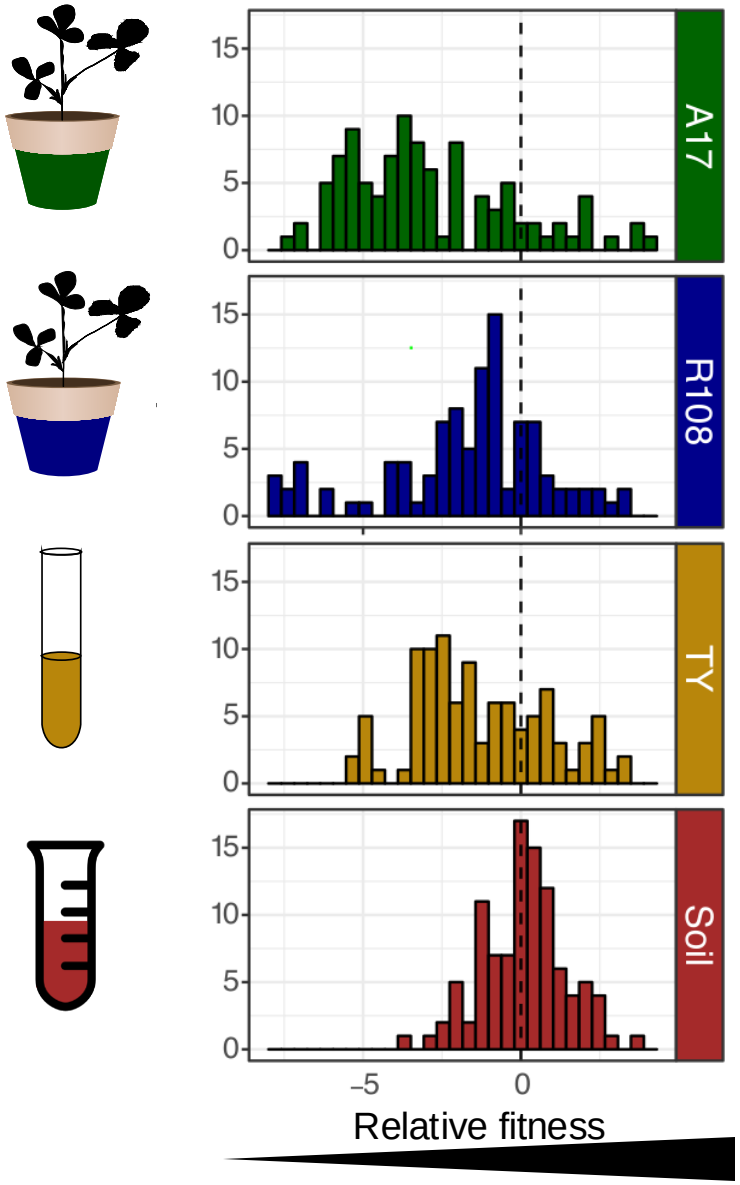
Convert strain frequencies into relative fitness





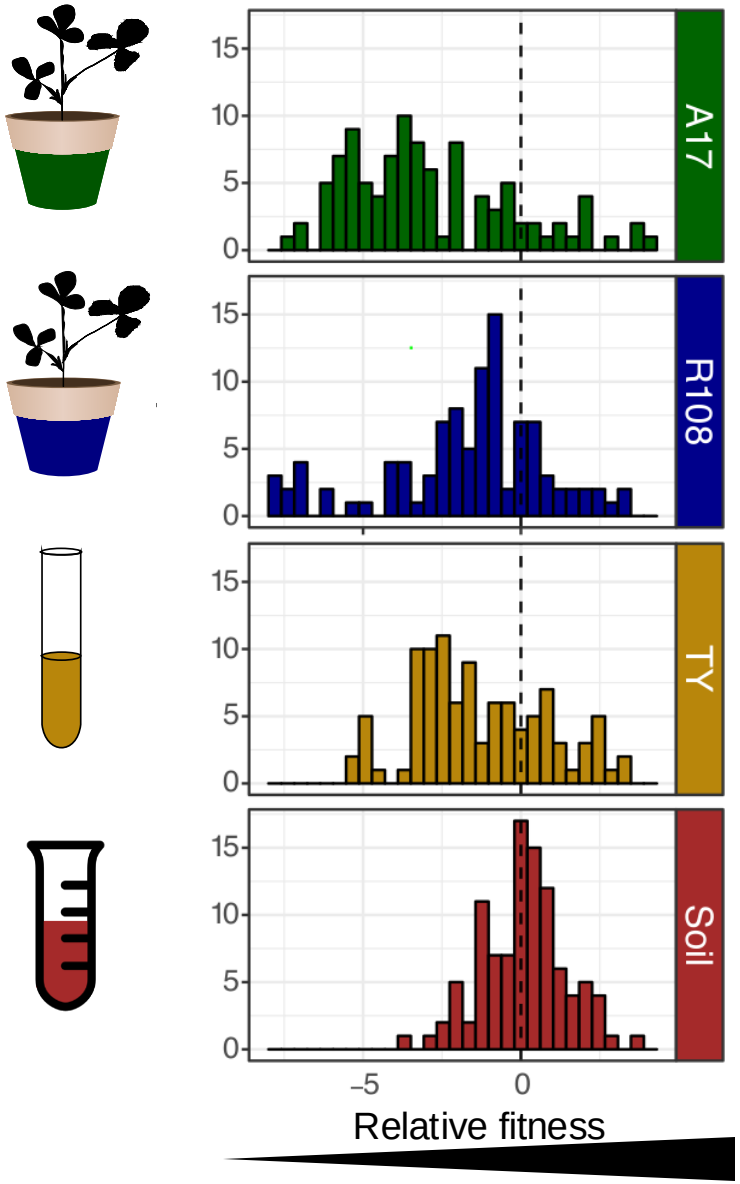
Burghardt et al. 2018, *PNAS*

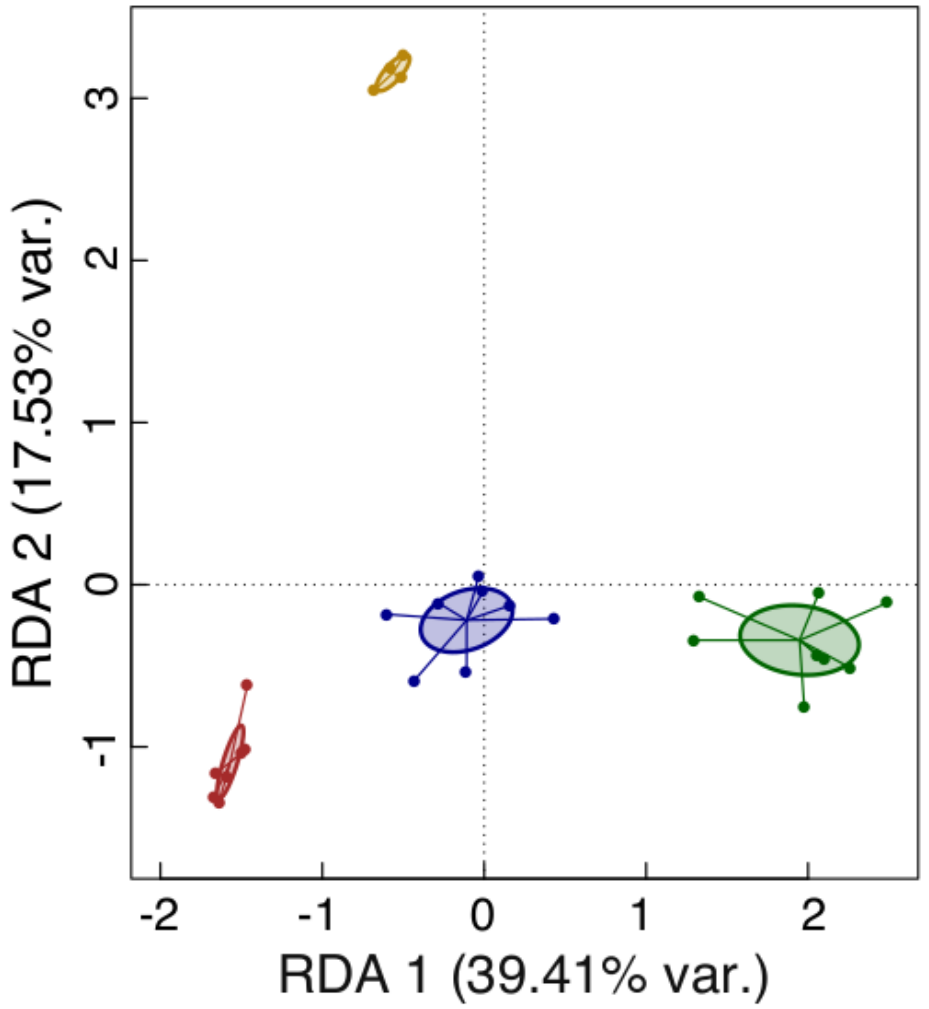
Hosts impose strong selection, reducing diversity



Burghardt et al. 2018, *PNAS*

Strains exhibit environment-specific fitness

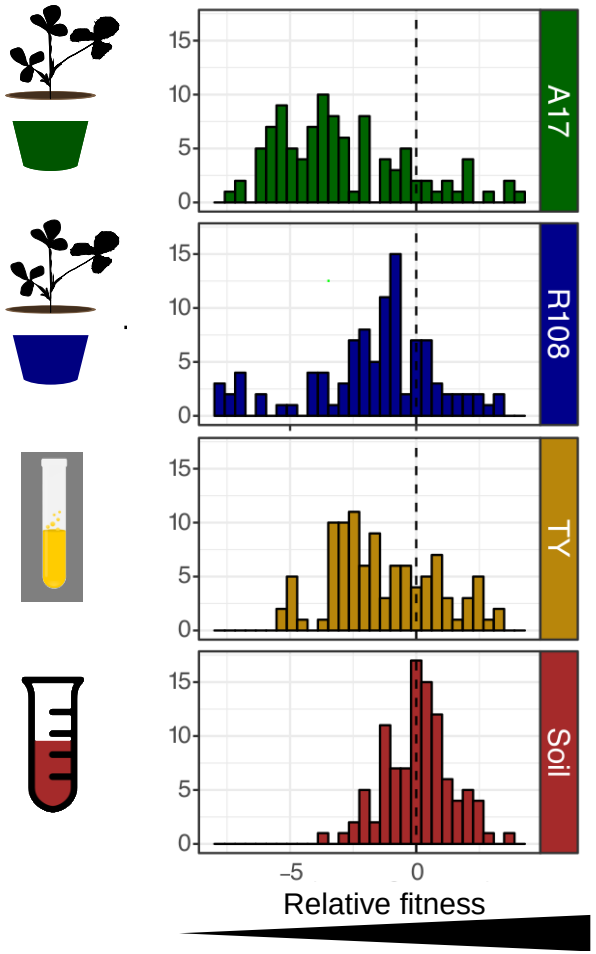




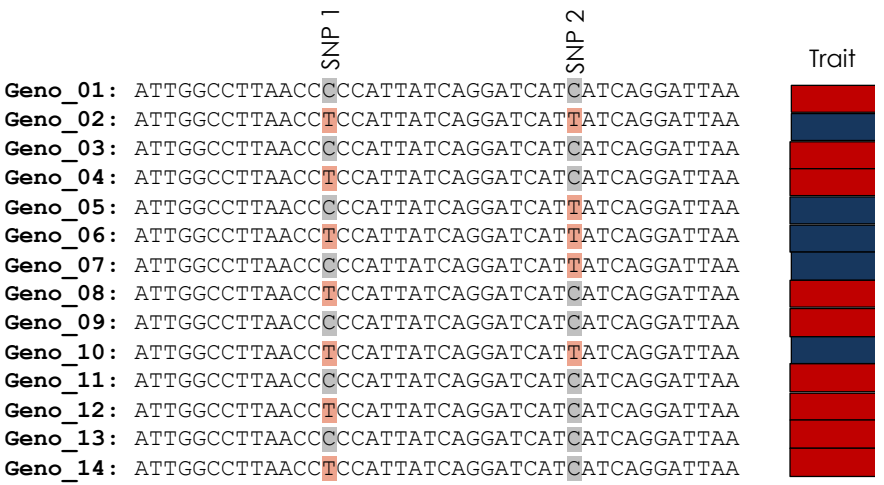
Burghardt et al. 2018, *PNAS*

Select & Resequence data allow downstream analysis

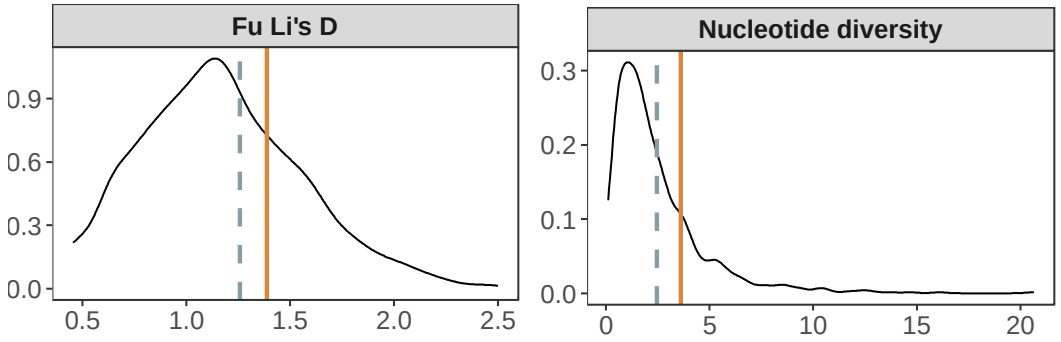
1. Measure Fitness



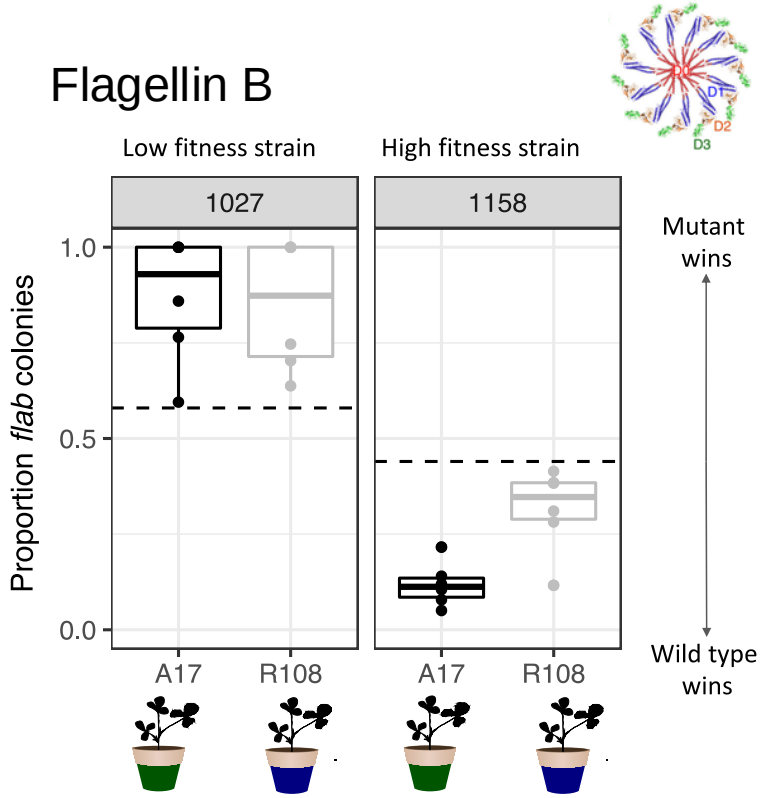
2. Genome wide association studies



3. Population Genetic Analysis



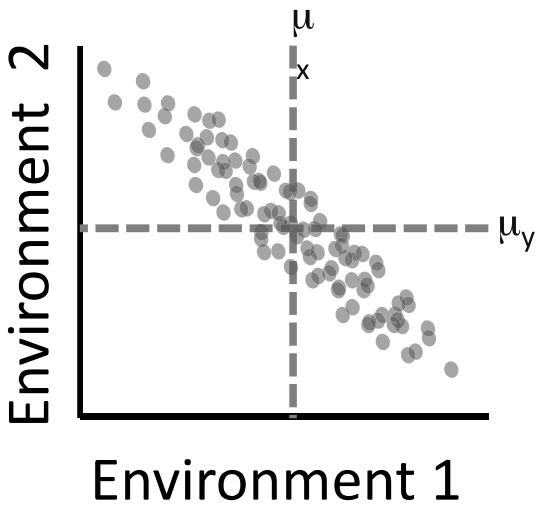
4. Validate Candidate Genes



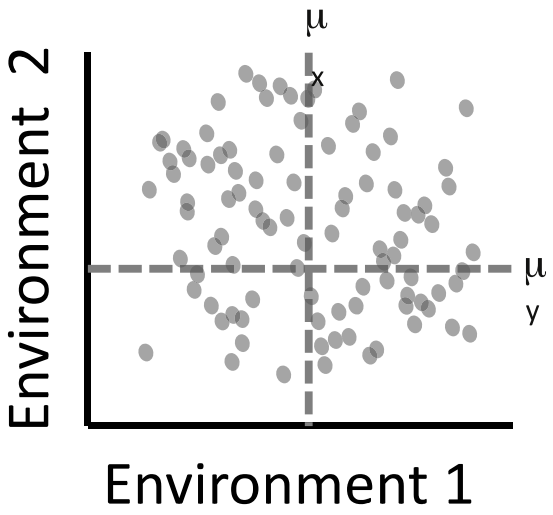
Burghardt *PNAS* 2018
Epstein *mSphere* 2018
Burghardt *Evolution* 2019
Burghardt 2020 *Plant Physiology*
Batstone et.al 2022 *Proc B*
Burghardt et al 2022 *AEM*
Epstein… Tiffin 2022 *Molecular Ecology*

What were rhizobial fitness correlations between WT and mutants?

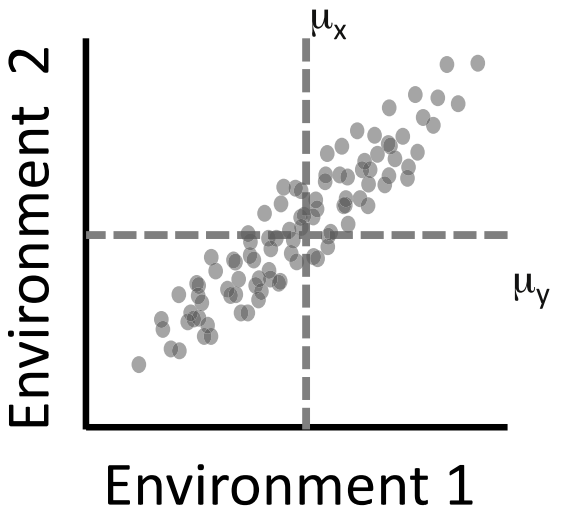
Constraint



Independence

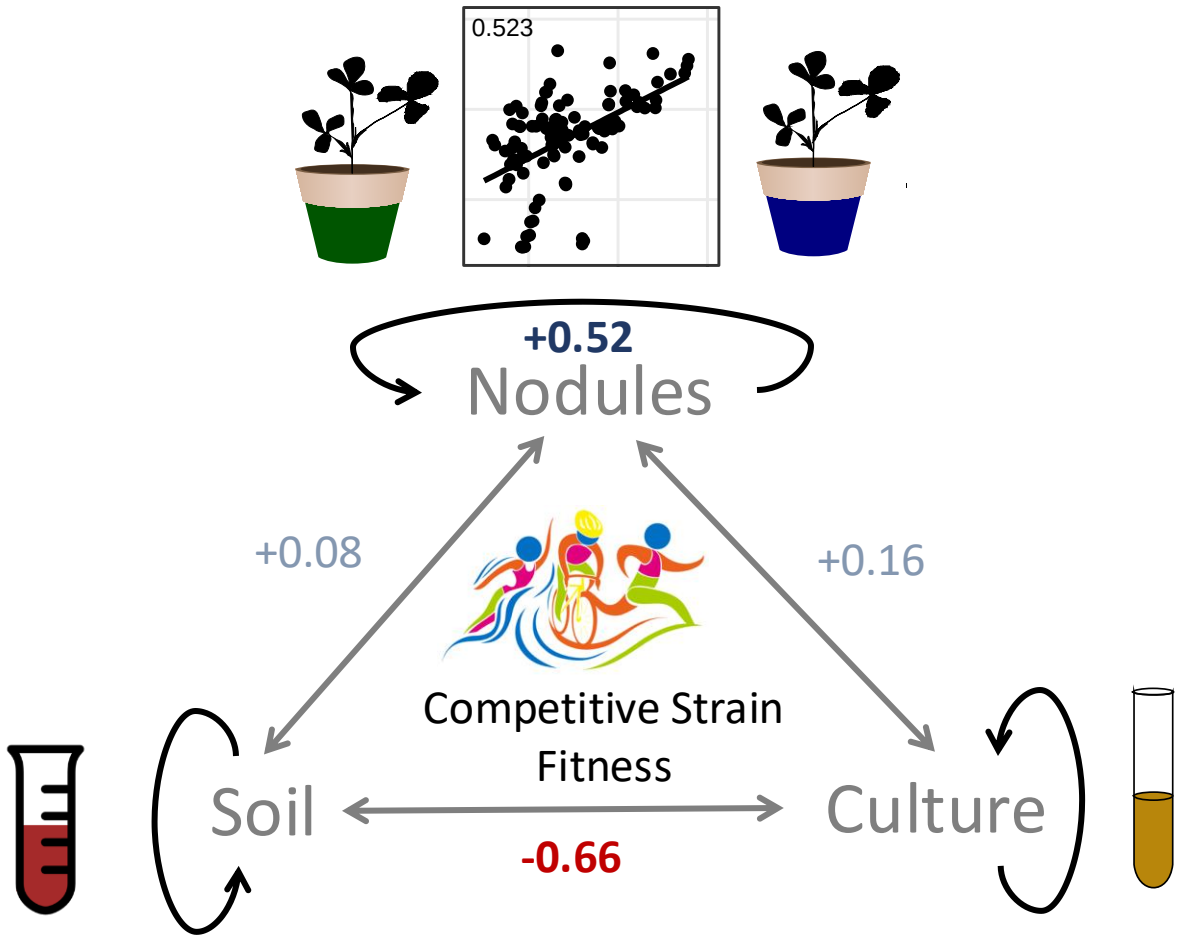


Facilitation



(Graphs: Salz et. al. 2017)

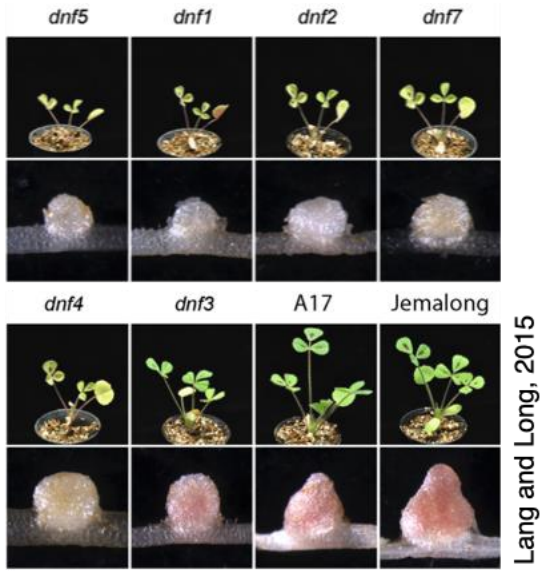
Fitness correlations across environments



Burghardt et al. 2018, *PNAS*

What scale of host genetic variation matters to rhizobial fitness?

1. Mutations



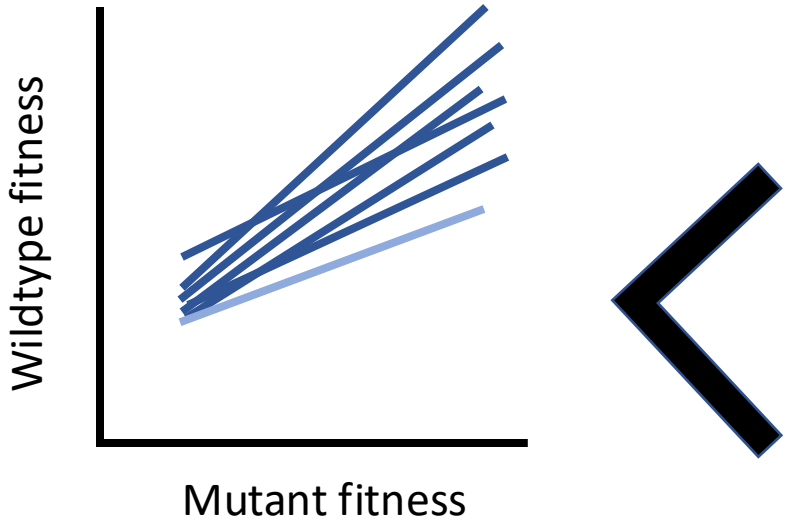
2. Accessions

3. Species

(Germplasm thanks: Nevin Young, HapMap Collab, Noble Foundation)

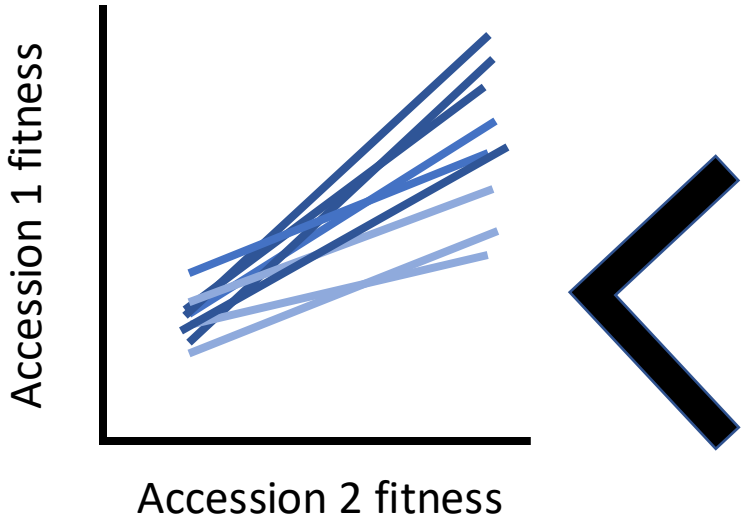
Only at the species-level do we observe negative rhizobial fitness correlations (evolutionary constraint)

1. Mutations



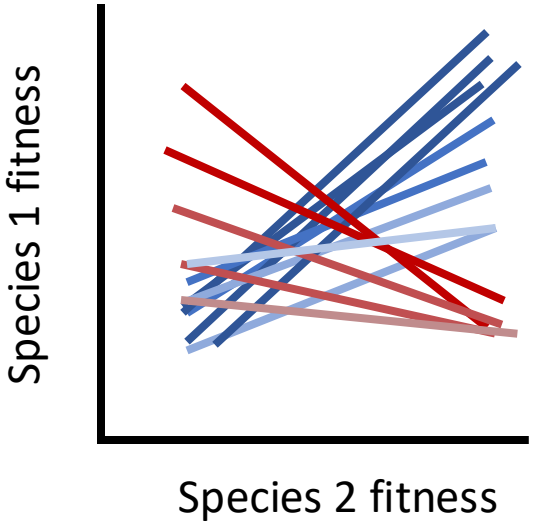
36 mutants
R ~ +0.76
[strongly positive]

2. Accessions



202 accessions
R ~ .74
[always positive]

3. Species



15 species
R = -0.60 to +0.95
[highly variable]

Can use GWAS tools in BOTH hosts simultaneously

FROM THE COVER

MOLECULAR ECOLOGY WILEY

Combining GWAS and population genomic analyses to characterize coevolution in a legume-rhizobia symbiosis

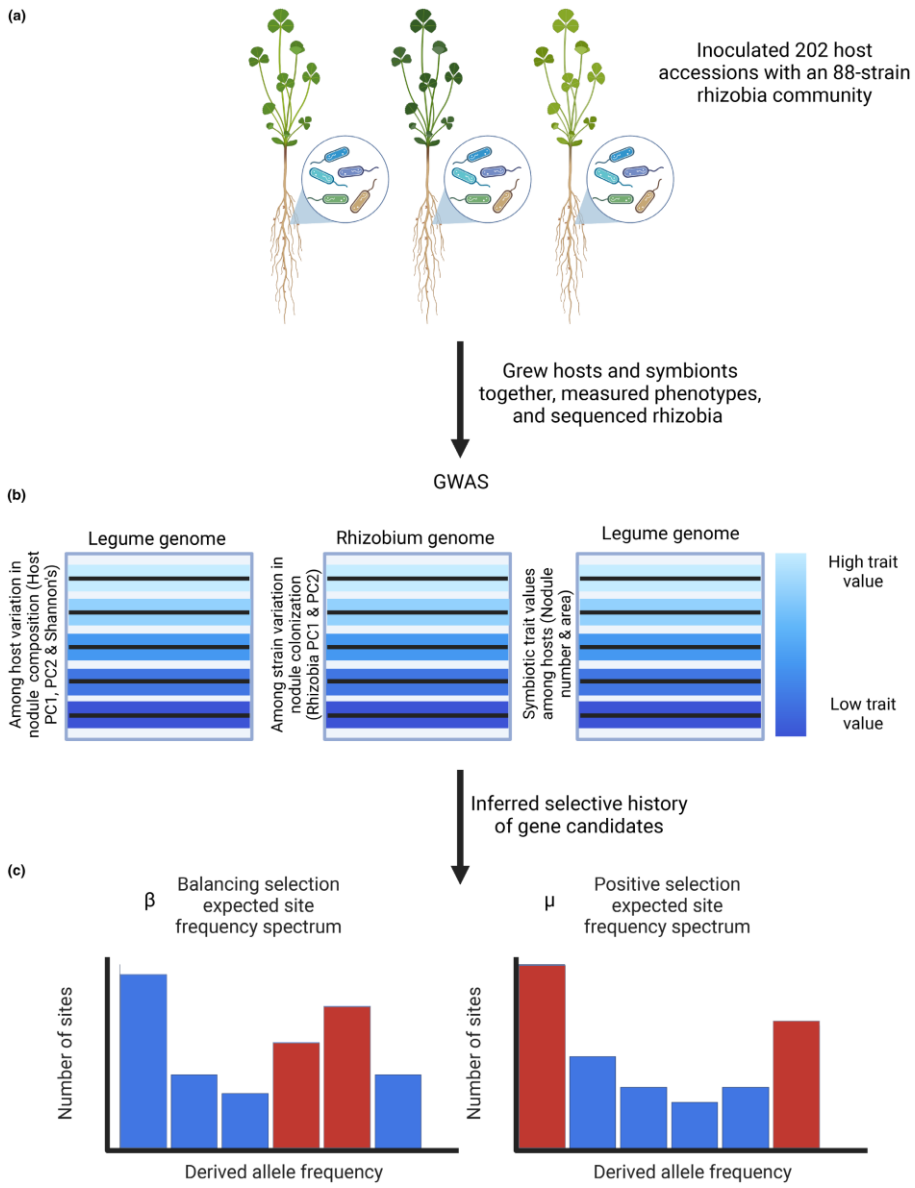
Brendan Epstein[1] | Liana T. Burghardt[2] | Katy D. Heath[3,4] | Michael A. Grillo[5] | Adam Kostanecki[1] | Tuomas Hämälä[1] | Nevin D. Young[1,6] | Peter Tiffin[1]

PERSPECTIVE

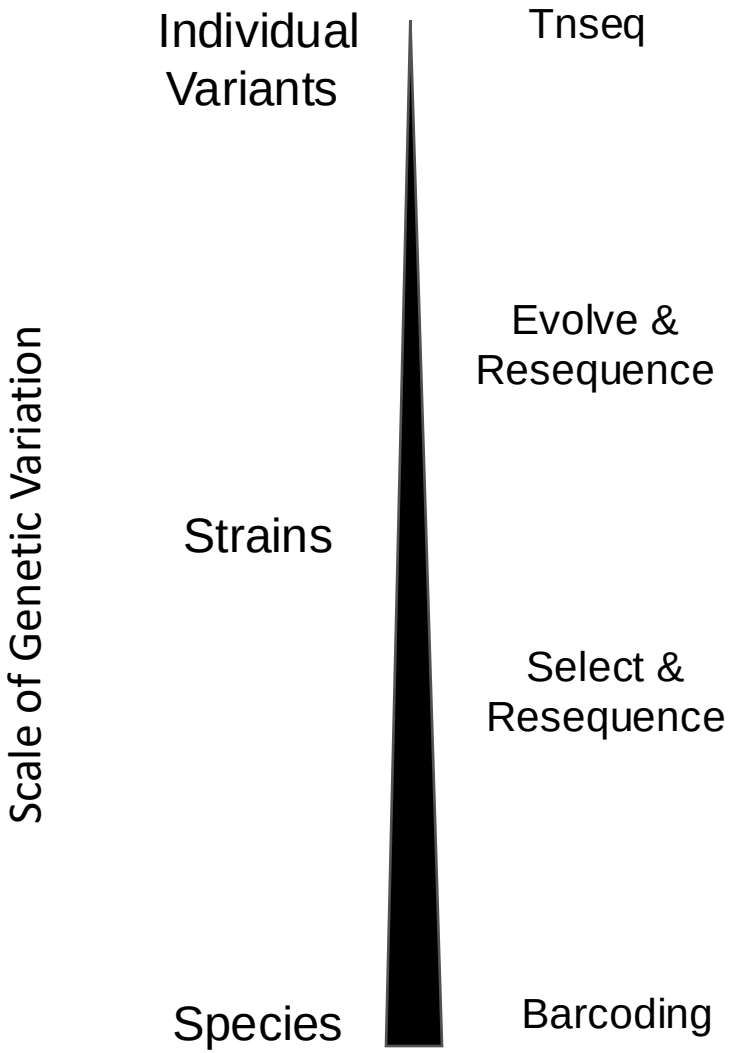
MOLECULAR ECOLOGY WILEY

An emerging view of coevolution in the legume–rhizobium mutualism

Christopher Carlson | Megan E. Frederickson



Whole genome shotgun sequencing allows measurement of microbial symbiont fitness at many different levels of genetic variation



ADAPTATION

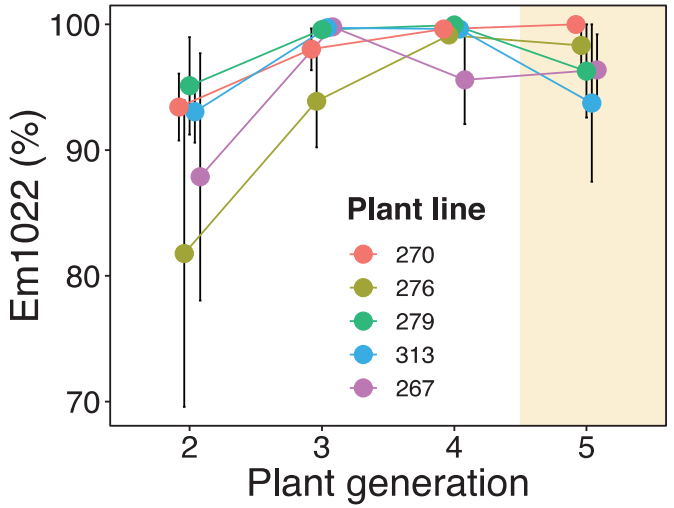
Experimental evolution makes microbes more cooperative with their local host genotype

Rebecca T. Batstone[1,2*], Anna M. O'Brien[1,3], Tia L. Harrison[1], Megan E. Frederickson[1,4,5]

Experimental evolution makes microbes more cooperative with their local host genotype

Science

1. Evolve two strains for host generation on 5 host genotypes



High quality microbial partner spread to near fixation

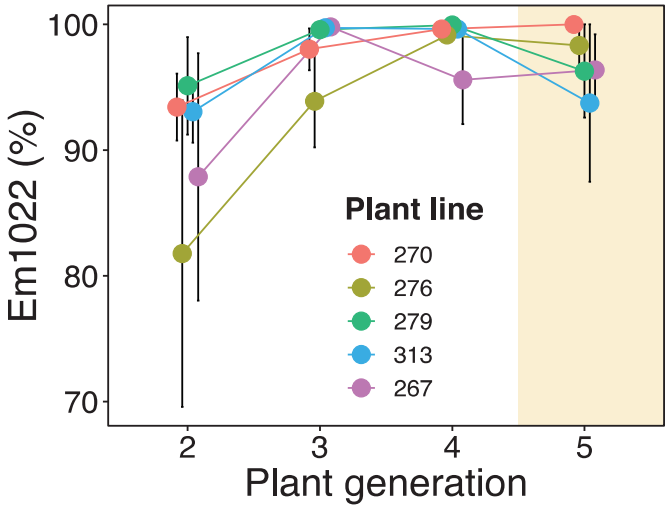
Experimental evolution makes microbes more cooperative with their local host genotype

Science

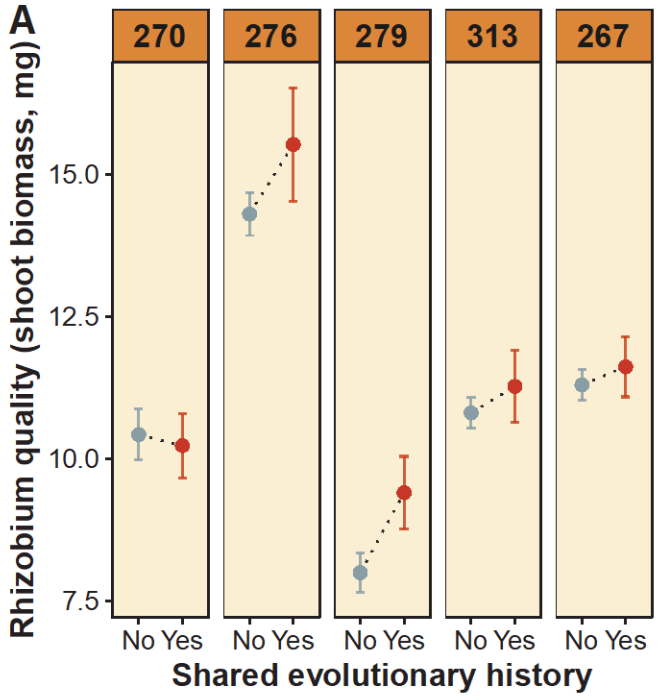
1. Evolve two strains for host generation on 5 host genotypes

2. Isolate evolved strains and sequence

3. Assess phenotypic consequences of evolution



High quality microbial partner spread to near fixation

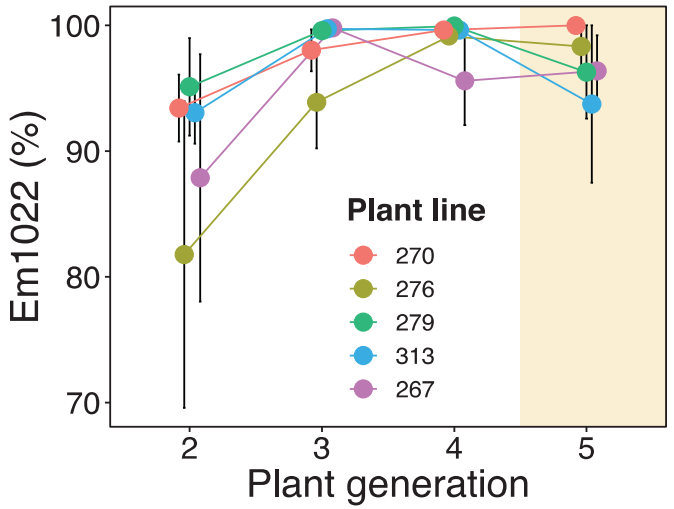


Isolates are most helpful to the hosts they evolve on

Experimental evolution makes microbes more cooperative with their local host genotype

Science

1. Evolve two strains for host generation on 5 host genotypes



High quality microbial partner spread to near fixation

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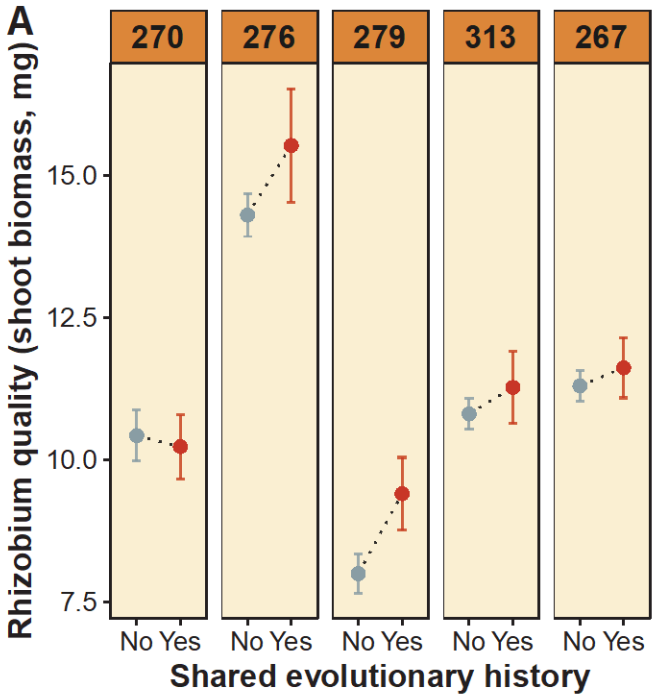
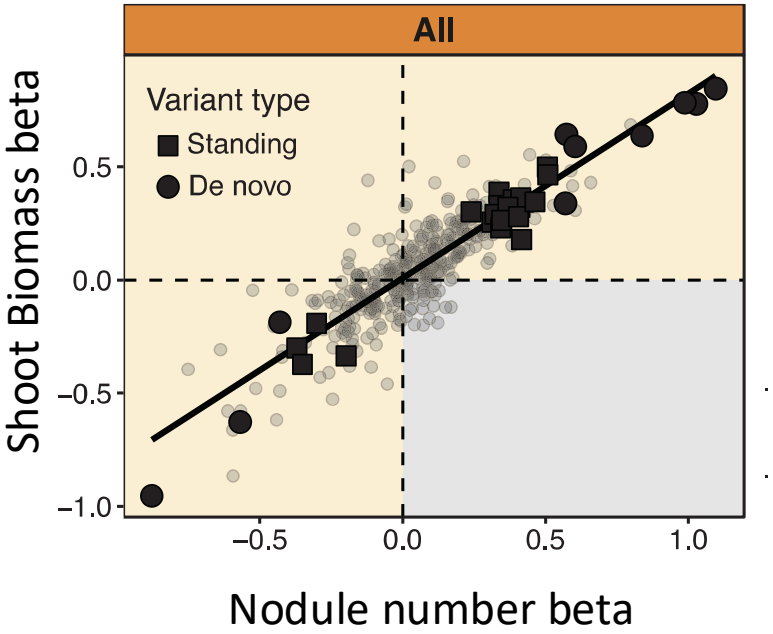


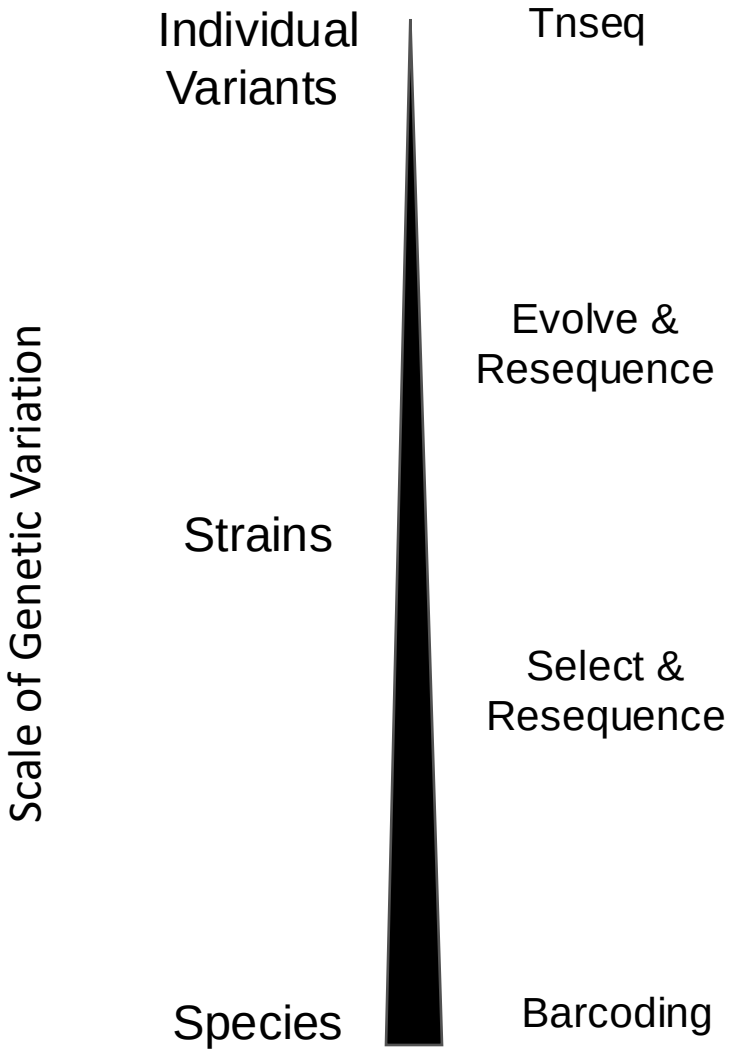
Isolates are most helpful to the hosts they evolve on

4. Assess genomic patterns of evolution in response to hosts



Both standing and de novo genetic variation helped both partners

Whole genome shotgun sequencing allows measurement of microbial symbiont fitness at many different levels of genetic variation



Lifestyle adaptations of *Rhizobium* from rhizosphere to symbiosis

Rachel M. Wheatley[a,1], Brandon L. Ford[a,1], Li Li[b,1], Samuel T. N. Aroney[a], Hayley E. Knights[a], Raphael Ledermann[a], Alison K. East[a], Vinoy K. Ramachandran[a,2], and Philip S. Poole[a,2]

Science

Transposon and insertion sequencing is increasingly used to study symbiosis…

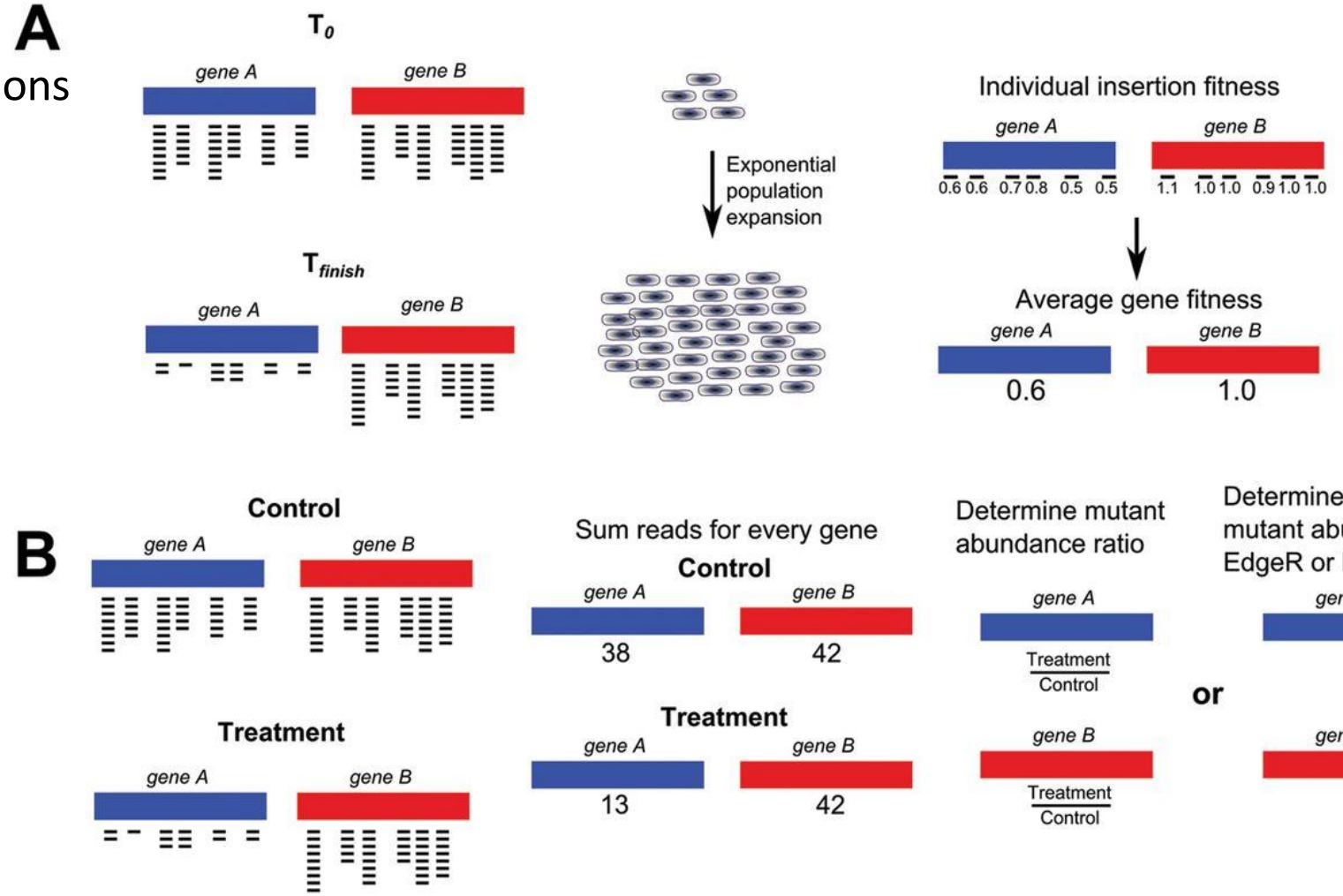
1. Create library filed with mutations

2. Expose to selection

3. Whole genome sequencing

Inference: if genetic disruptions stick around… the gene is not essential in that environment

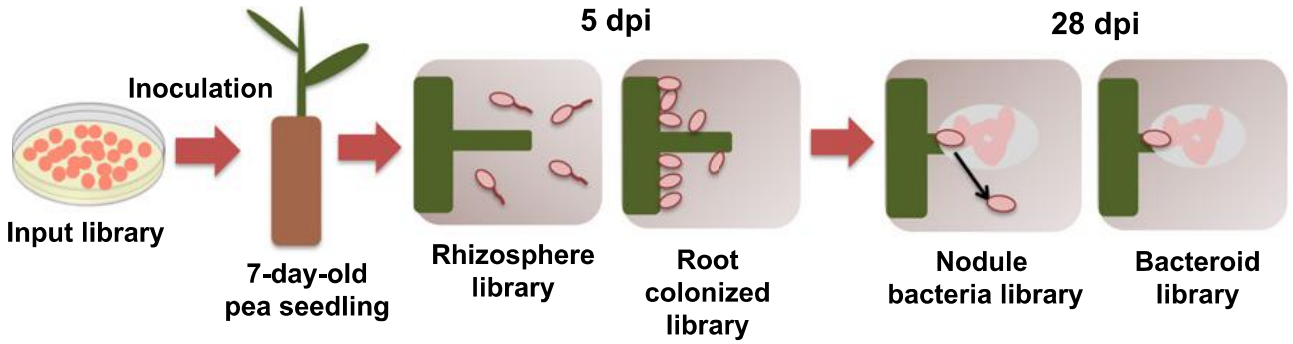
Limitations: Only screens knockout disruptions, not quantitative variation + can't have huge bottlenecks

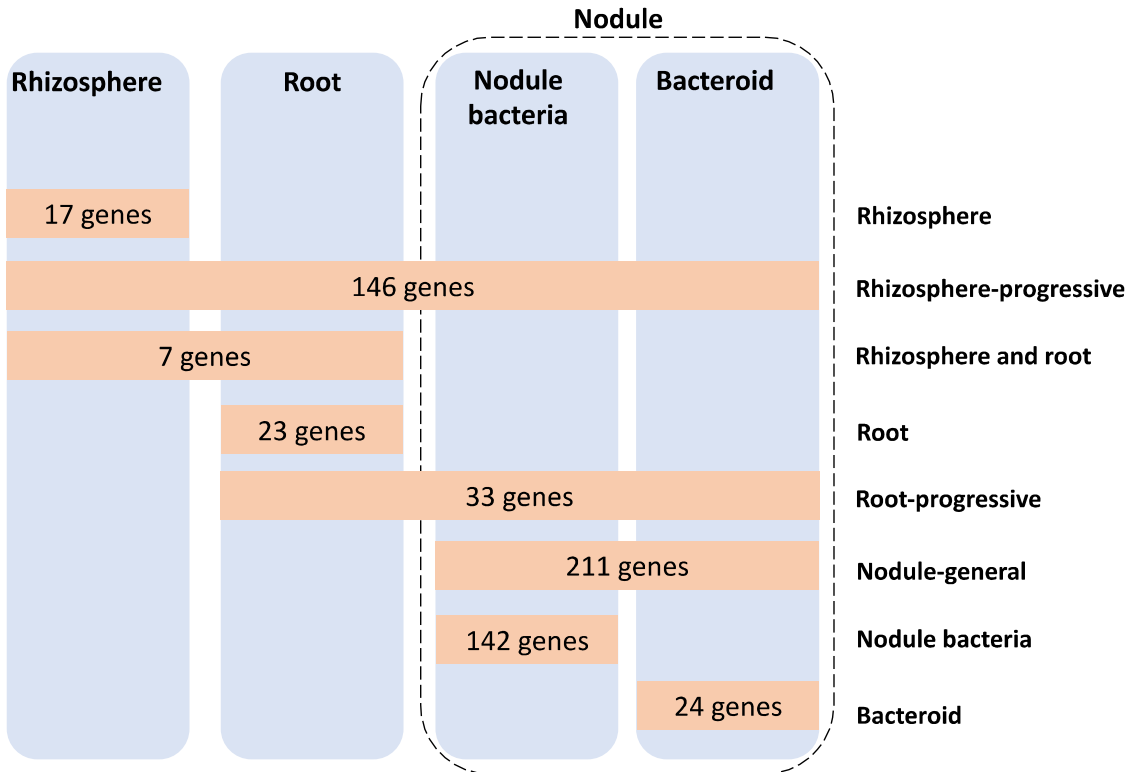


Implementation and Data Analysis of Tn-seq, Whole-Genome Resequencing, and Single-Molecule Real-Time Sequencing for Bacterial Genetics

Authors: Peter E. Burby, Taylor M. Nye, Jeremy W. Schroeder, Lyle A. Simmons

Transposon/Insertion Sequencing: Lifestyle adaptations of Rhizobium from rhizosphere to symbiosis

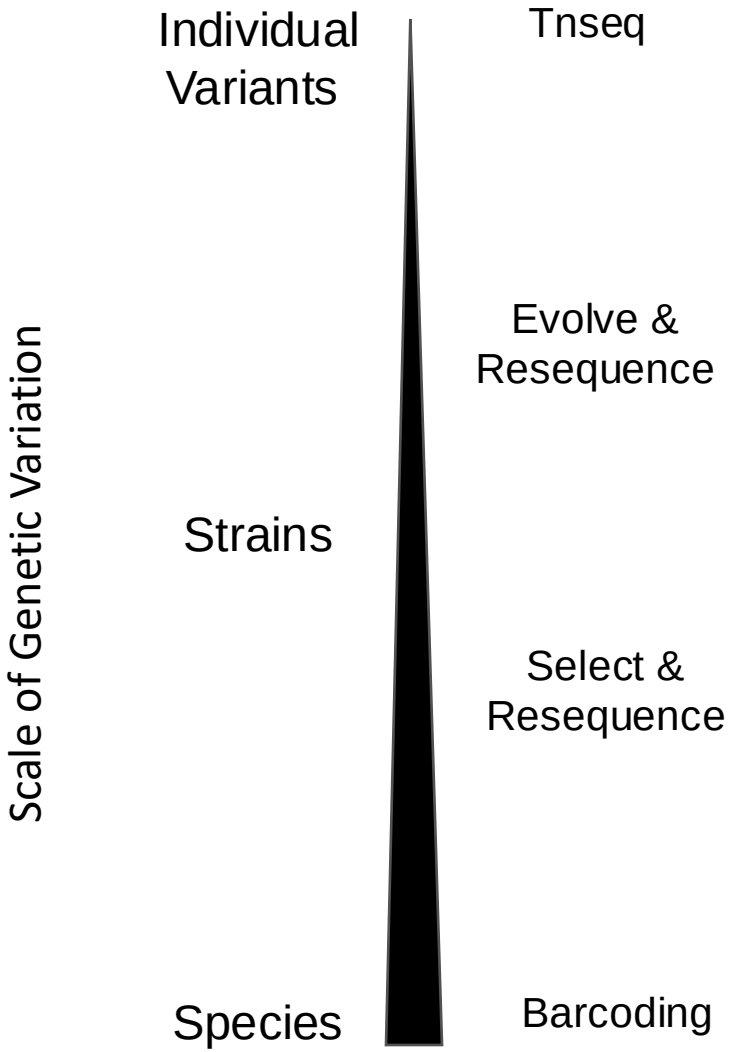




- Catagorize genes with essential functions across one or more rhizobial environments

Wheatley, PNAS

Whole genome shotgun sequencing allows measurement of microbial symbiont fitness at many different levels of genetic variation



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ADAPTATION

Science

Experimental evolution makes microbes more cooperative with their local host genotype

Rebecca T. Batstone[1,2*], Anna M. O'Brien[1,3], Tia L. Harrison[1], Megan E. Frederickson[1,4,5]

Select and resequence reveals relative fitness of bacteria in symbiotic and free-living environments

Liana T. Burghardt[a], Brendan Epstein[a], Joseph Guhlin[a], Matt S. Nelson[b], Margaret R. Taylor[b], Nevin D. Young[a,c], Michael J. Sadowsky[a,b,d], and Peter Tiffin[a,1]

ISME

Variation in rhizosphere microbial communities and its association with the symbiotic efficiency of rhizobia in soybean

Qin Han[1] · Qun Ma[1] · Yong Chen[1] · Bing Tian[1] · Lanxi Xu[1] · Yang Bai[2] · Wenfeng Chen[3] · Xia Li[1]

Learning objectives

- Define symbiosis and understand variation in usage
- Understand basic categories of symbiosis and brainstorm examples
- Define holobiont and hologenome concepts
- Articulate how to use shotgun sequencing to measure symbiont fitness at multiple scales of genetic variation
- Fall in love with legumes and rhizobia

"Our task now is to resynthesize biology; put the organism back into its environment; connect it again to its evolutionary past; and let us feel that complex flow that is organism, evolution, and environment united."-

Carl Woese 2003

Questions?

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Twitter@lianaburghardt
lianaburghardtlab.com

Image courtesy of the Soil and Terrestrial Environmental Physics group of Dani Or at ETH Zurich (www.step.ethz.ch). SEM image acquisition and coloring by Anne Greet Bittermann, ETH Zurich – ScopeM

Dual transcriptomics (DualSeq): modified shotgun sequencing from both species simultaneously

Homogenize tissue

Isolate RNA

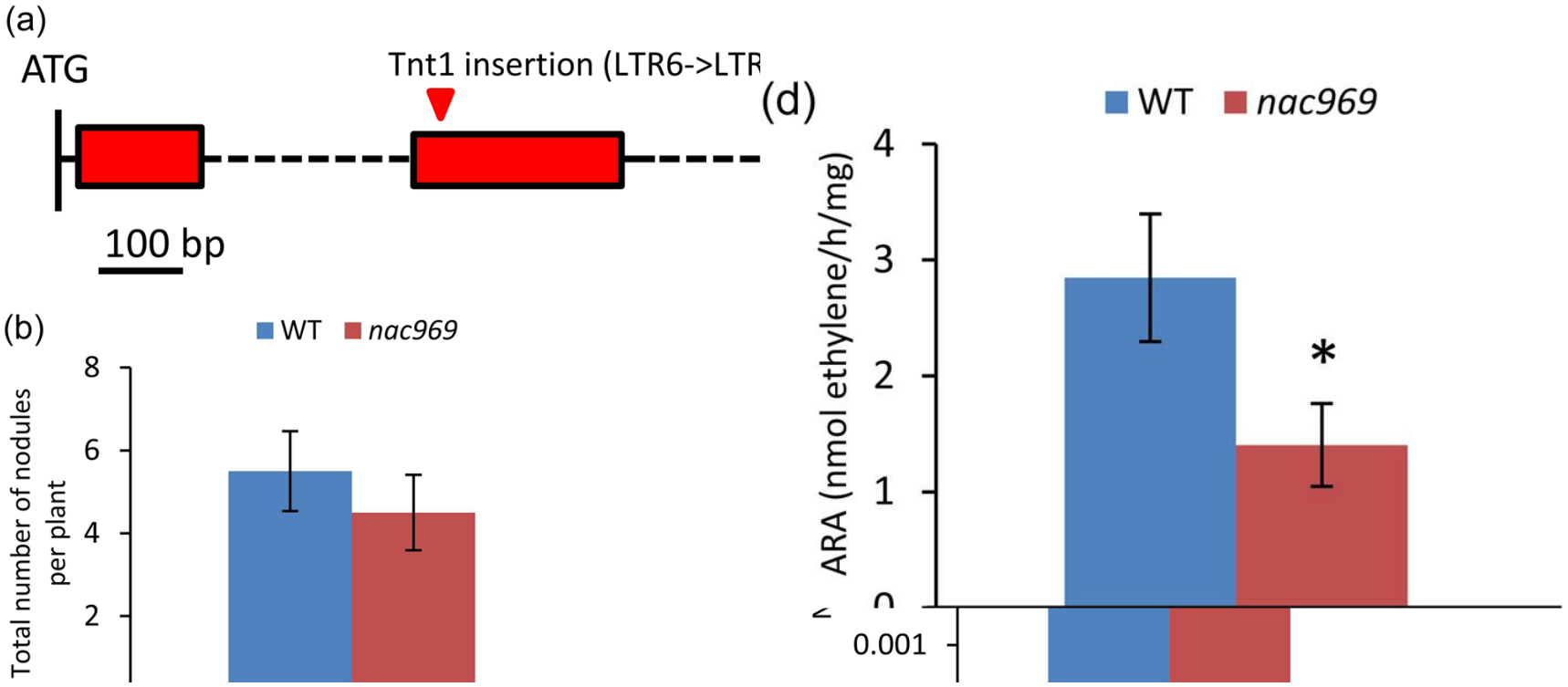
rRNA depletion and cDNA library creation

Short read sequencing

Map reads to concatenated genomes

Comparative transcriptomics
Coexpression modules
Pathway Analysis

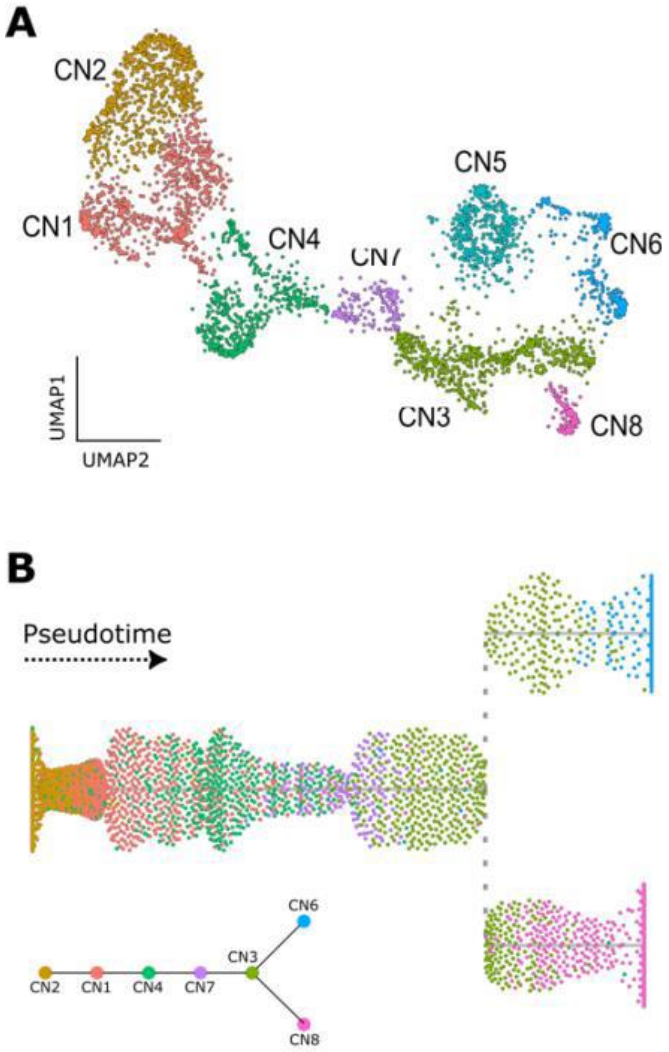
A dual legume-rhizobium transcriptome of symbiotic nodulesenescence reveals coordinated plant and bacterial responses- Saviac 2022

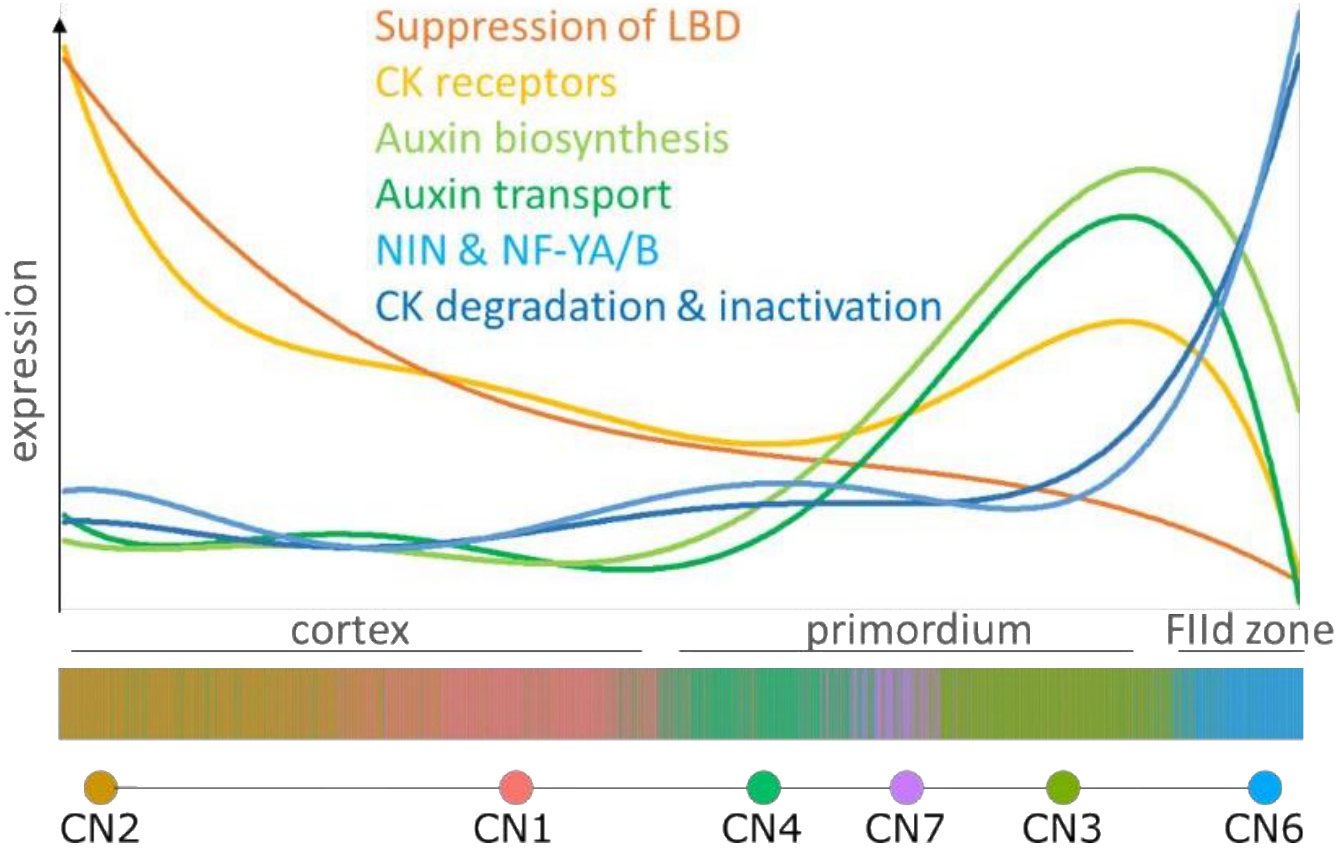


Thomas Wolf, et al. Two's company: studying interspecies relationships with dual RNA-seq, Current Opinion in Microbiology,2018 https://doi.org/10.1016/j.mib.2017.09.001.

Moving to Single Cell Transcriptomics… is still fancy shotgun sequencing

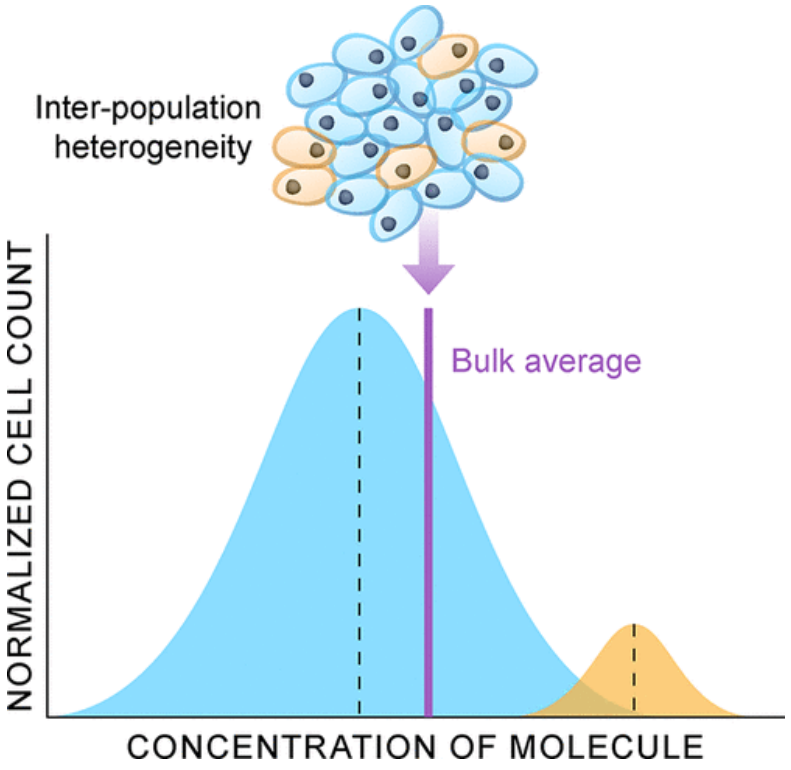
The Single-Cell Transcriptome Program of Nodule Development Cellular Lineages in *Medicago truncatula*

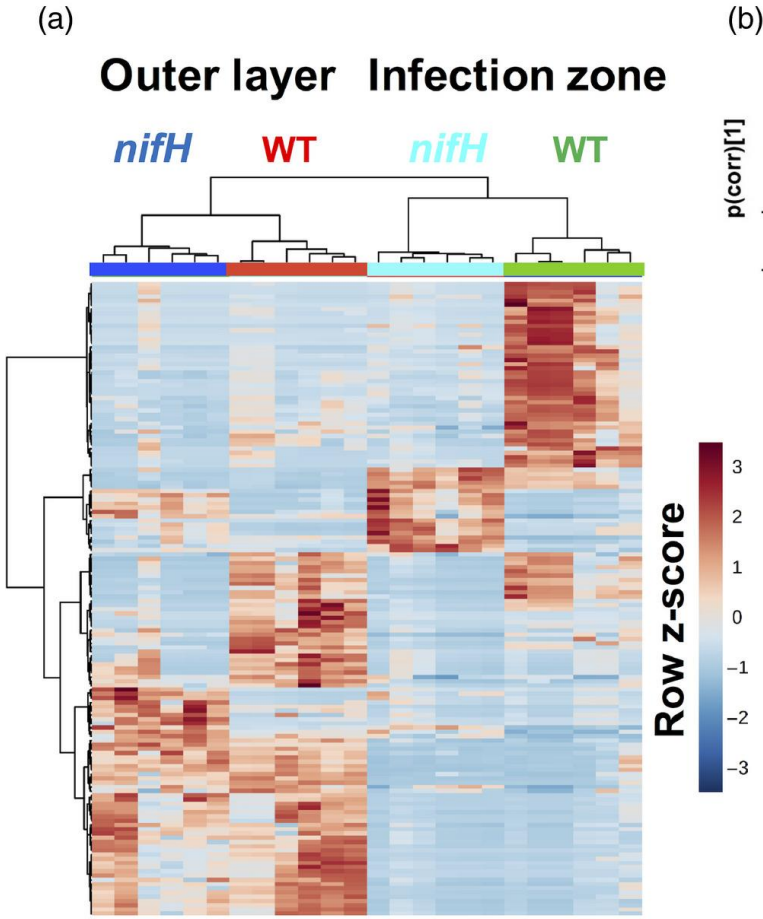


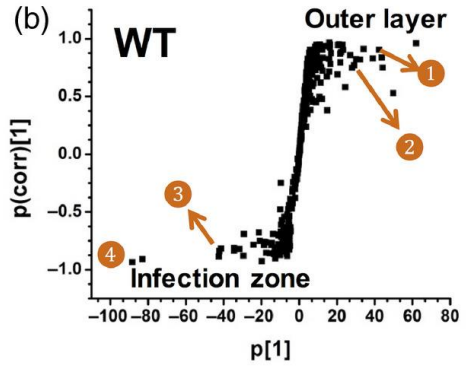


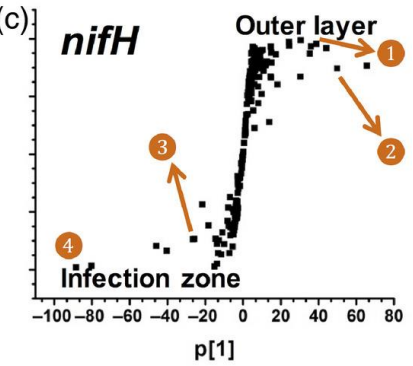
Metabolomic profiling of soybeans with functional and nonfunctional symbiotic partners

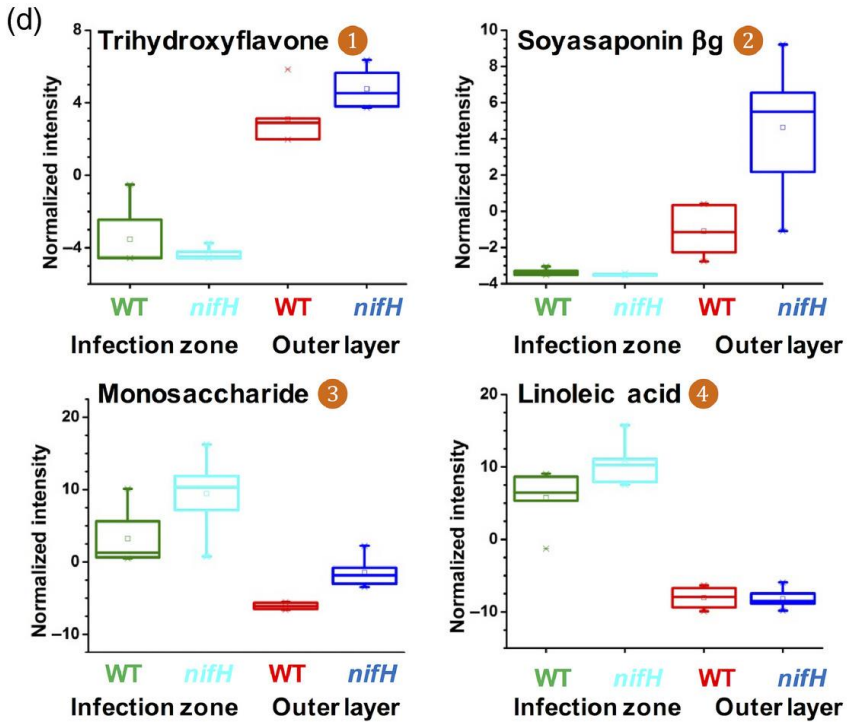
Metabolomic profiling of wild-type and mutant soybean root nodules using laser-ablation electrospray ionization mass spectrometry reveals altered metabolism









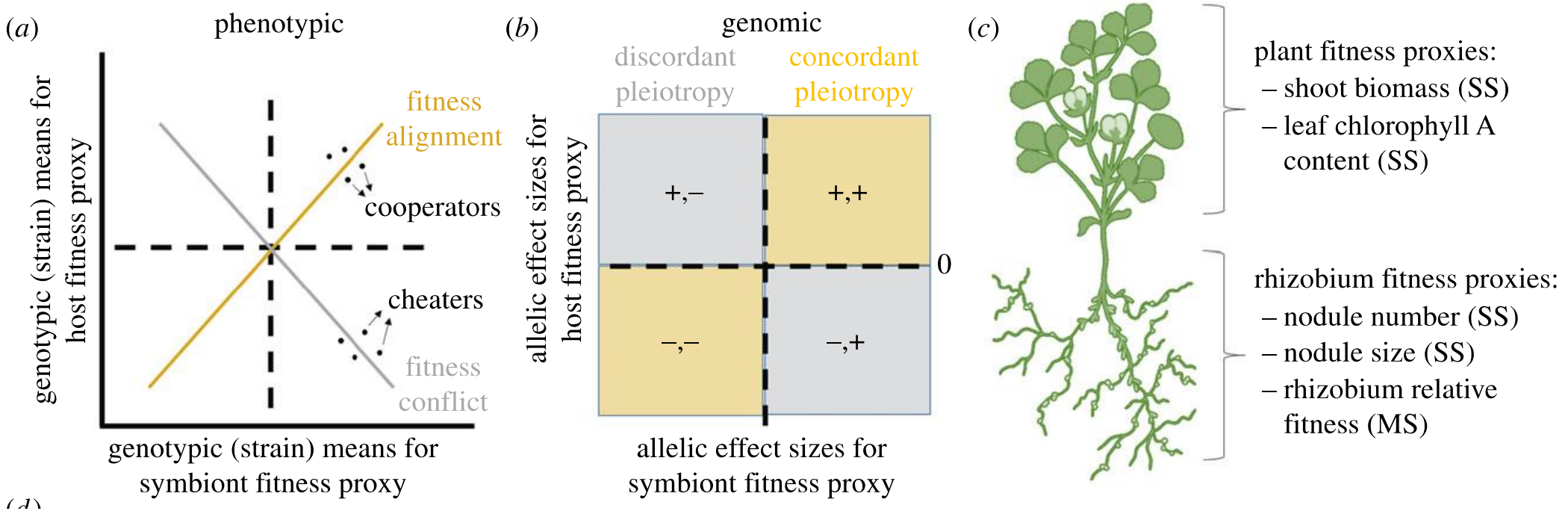


The Plant Journal, Volume: 103, Issue: 5, Pages: 1937-1958, First published: 15 May 2020, DOI: (10.1111/tpj.14815)

Ask if genes effecting symbiont fitness also effect host fitness…

Phenotypic and genomic signatures of interspecies cooperation and conflict in naturally occurring isolates of a model plant symbiont

Rebecca T. Batstone[1], Liana T. Burghardt[2] and Katy D. Heath[3]



Ask if genes effecting symbiont fitness also effect host fitness…