Macroevolution to Microbiomes: Piecing Together the Puzzle to Understand the Evolution of the Ants

Image from www.imaginetoys.com

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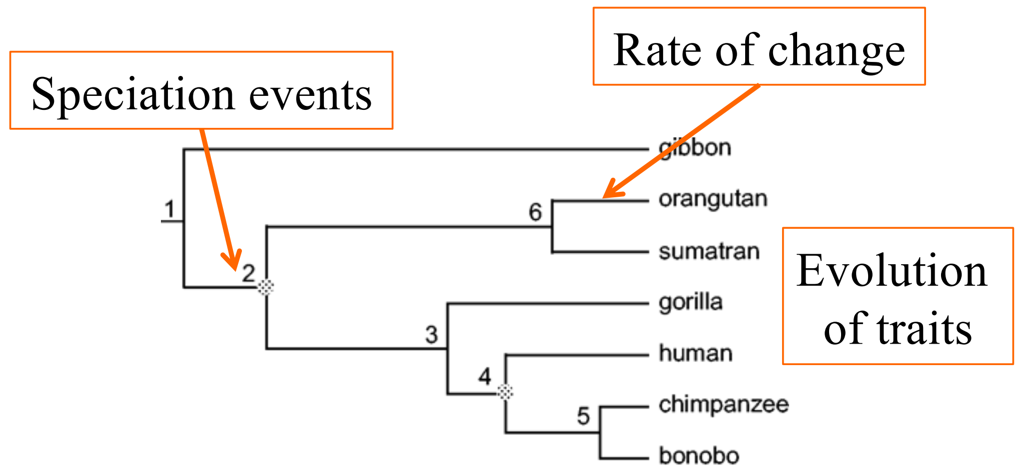
CUIC CORNELL UNIVERSITY INSECT COLLECTION

MOREAU LAB

What processes generate biodiversity?

Abiotic and/or biotic interactions drive diversity and adaptation.
What influence does symbiosis have on the ecology and evolution of interacting partners?

Photographs Corrie S. Moreau



"The little things that run the world."

-- E.O. Wilson (1987)

E.O. Wilson visits Moreau Lab
Photograph Karen Bean

Ants engage in symbioses with many other organisms (animals, plants, fungi, & bacteria)

"the little things that run the little things that run the world!"
-- C.S. Moreau (2020) in *Current Opinions in Insect Science* 39: 1-5.

Photograph © Alex Wild

Photograph © Alex Wild

Photograph © Alex Wild

Ant-plant interactions evolved through increasing interdependence

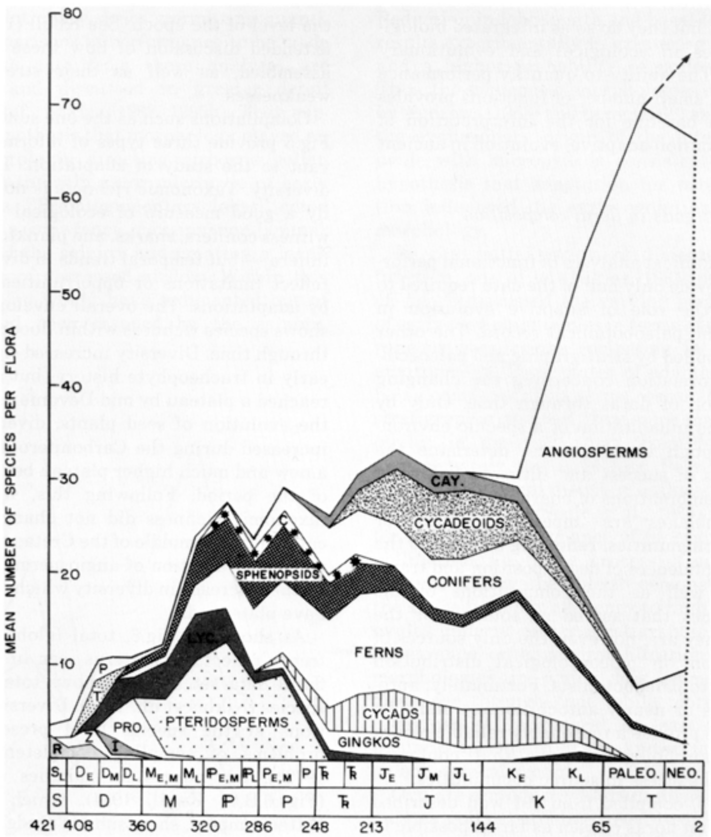


Image from: Niklas et al. (1980) *Evol Biol* 12: 1-89.

Plant photographs from:
Chanderbali et al. (2016) *Genetics* 202(4): 1255-1265.

Beetles photograph Mark Wilson;
Staghorn fern photograph http://wikimediia.org

Numerous clades linked to angiosperm-forests

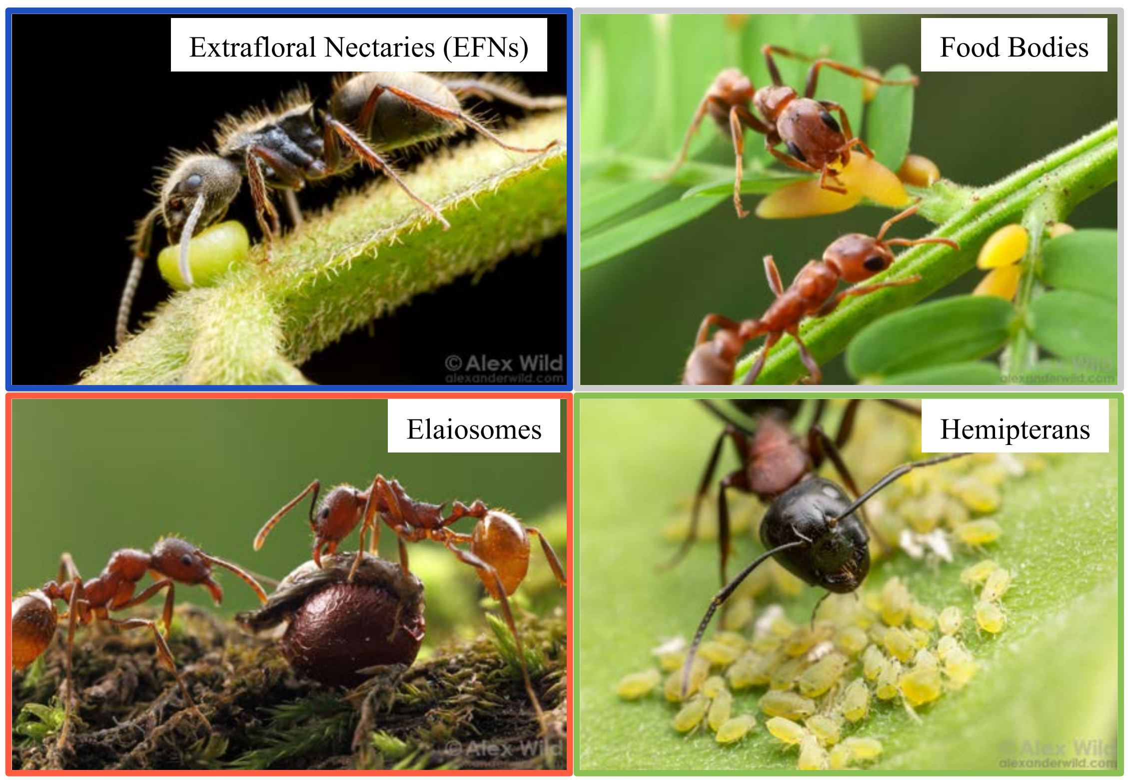
- Major ant groups diversified with rise of angiosperm-forests

  Moreau et al. (2006) *Science*; Moreau & Bell (2013) *Evolution*

Ant photographs © Alex Wild

Numerous clades linked to angiosperm-forests

- Major ant groups diversified with rise of angiosperm-forests
  Moreau et al. (2006) *Science*; Moreau & Bell (2013) *Evolution*
- Plant-derived food sources enabled ecological expansion
  Wilson & Hölldobler (2005) *PNAS*



Ant photographs © Alex Wild

Are plant and ant evolution linked?

How did associations between ants and plants evolve?

- Ages of ant-associated plant structures vs. ant utilization of plants for food, foraging, nesting
- Is there an evolutionary sequence by which ants became reliant on plants?

Ant photographs © Alex Wild

Plant evolution

Inferred and time-scaled phylogeny of 10,785 vascular plant genera
(from Hinchliff & Smith (2014) *PLOS ONE)*

Ancestral state reconstruction on ant-associated trait data

Plant photographs from:
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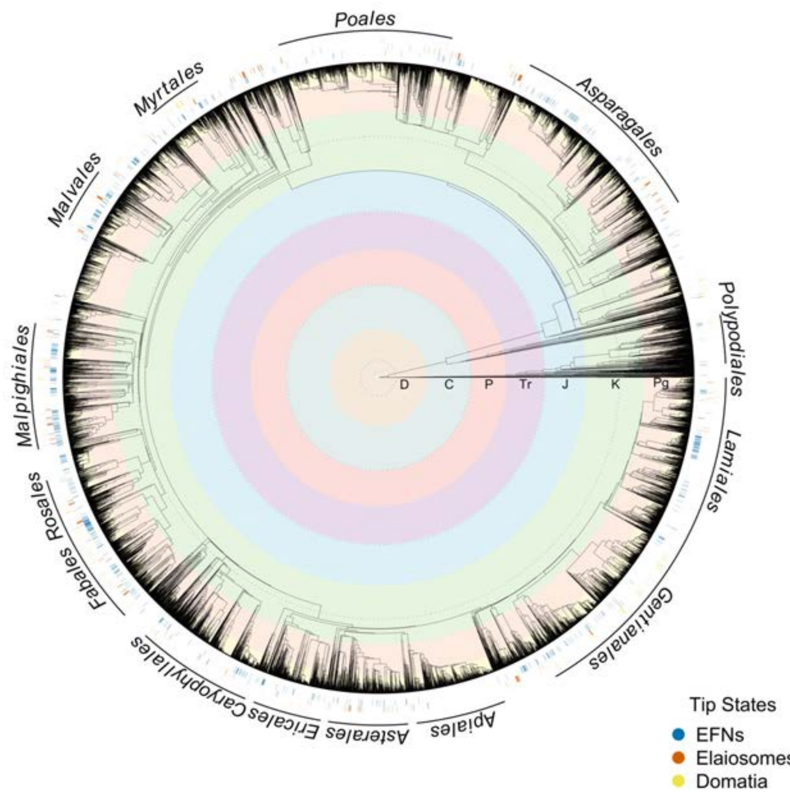


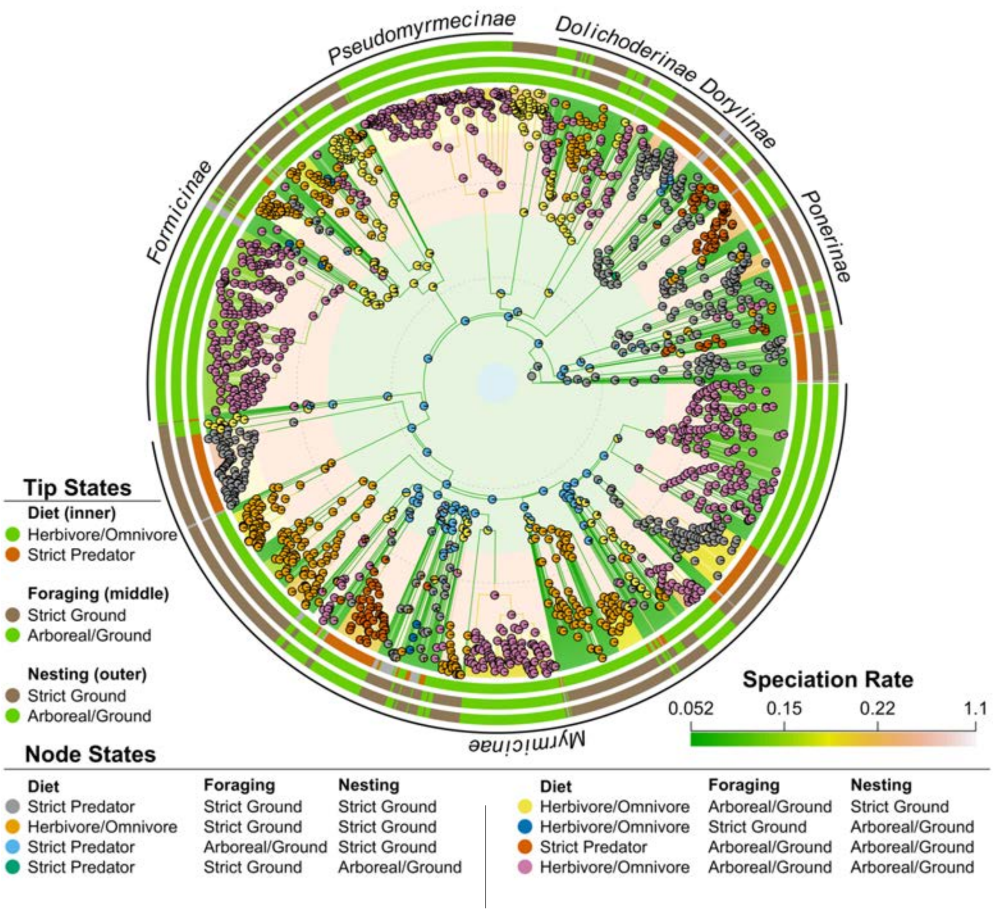
Nelsen, Ree, & Moreau (2018) *PNAS* 115(48): 12253-12258.

Matt Nelsen

Ant evolution

Inferred and time-scaled phylogeny of 1,730 ant species

Ancestral state reconstruction on diet and foraging trait data



Ant photograph © Alex Wild

Nelsen, Ree, & Moreau (2018) *PNAS* 115(48): 12253-12258.

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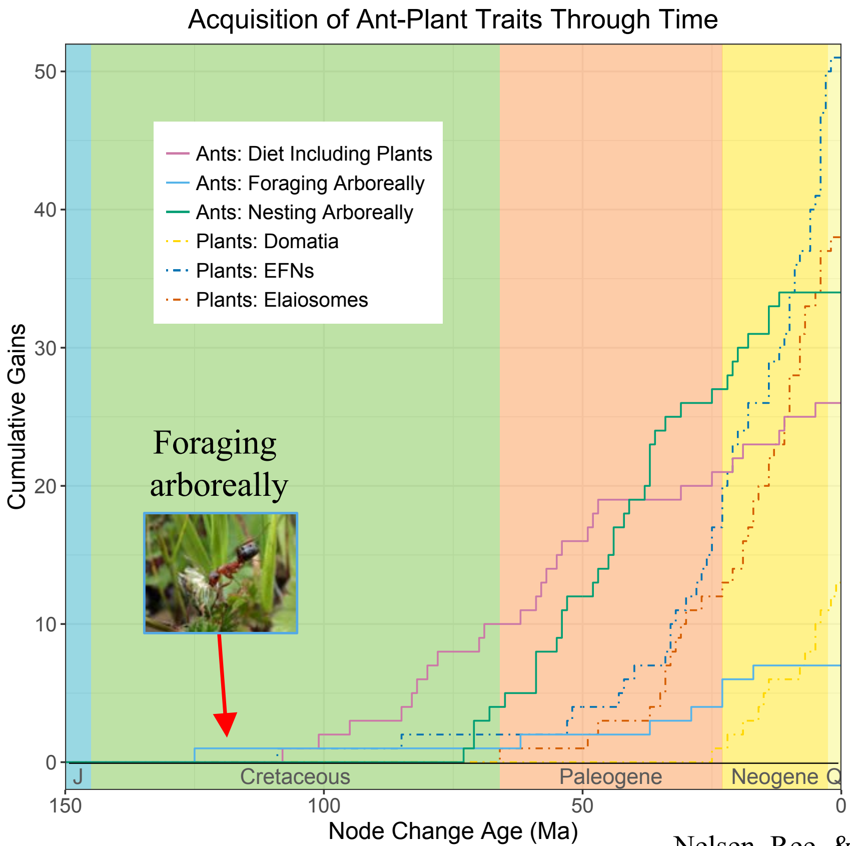
Ground foraging and nesting



Ant photographs © Alex Wild & Staab et al. (2017)

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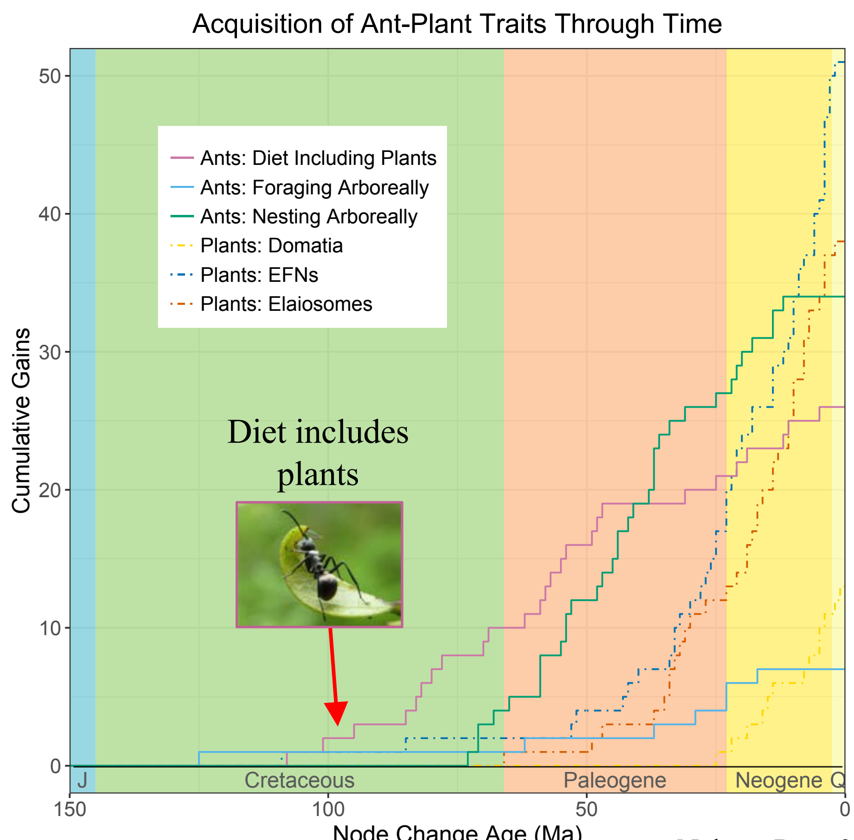

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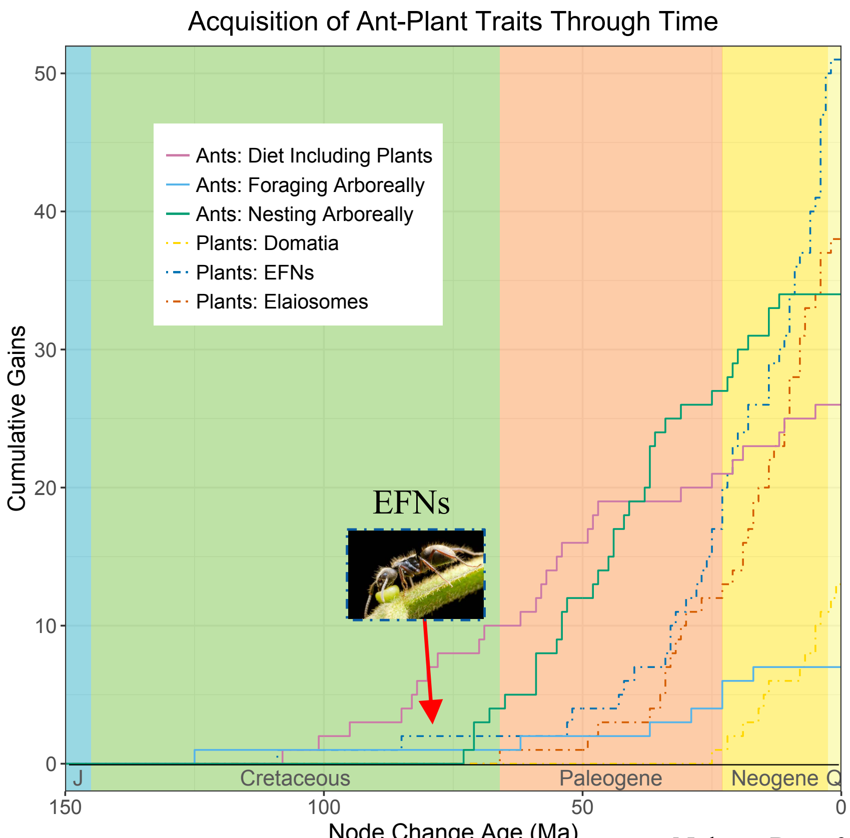

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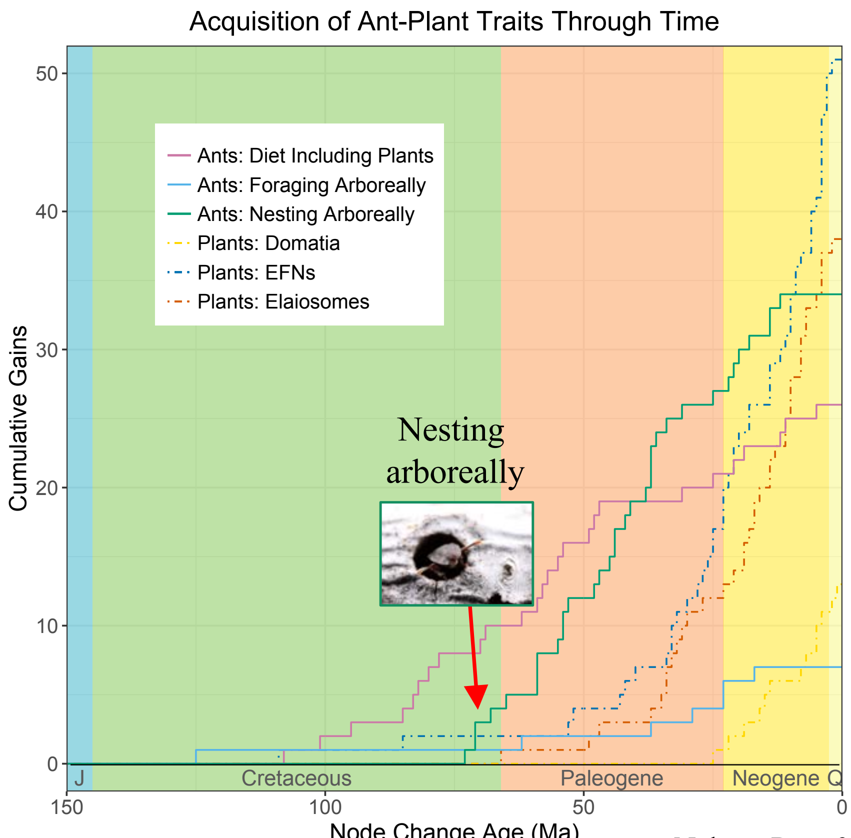

Ant photographs © Alex Wild & Staab et al. (2017)

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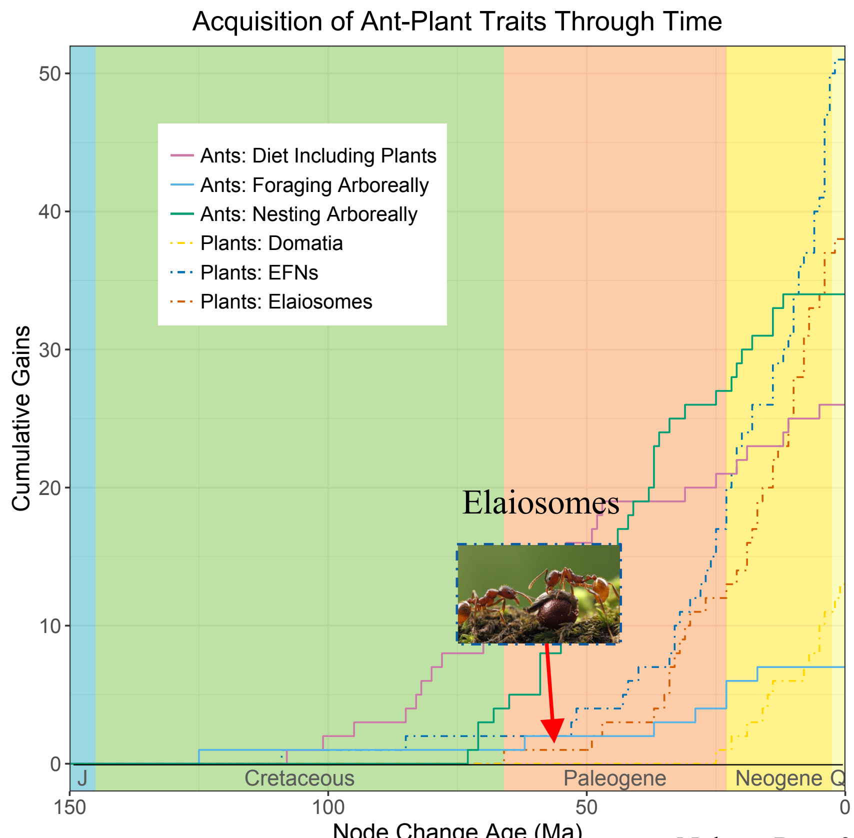

Ant photographs © Alex Wild & Staab et al. (2017)

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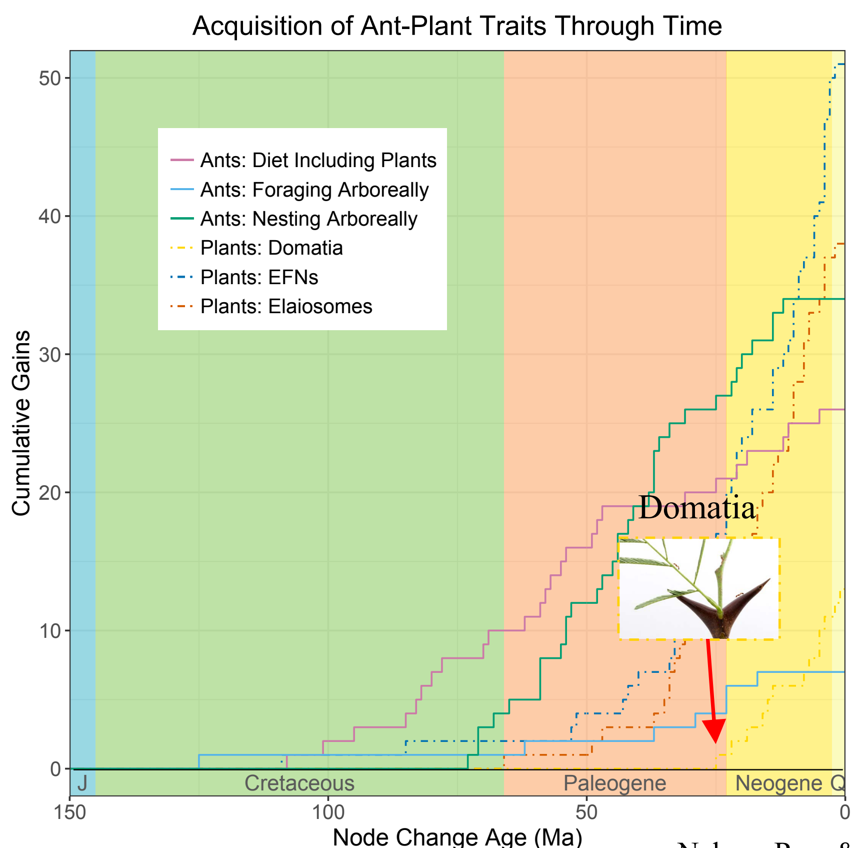

Ant photographs © Alex Wild & Staab et al. (2017)

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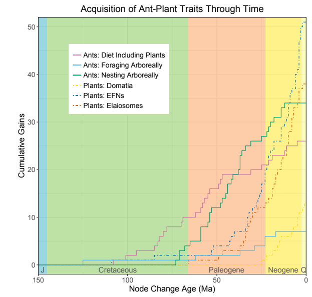
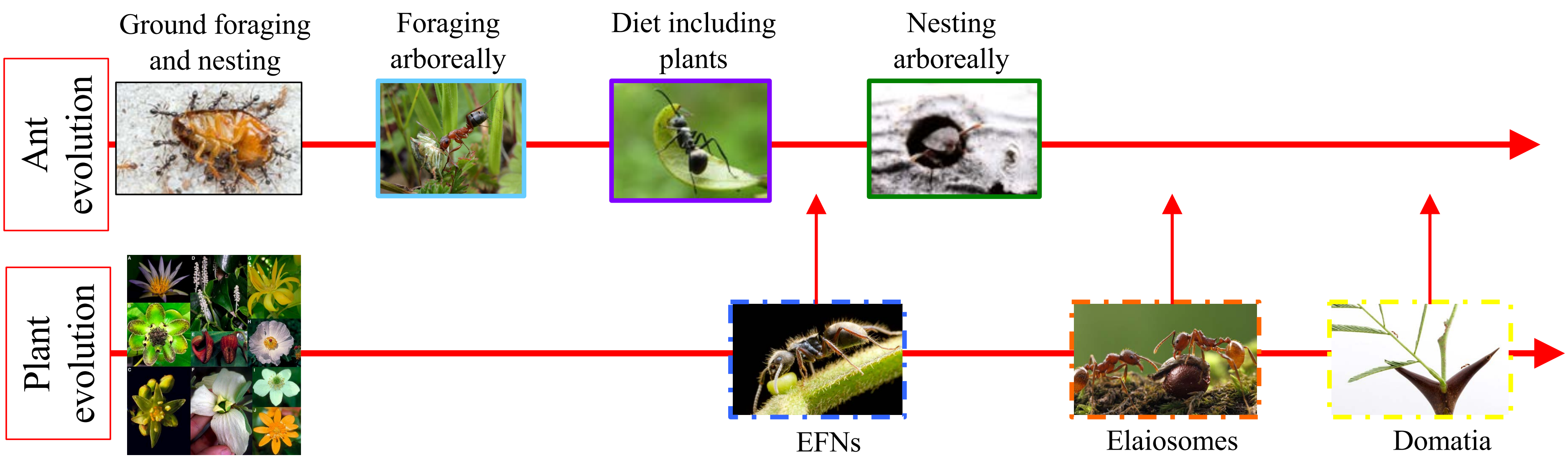
Ant-plant interactions evolved through increasing interdependence



- Ants utilized plants long before plants evolved specialized ant-associated structures
- Incremental sequence of evolutionary events led to ant reliance on plants
- To become fully herbivorous ants had to transition from predatory to omnivorous to herbivorous



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Ant-plant mutualisms and comparative genomics

Why do ant-plant mutualisms evolve and persist through time?

Plants need ants like a dog needs fleas – Richard Spruce 1873

But, we know ant-plant mutualisms have evolved multiple times across the ants and across the plants

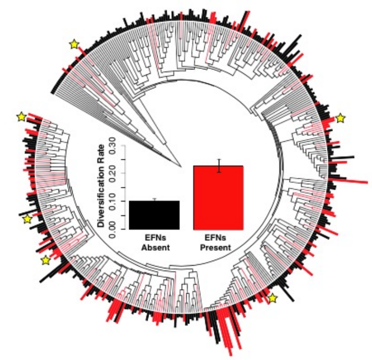
Photograph © Alex Wild

Plants that participate in ant-plant mutualisms (and other defensive mutualisms) have twofold higher rates of diversification

Defense mutualisms enhance plant diversification

Marjorie G. Weber[1,2] and Anurag A. Agrawal

Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853



Symbiosis—the close and often long-term interaction of two or more species—is predicted to drive genome evolution in a variety of ways.

Red Queen's statement to Alice in Through the Looking-Glass
"it takes all the running you can do, to keep in the same place."

Red Queen hypothesis is an evolutionary hypothesis which proposes organisms must constantly adapt to survive when pitted against ever-evolving opposing organisms in an ever-changing environment – Van Valen 1973

For example, parasitic (antagonistic) interactions have been shown to increase rates of molecular evolution
(i.e. the Red Queen; Van Valen 1973; Paterson *et al*. 2010; Bromham *et al*. 2013; Yoshizawa & Johnson 2003)

But, mutualisms are predicted to reduce rates of change by decreasing the fitness of uncommon genotypes and reducing the rate of evolution
(i.e. the Red King effect; Law and Lewis, 1983; Law, 1985; Doebeli & Knowlton, 1998; Bergstrom & Lachmann, 2003; Damore & Gore, 2011)

Pseudomyrmex ant-plant mutualisms: acacia-ants

Nesting space

Extrafloral nectar

Food bodies

Defense

Mutualists

Very similar ant behavior (patrol plants 24 hours per day, highly aggressive, large colonies)

Triplaris, Acacia, Tachigali plants

~40 species

Generalists/Parasites

Nest non-specifically in existing hollow twigs

Non-aggressive defensive behavior

~260 species

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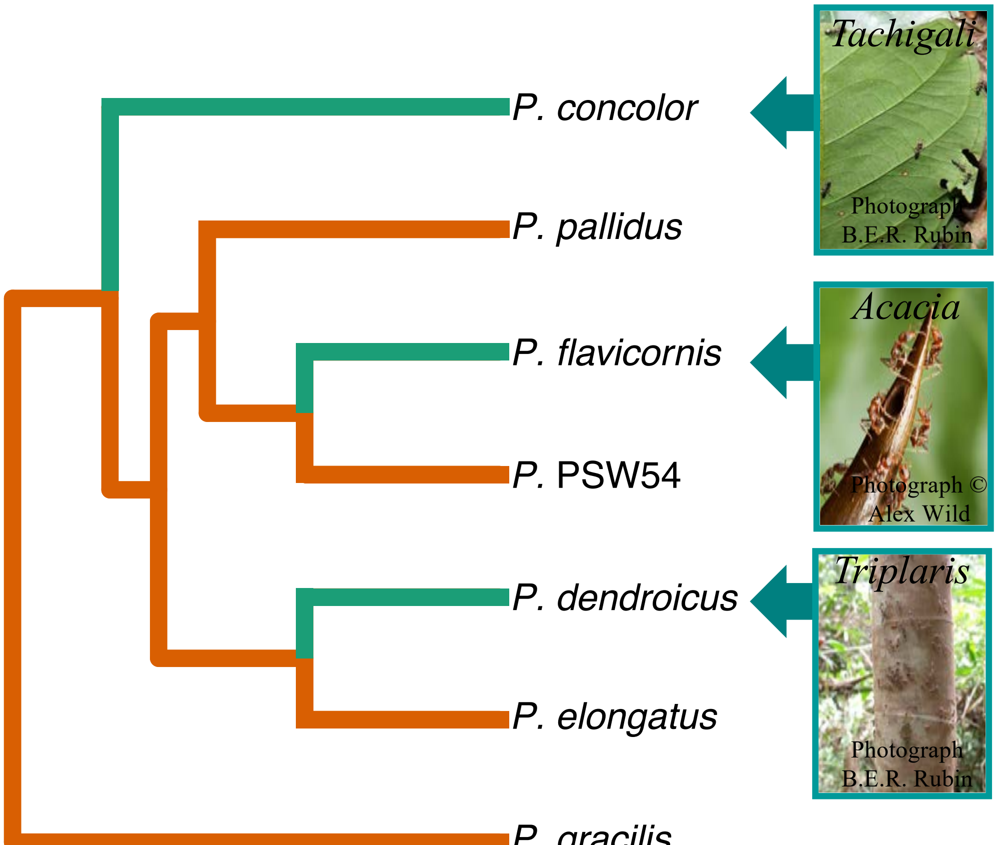
Convergent evolution of mutualism



= mutualist = generalist

Natural evolutionary experiment to investigate the role of mutualisms on genomes

Although there are were 8 ant genomes sequenced the closest relative to *Pseudomyrmex* is very distantly related

Phylogeny modified from Ward & Downie (2005)

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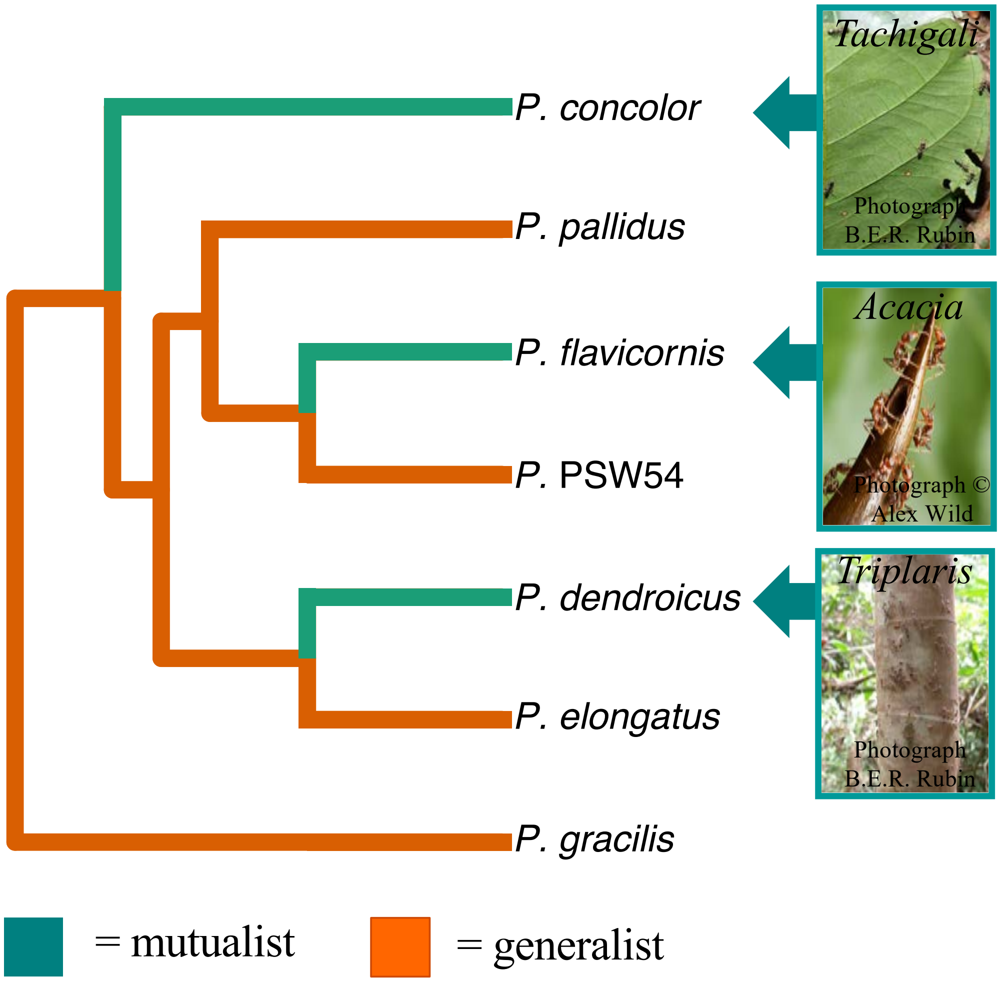
Genome evolution of ant-plant mutualisms



Genome sequencing *<u>coverage completed</u>*

65X

65X

65X

65X

65X

65X

~90X (reference)

<u>Summary of findings:</u>

- Convergent positive selection in nervous system genes
- Faster overall rates of genome evolution in the three mutualists

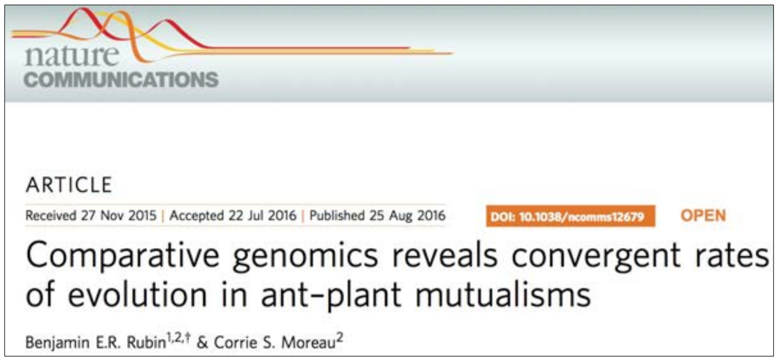
<u>Reference genome: *P. gracilis*</u>

- Haploid male
- Two 100bp paired-end Illumina HiSeq lanes (~150 base and 2,500 base inserts)
- ALLPATHS-LG assembly
- One 100bp paired-end Illumina RNA-seq lane
- N50 contig = 30 kb
- 99% CEGMA genes recovered in assembly
- 16,069 genes with transcript support inferred by MAKER
- Includes 77 of 79 expected cytoplasmic ribosomal proteins
- Genotyping-By-Sequencing of 47 sisters to create 38 linkage groups of 185 scaffolds

Rubin & Moreau (2016)
Nature Communications 7: e12679.

Benjamin Rubin

Genome evolution of ant-plant mutualisms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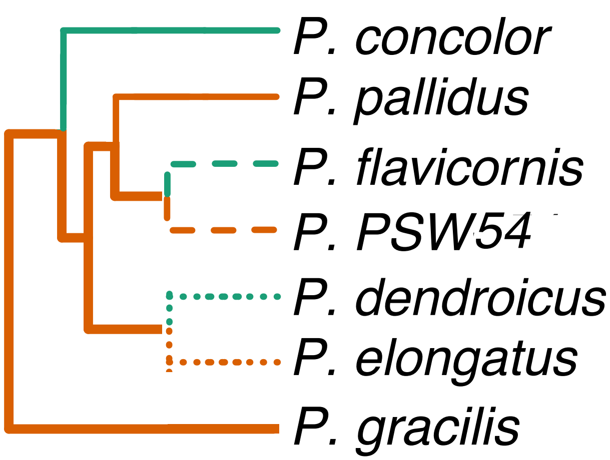
ARTICLE

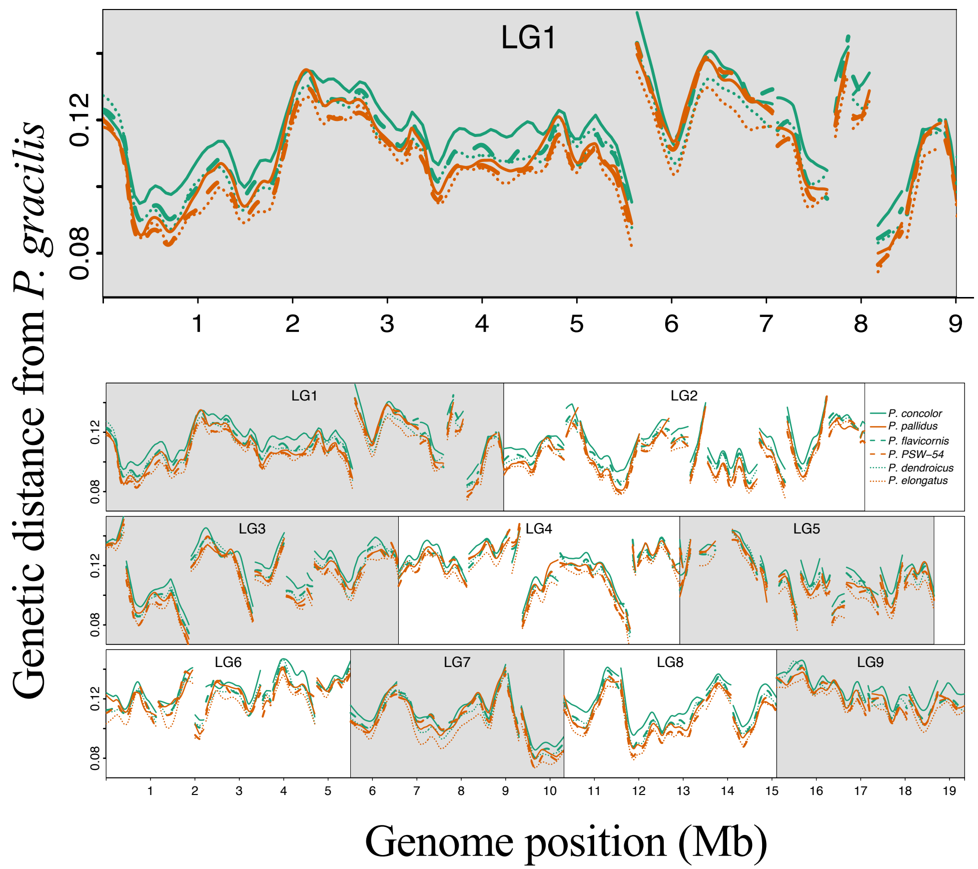
Received 27 Nov 2015 | Accepted 22 Jul 2016 | Published 25 Aug 2016 DOI: 10.1038/ncomms12679 OPEN

Comparative genomics reveals convergent rates of evolution in ant-plant mutualisms

Benjamin E.R. Rubin[1,2,†] & Corrie S. Moreau[2]



Genetic distance from *Pseudomyrmex gracilis*



Rubin & Moreau (2016)
Nature Communications 7: e12679.

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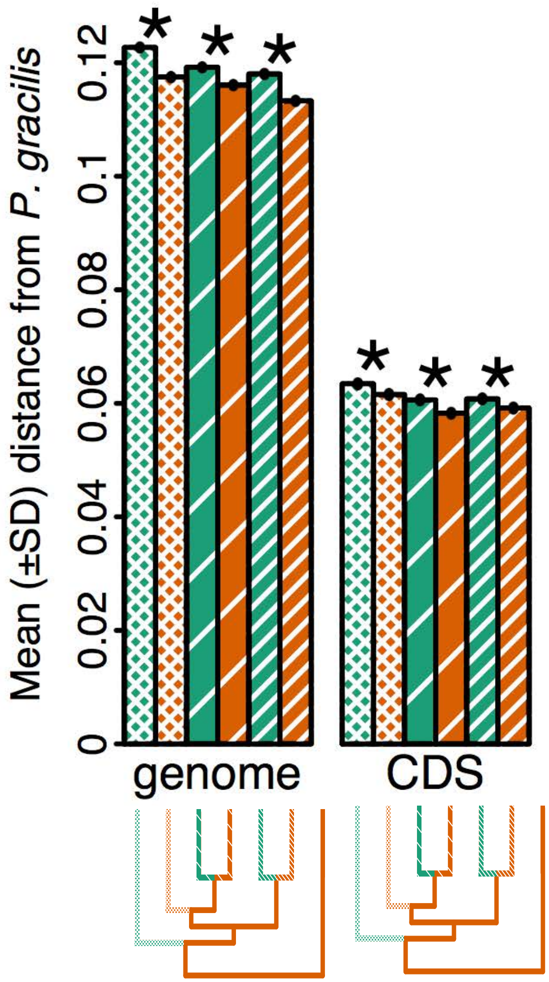
Genome evolution of ant-plant mutualisms



(Significance determined with bonferroni corrected paired t-tests of 25kb sliding windows.)

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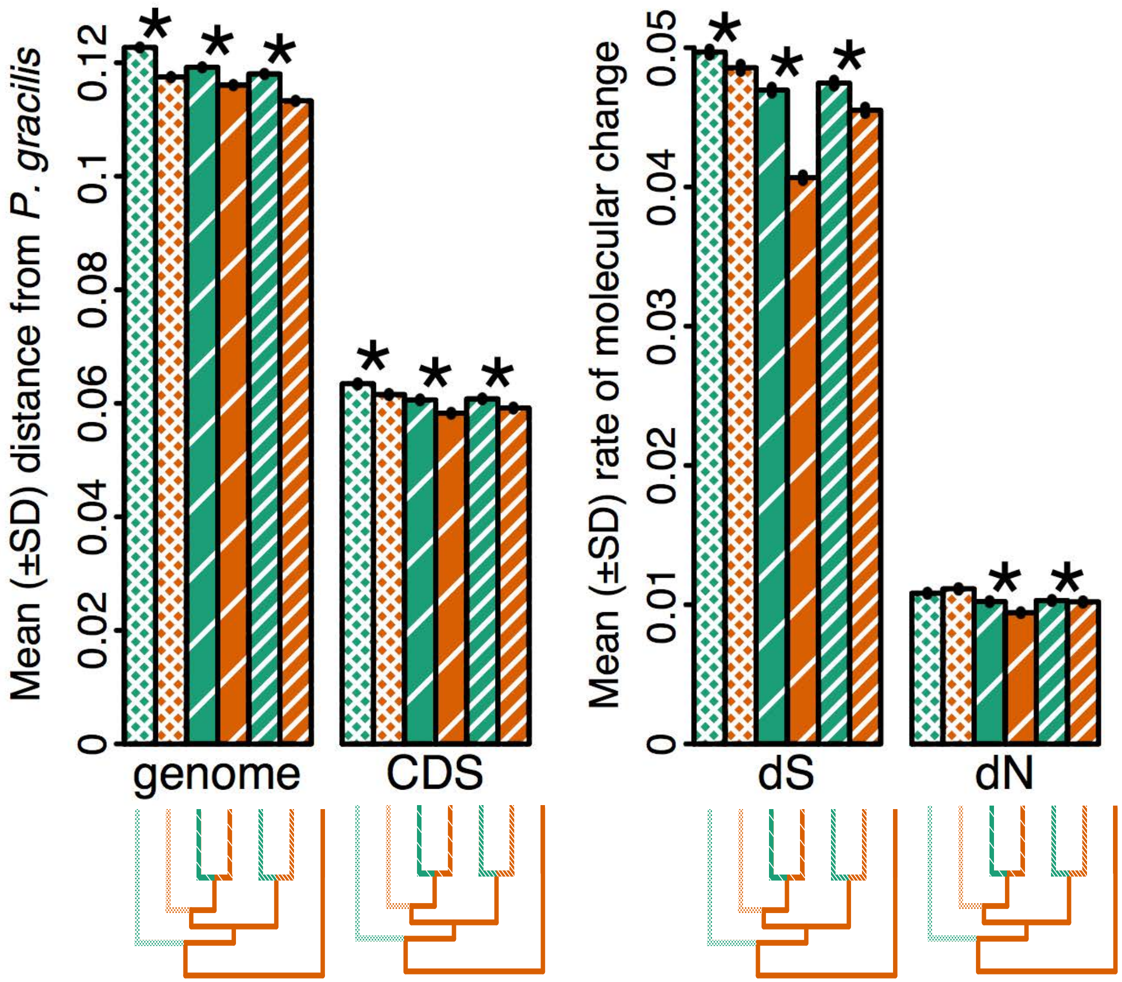

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Difference in rates not due to differences in generation time

1. Generation time

Prediction: Mutualists have consistently shorter generation times

Photograph © Alex Wild

Rubin & Moreau (2016)
Nature Communications 7: e12679.

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Difference in rates not due to differences in generation time

1. Generation time

Prediction: Mutualists have consistently shorter generation times

Result: Mutualists have consistently longer generation times

Generalists: 2 months to reproduce

Mutualists: 1.5 years to reproduce (Janzen, 1975)

Photograph © Alex Wild

Rubin & Moreau (2016)
Nature Communications 7: e12679.

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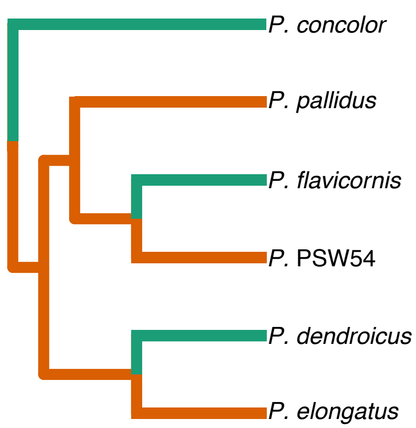
Difference in rates not due to differences in population size

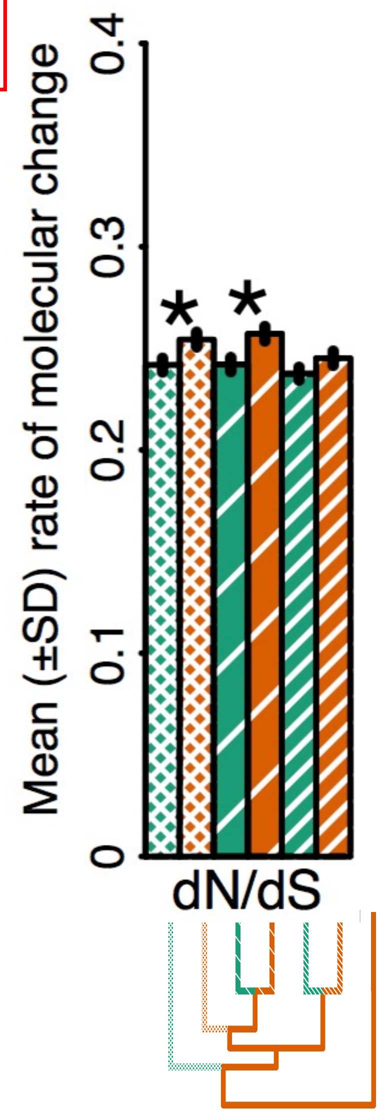
~~1. Generation time~~

2. Population size

Prediction: dN/dS ratios higher in mutualists and smaller estimated population sizes



Scaled theta (GPhoCS)
20.7
20.2
15.1
16.9
20.2
17.1



Rubin & Moreau (2016)
Nature Communications 7: e12679.

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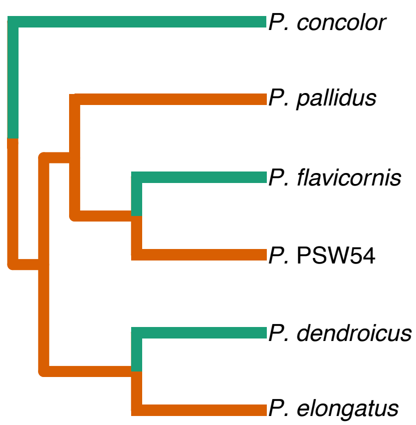
Difference in rates not due to differences in population size

~~1. Generation time~~

2. Population size

Prediction: dN/dS ratios higher in mutualists and smaller estimated population sizes

Result: No consistent differences in dN/dS ratios or smaller estimated population sizes



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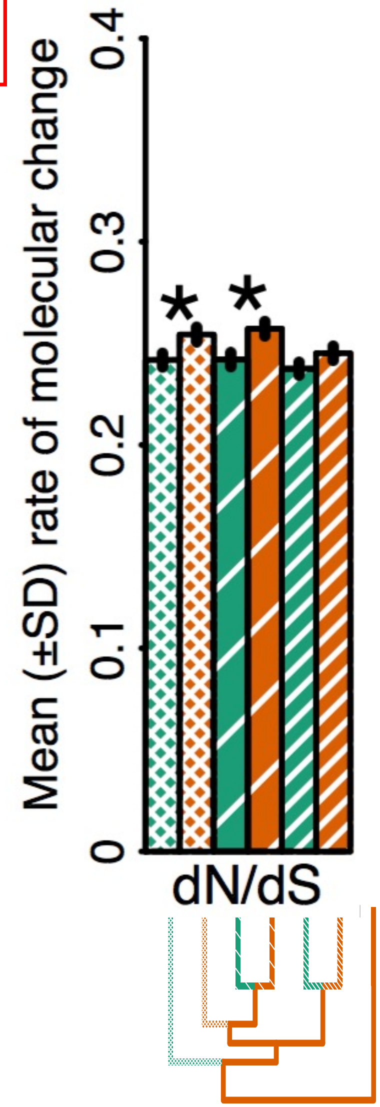

Rubin & Moreau (2016)
Nature Communications 7: e12679.

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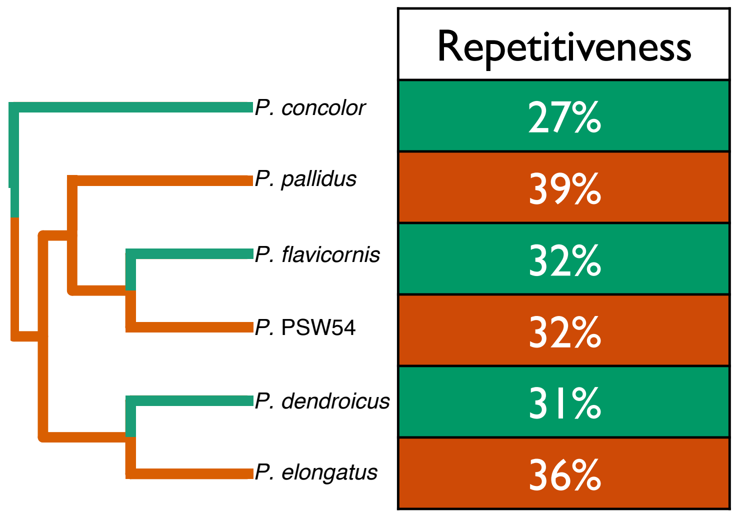
Difference in rates may be due to relaxed selection

~~1. Generation time~~

~~2. Population size~~

3. Relaxed selection

Prediction: Transposable elements increase in frequency in mutualists



Rubin & Moreau (2016)
Nature Communications 7: e12679.

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Difference in rates may be due to relaxed selection

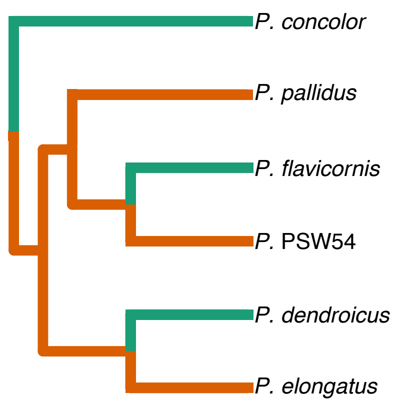
~~1. Generation time~~

~~2. Population size~~

3. Relaxed selection

Prediction: Transposable elements increase in frequency in mutualists

Result: Repeat content not consistently different



Repetitiveness
27%
39%
32%
32%
31%
36%

Rubin & Moreau (2016)
Nature Communications 7: e12679.

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Difference in rates may be due to higher mutation rates

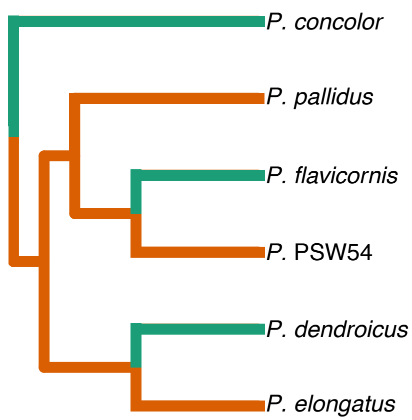
~~1. Generation time~~

~~2. Population size~~

3. Relaxed selection?

4. Elevated mutation rate

Prediction: Higher frequency of SNPs (heterozygosity) in mutualists



SNP rate (kmer prediction)
1/755
1/612
1/576
1/930
1/550
1/1185

Rubin & Moreau (2016)
Nature Communications 7: e12679.

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Difference in rates may be due to higher mutation rates

~~1. Generation time~~

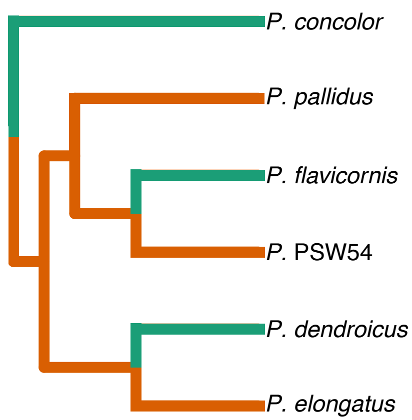
~~2. Population size~~

3. Relaxed selection?

4. Elevated mutation rate?

Prediction: Higher frequency of SNPs (heterozygosity) in mutualists

Result: Trend of higher rates in mutualists



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~~2. Population size~~

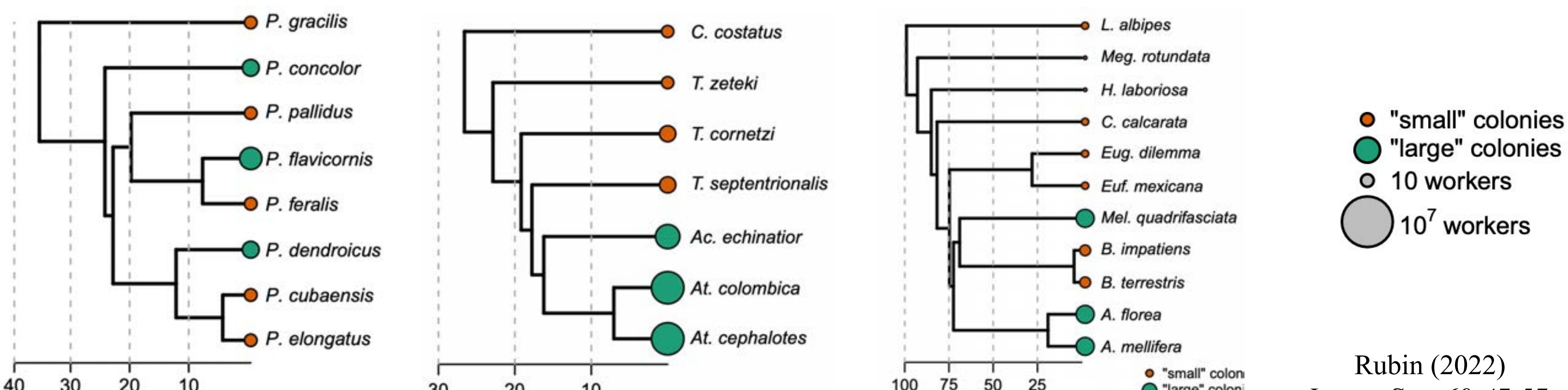
3. Relaxed selection?

4. Elevated mutation rate?

5. Colony size (comparing whole genomes across multiple ant and bee lineages)

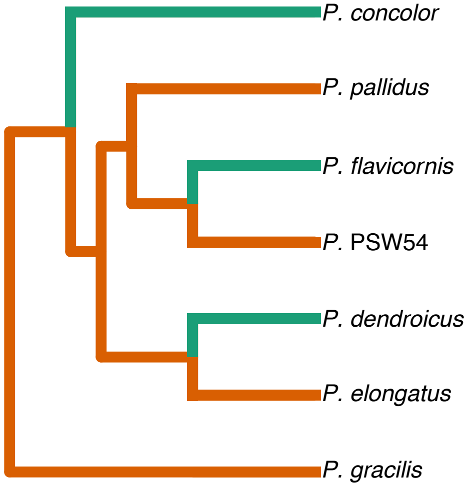
Prediction: In large colonies, queens produces hundreds of millions of offspring, requiring more divisions in germline stem cells leading to mutation accumulation

Result: Higher rates in species with large colonies of multiple lineages of ants and bees



Rubin (2022)
Insect. Soc. 69:47-57.

Convergent genome evolution due to life history



There are many factors that can impact genome evolution in social organisms and we need more comparative studies to understand how genomes shape sociality, but also how sociality shapes genomes evolution.

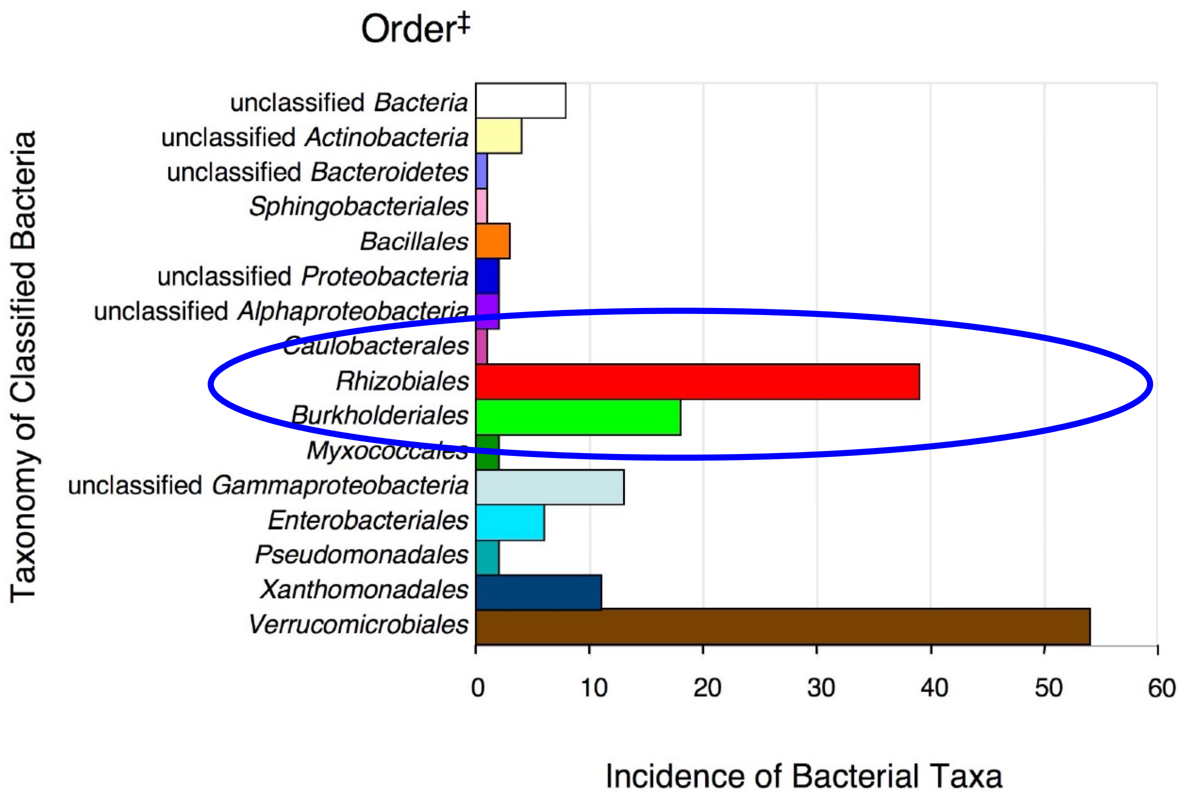
Symbiotic bacteria across the ants and the evolution of herbivory

Why gut microbes of ants?

The ants provide an excellent system to study the diversity, distribution, and influence of gut-associated bacteria:

- **Diet & Nutrition:** How does diet influences associations?
- **Transmission:** How are microbes transmitted among individuals and through time?
- **Host-symbiont coevolution:** What is the role of shared evolutionary history and/or convergence?
- **Geography:** Are microbes acquired from the environment?

Ants have diverse bacterial communities



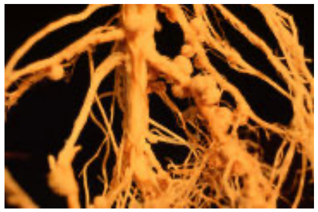
Results of using general and targeted bacterial sequencing across <u>398 individual</u> ants from <u>148 genera</u>.

Russell, *et al*. (2009) *Evolution* 63(3): 624-640.

Russell, Moreau *et al*. (2009) *PNAS* 106(50): 21236-21241.

Nitrogen fixation by ant gut symbionts?

$N_2 \longrightarrow$



$\xrightarrow{NH_4^+}$

$\xleftarrow[\text{carbohydrates}]{}$

Images from http://MicrobeWiki.kenyon.edu

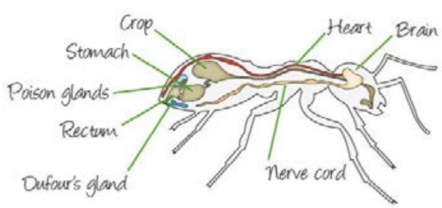


Image from www.antark.net

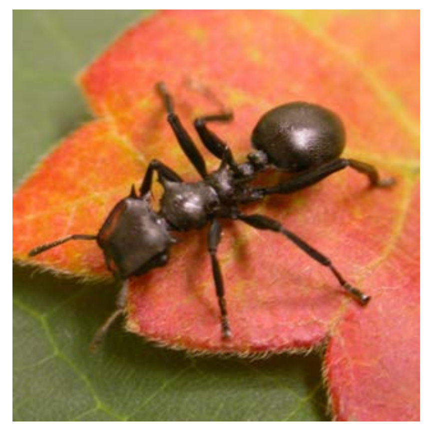
$N_2 \longrightarrow$

Image from http://MicrobeWiki.kenyon.edu

?

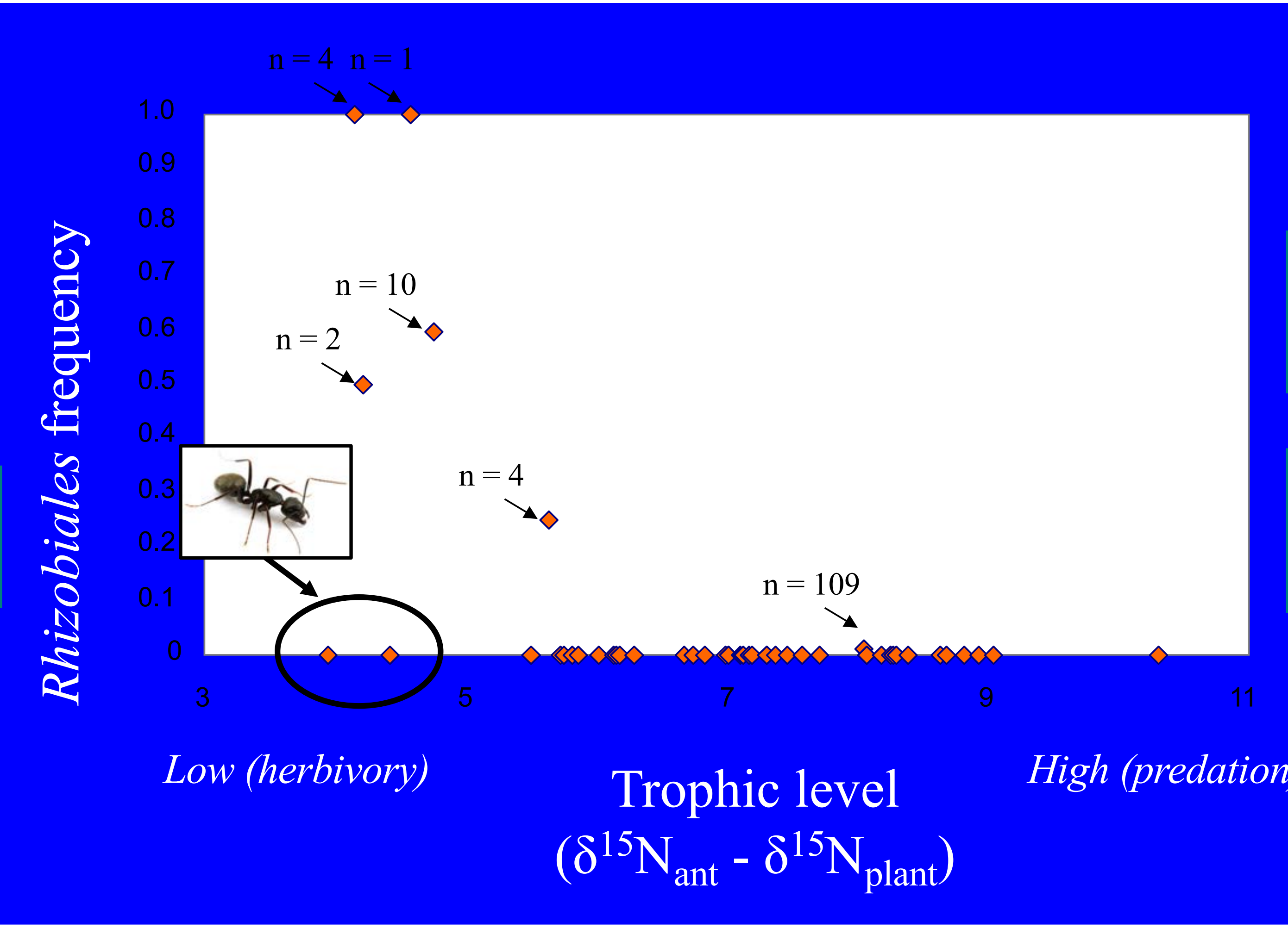
$\xrightarrow{NH_4^+}$

$\xleftarrow[\text{carbohydrates}]{}$



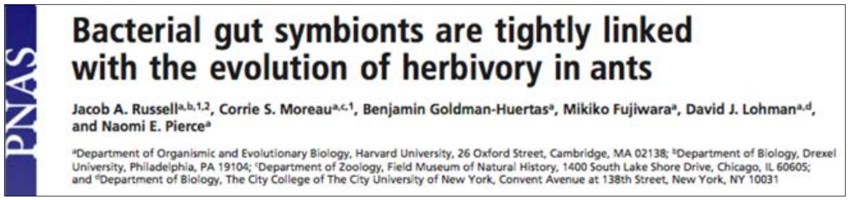
Photograph C.S. Moreau

Rhizobiales bacteria are common at low trophic levels



Russell, Moreau *et al*. (2009)
PNAS 106(50): 21236-21241.

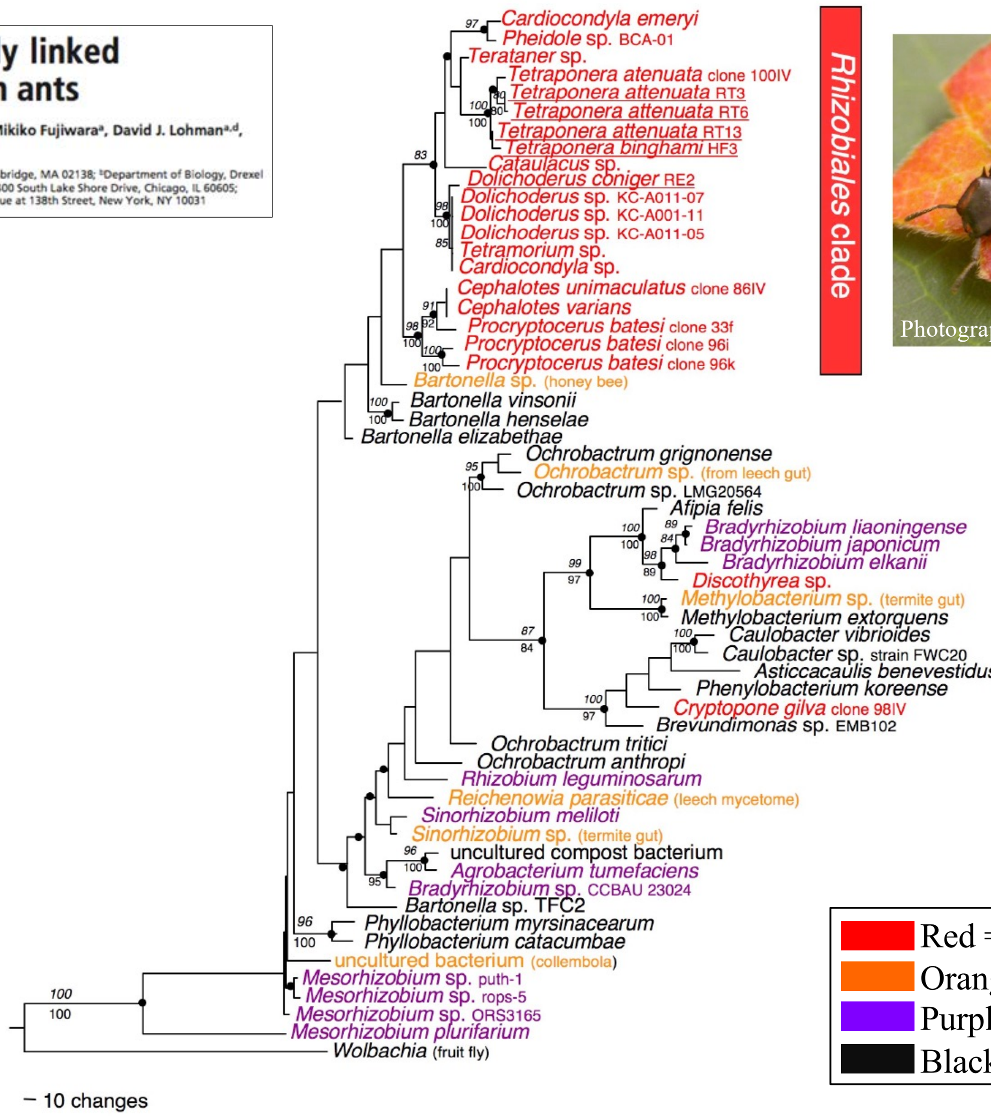
Bacteria phylogeny:
16S rRNA phylogeny of ant-associated *Rhizobiales* bacteria and their GenBank relatives


PNAS

Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants

Jacob A. Russell[a,b,1,2], Corrie S. Moreau[a,c,1], Benjamin Goldman-Huertas[a], Mikiko Fujiwara[a], David J. Lohman[a,d], and Naomi E. Pierce[a]

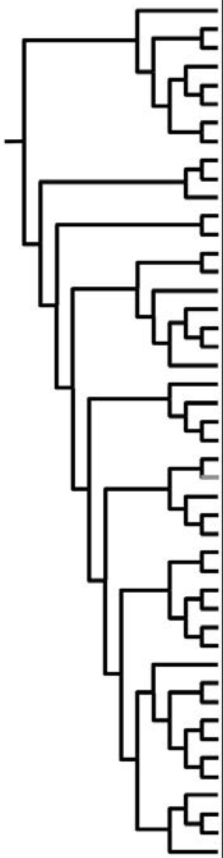
[a]Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138; [b]Department of Biology, Drexel University, Philadelphia, PA 19104; [c]Department of Zoology, Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605; and [d]Department of Biology, The City College of The City University of New York, Convent Avenue at 138th Street, New York, NY 10031



Red	Red = ant associates
Orange	Orange = animal associates
Purple	Purple = plant associates
Black	Black = other or unspecified

Russell, Moreau *et al*. (2009)
PNAS 106(50): 21236-21241.

Ant phylogeny:
Rhizobiales symbionts have independently evolved associations with arboreal and herbivorous ants on at least five occasions



Ant genus	*Rhizobiales*	$\delta^{15}N_{ant} - \delta^{15}N_{plant}$	Ant subfamily
Platythyrea	0/2	4.314	
Pachycondyla	0/4	4.619	
Diacamma	0/3	5.330	
Odontoponera	0/3	5.922	Ponerinae
Anochetus	0/1	5.664	
Odontomachus	0/5	5.459	
Myopias	0/1	4.035	
Leptogenys	0/15	6.604	
Eciton	0/1	5.817	Ecitoninae
Labidus	0/1	7.312	
Aenictus	0/7	6.328	Aenictinae
Pseudomyrmex	0/1	2.504	Pseudomyrmecinae
Tetraponera	1/2	1.871	
Tapinoma	0/1	3.695	
Technoyrmex	0/4	3.687	
Dolichoderus	7/10	2.005	
Azteca	0/1	3.118	Dolichoderinae
Anonychomyrma	0/1	3.328	
Linepithema	0/1	5.402	
Leptomyrmex	0/1	4.936	
Heteroponera	0/2	4.672	Heteroponerinae
Gnamptogenys	0/2	6.160	
Rhytidoponera	0/1	4.755	Ectatomminae
Ectatomma	0/1	4.131	
Myrmelachista	0/1	5.086	
Paratrechina	0/1	4.619	Formicinae
Oecophylla	0/2	4.400	
Polyrhachis	0/5	1.607	
Camponotus	0/10	1.780	
Pheidole	1/109	5.462	
Cephalotes	4/4	1.154	
Apterostigma	0/1	5.649	
Strumigenys	0/1	5.033	
Cyphomyrmex	0/1	3.997	
Atta	0/1	2.873	
Cataulacus	[illegible]	2.316	
Crematogaster	0/4	3.154	
Tetramorium	1/4	3.196	Myrmicinae
Monomorium	0/2	4.735	
Solenopsis	0/3	5.274	
Meranoplus	0/1	3.561	
Lophomyrmex	0/6	4.767	
Wasmannia	0/1	5.090	
Pristomyrmex	0/1	3.892	
Vollenhovia	0/2	5.046	
Myrmicaria	0/2	4.413	

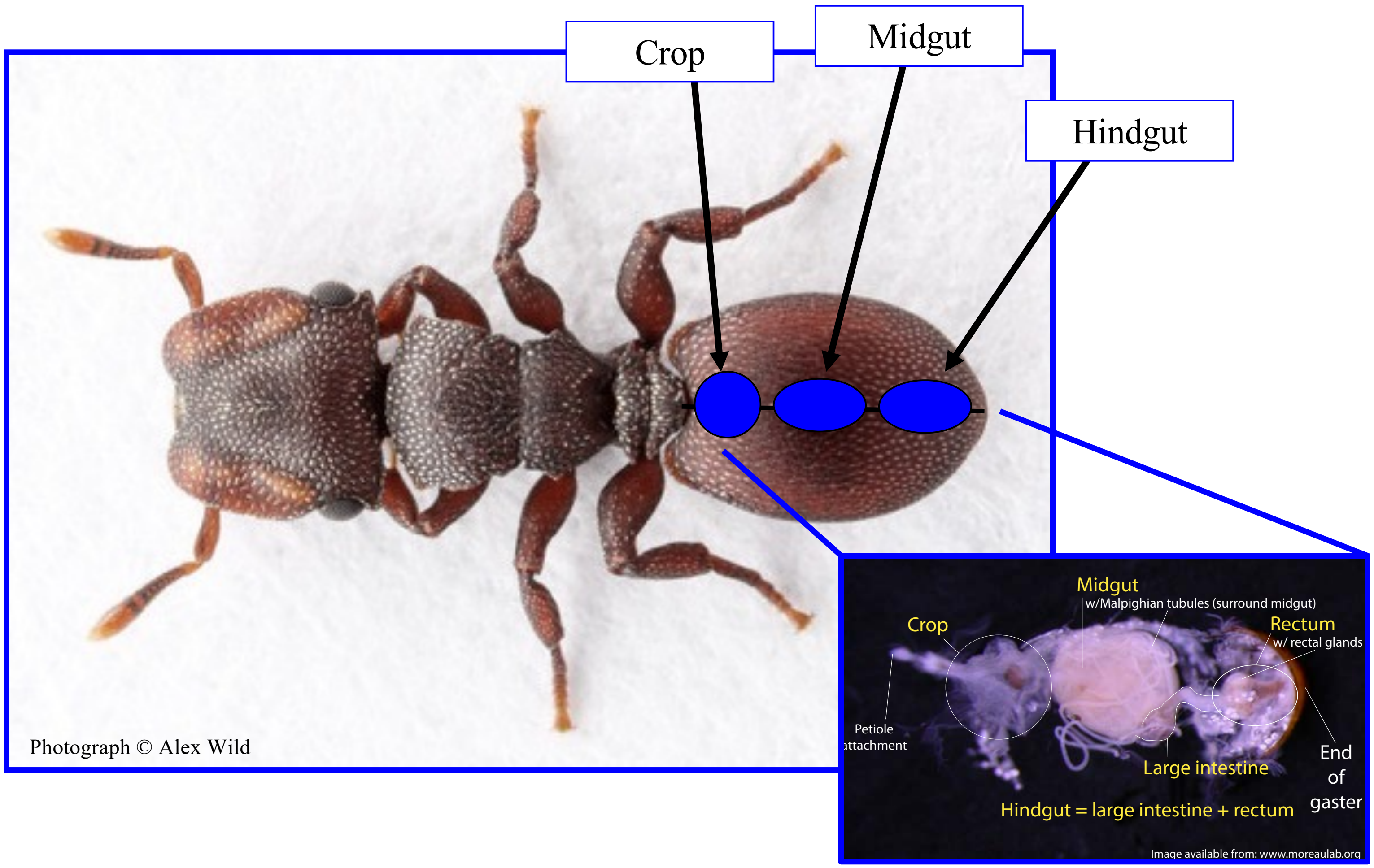
Concentrated changes test (Maddison 1990) provided strong support for the correlated evolution between the presence of *Rhizobiales* symbionts and herbivorous diets across the ant phylogeny (P = 0.001)

Photograph C.S. Moreau

Russell, Moreau *et al*. (2009)
PNAS 106(50): 21236-21241.

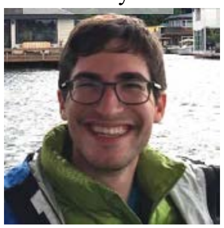
Anderson *et al*. (2012)
Mol. Ecol. 21: 2282-2296.

Tissue specificity: Bacterial screening of individual body parts

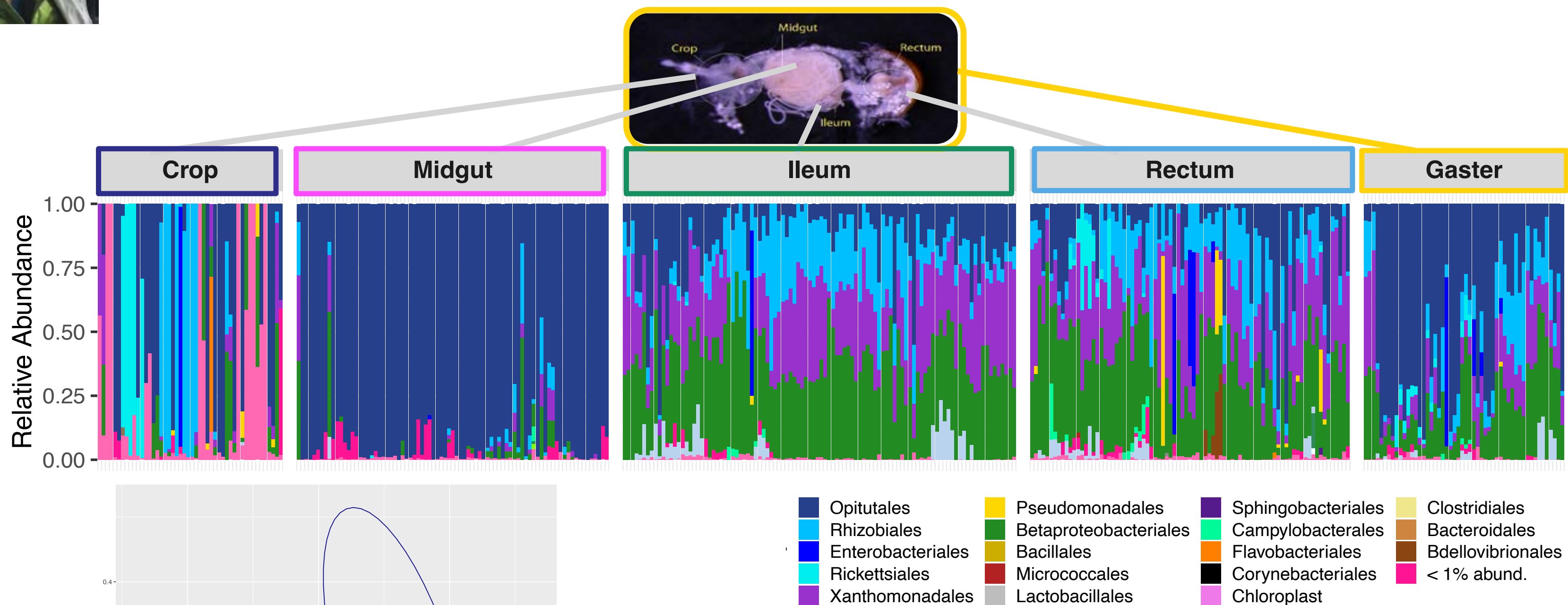


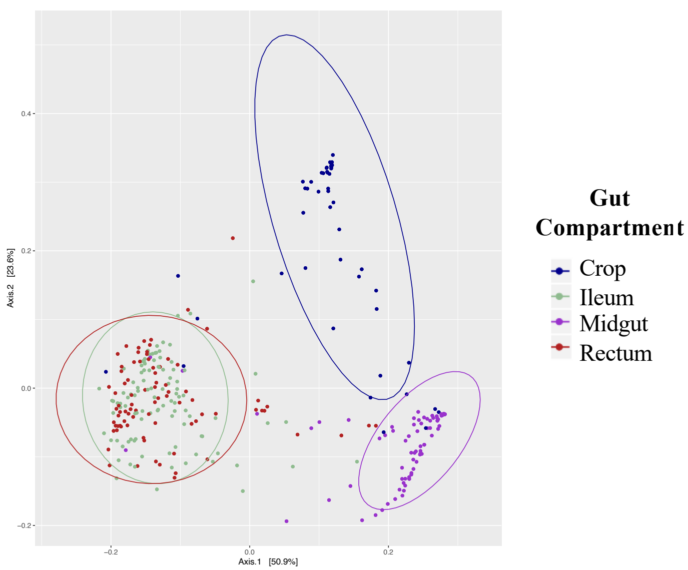
Photograph by C.S. Moreau

Peter Flynn



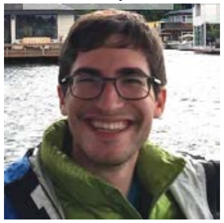
Are bacteria providing nutrition to ants? Indirect evidence



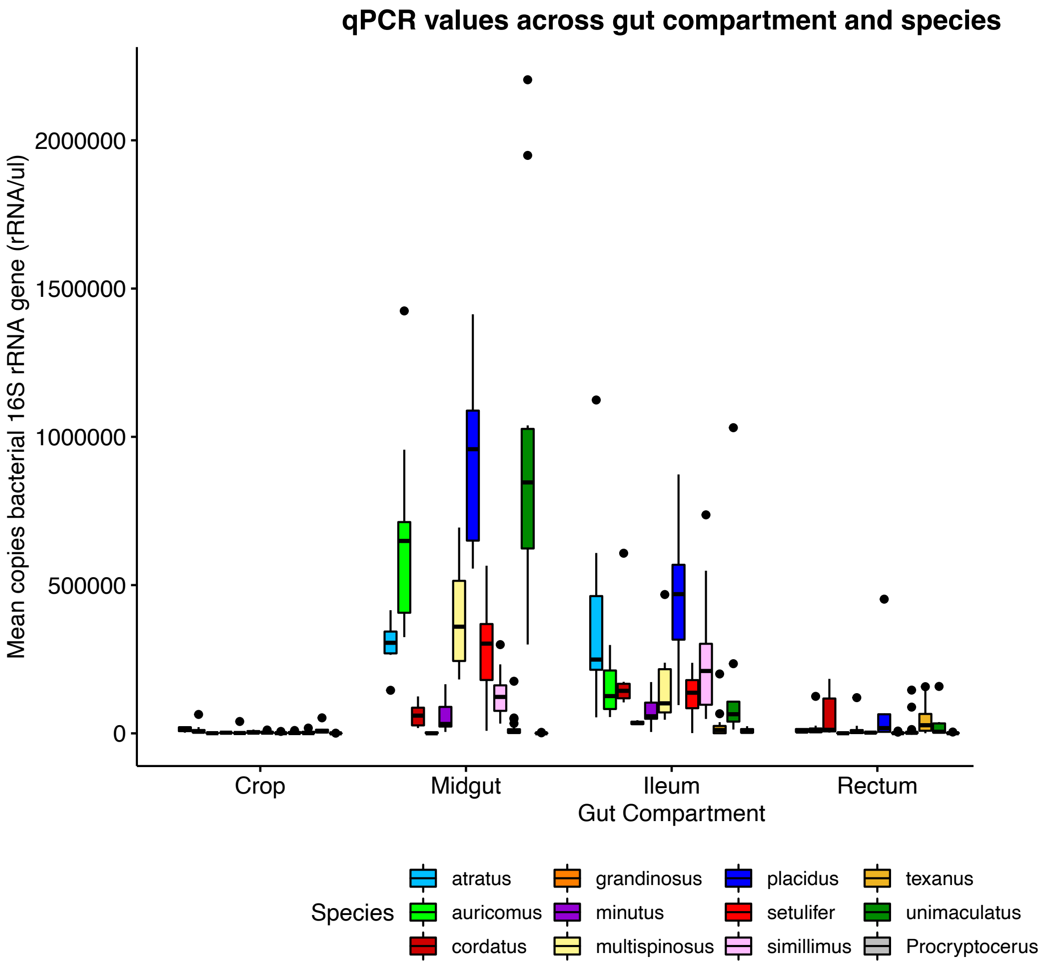


Flynn, D'Amelio, Sanders, Russell, & Moreau (2021)
Appl. Environ. Microbiol. 87(8): e02803-20

Peter Flynn



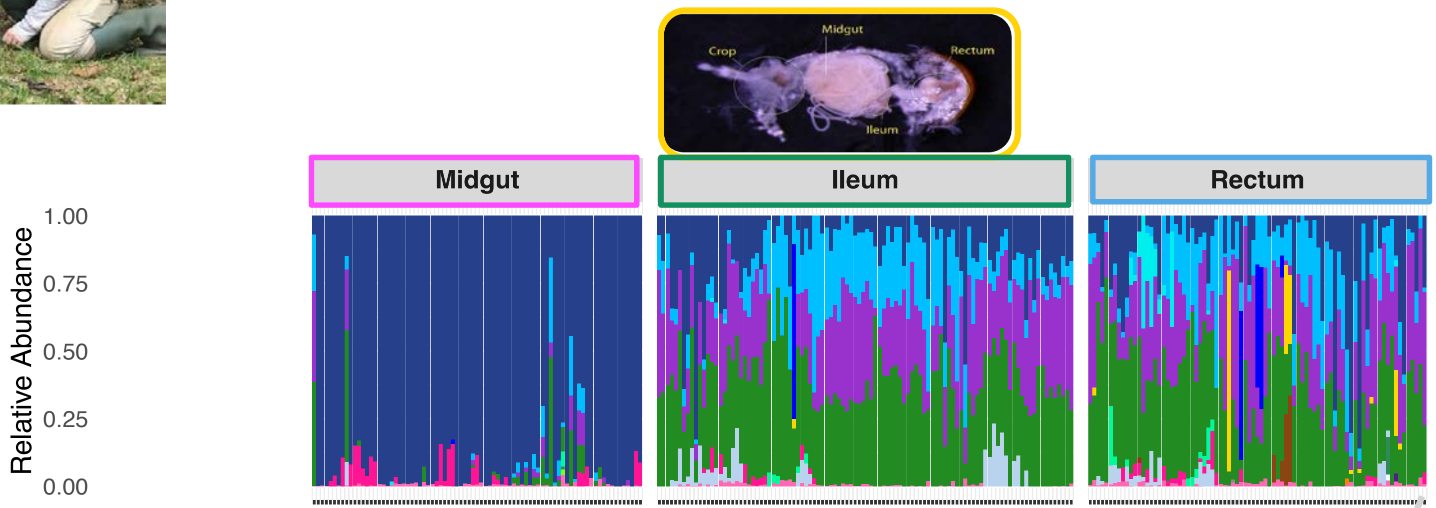
Are bacteria providing nutrition to ants? Indirect evidence



Flynn, D'Amelio, Sanders, Russell, & Moreau (2021)
Appl. Environ. Microbiol. 87(8): e02803-20

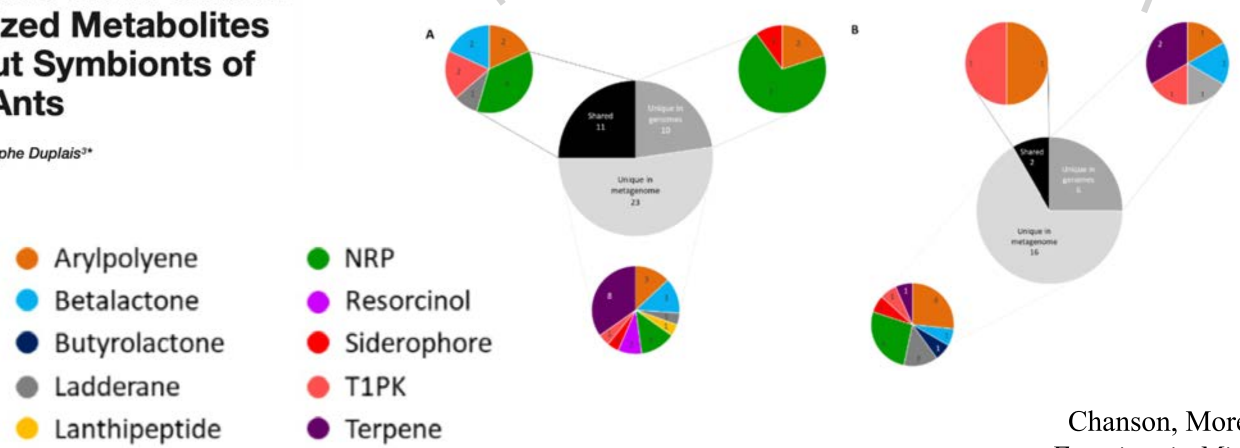
Anais Chanson

Bacteria are also interacting within the gut



Assessing Biosynthetic Gene Cluster Diversity of Specialized Metabolites in the Conserved Gut Symbionts of Herbivorous Turtle Ants

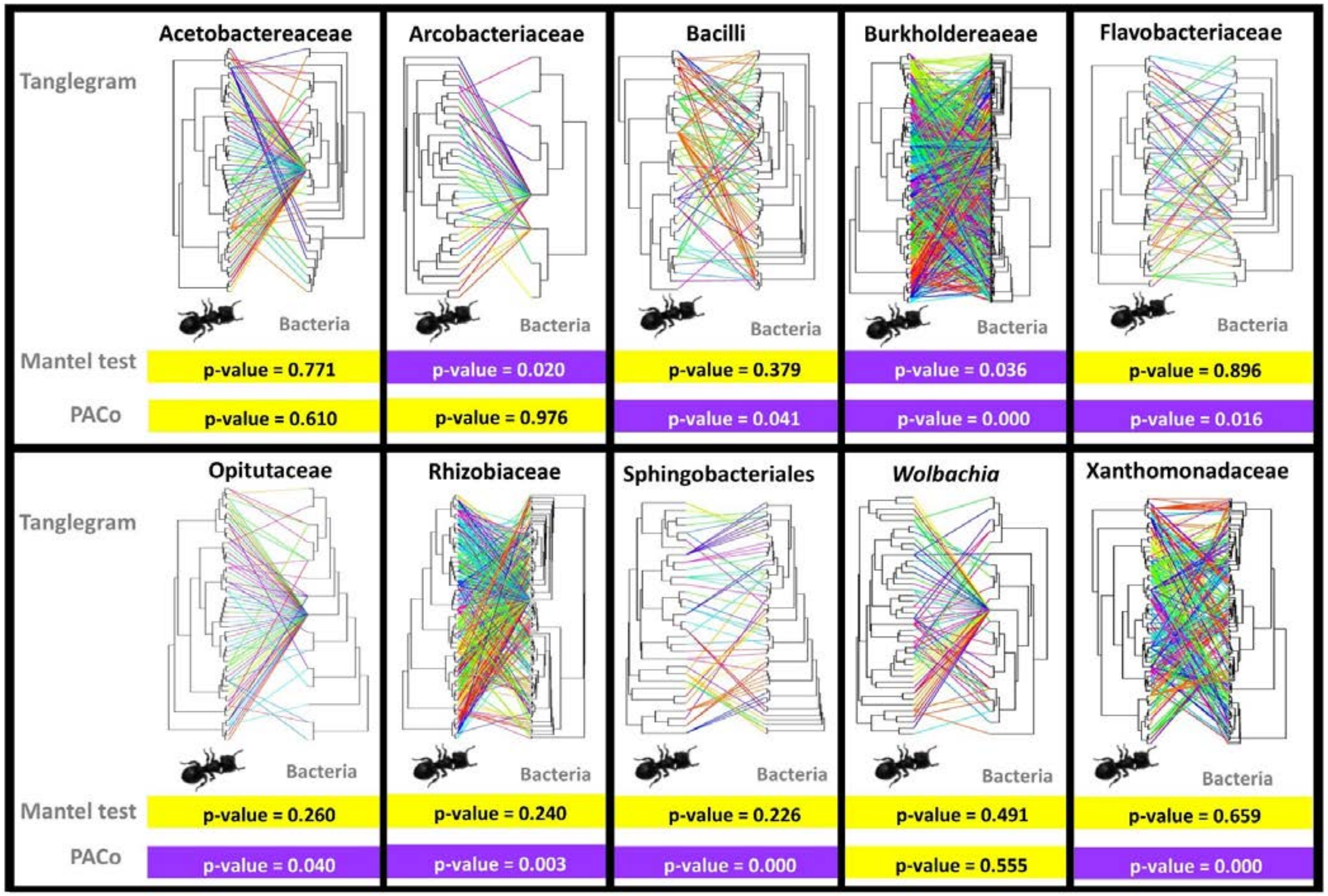
*Anaïs Chanson[1], Corrie S. Moreau[2] and Christophe Duplais[3]**



Chanson, Moreau, & Duplais (2021)
Frontiers in Microbiology 12: e678100.

Manuela Ramalho

Some gut bacteria codiversify with their hosts



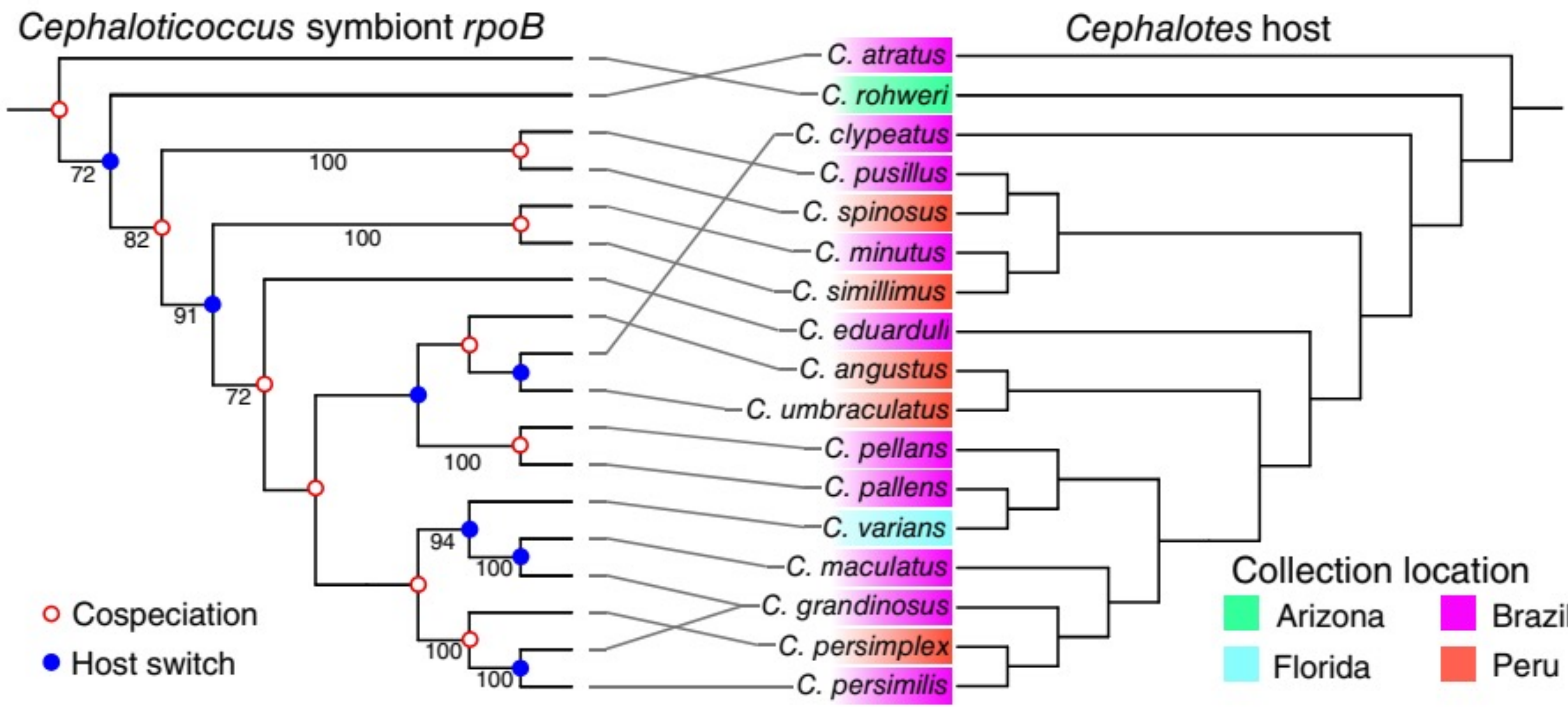
= codiversification

= no codiversification

Ramalho & Moreau (2023)
Animal Microbiome 5(1): 1-16.

Flynn, D'Amelio, Sanders, Russell, & Moreau (2021)
Appl. Environ. Microbiol. 87(8): e02803-20

Gut symbiont and host have codiversified over 50 million years



Hu et al. (2022) *Ecological Monographs. 2023;93:e1560.*

Are bacteria providing nutrition to ants? Indirect evidence

We have sequenced *nifH* genes from the following ants with *Rhizobiales*:

- *Cephalotes* (3 spp.)
- *Dolichoderus* (2 spp.)
- *Tetramorium* (1 sp.)
- *Procryptocerus* (1 sp.)
- and from gut tissue of *Cephalotes varians*

nif genes are responsible for the coding of proteins related and associated with the fixation of atmospheric nitrogen, but acetylene reduction assays have, thus far, failed to detect nitrogen-fixation activity in adult workers of turtle ants.

$$\text{Acetylene } (C_2H_2) \xrightarrow{\text{Nitrogenase}} \text{Ethylene } (C_2H_4)$$

	YH001		YH026		CSM2266		control	
Sampling time (h)	C_2H_2 (ppm)	C_2H_4 (ppm)	C_2H_2 (ppm)	C_2H_4 (ppm)	C_2H_2 (ppm)	C_2H_4 (ppm)	C_2H_2 (ppm)	C_2H_4 (ppm)
0h	22.8	0	27.3	0	27.7	0	13.3	0
1h	11.7	0	8.8	0	11.5	0	12.2	0
2h	12.7	0	15.9	0	10.6	0	12.9	0
4h	20.5	0	18.7	0	18.9	0	21.1	0
8h	24.7	0	13.6	0	22.4	0	26.6	0
16h	29	0	19.4	0	25.8	0	26.2	0

Russell, Moreau *et al*. (2009) *PNAS* 106(50): 21236-21241.

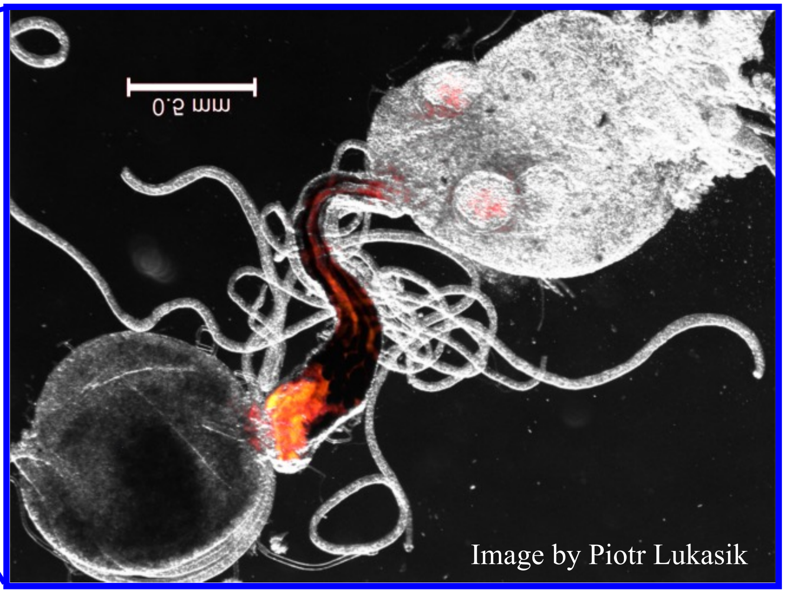
Anderson *et al*. (2012) *Mol. Ecol*. 21: 2282-2296.

Hu *et al*. (2018). *Nature Communications* 9: e964.

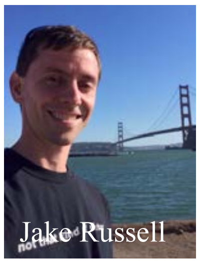
Are bacteria providing nutrition to ants? Indirect evidence

Gut bacteria of *Cephalotes* are concentrated in the anterior hindgut

This is where ant-derived N-wastes arrive via Malpighian tubules

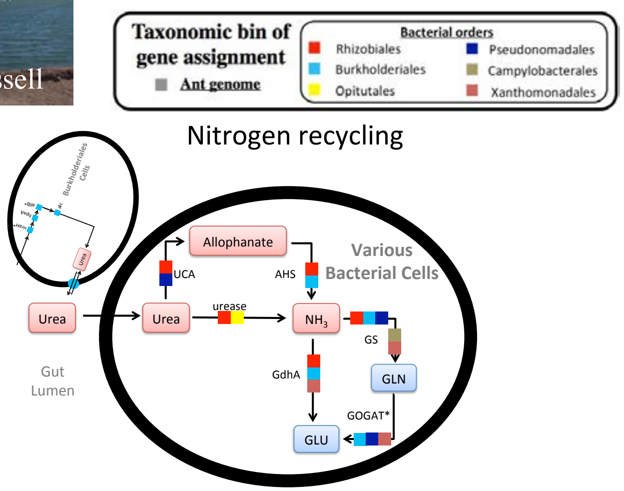


Fluorescent in situ hybridization with labeled probes targeting bacterial specific 16S rRNA



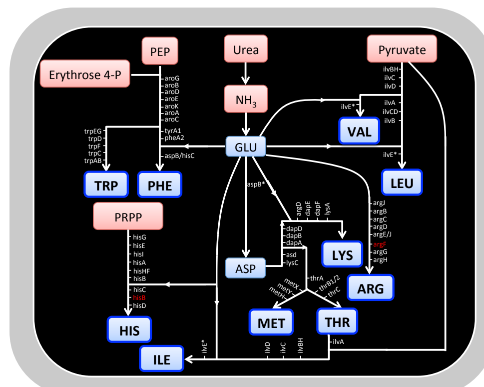
Are bacteria providing nutrition to ants? Direct evidence

Metagenomic insights into nitrogen recycling/upgrading:
Core bacteria from *Cephalotes* guts synthesize essential amino acids

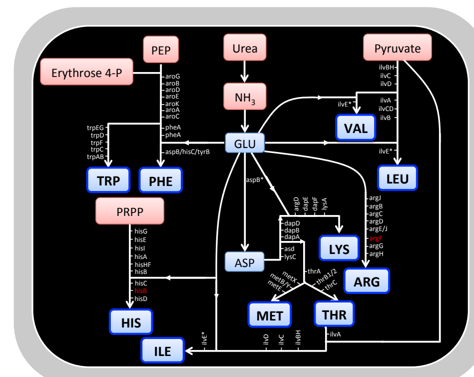


Predicted metabolic pathways for uric acid metabolism and nitrogen recycling

Rhizobiales



Burkholderiales



Predicted essential amino-acid biosynthetic pathways in metagenomes of *C. varians*

Taxonomic conservation across 18 *Cephalotes* metagenomes demonstrating N-recycling by the core symbionts across 46 million years

Hu *et al*. (2018).
Nature Communications 9: e964.

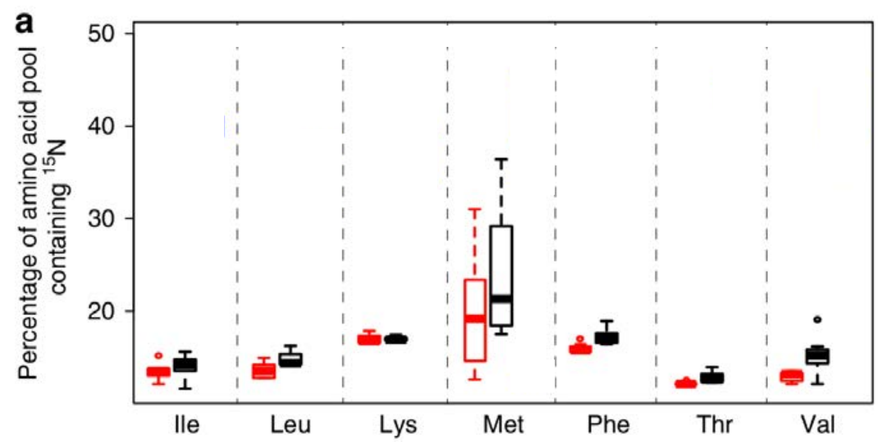
Are bacteria providing nutrition to ants? Direct evidence

Testing nutrient provisioning capabilities of gut bacteria in *C. varians*

Experimental Design: antibiotic-mediated symbiont removal

Experimental Design: diet		Cured ants
15N urea in diet	high 15N levels in essential amino acids	"naturally" low 15N levels in essential amino acids
14N urea in diet	"naturally" low 15N levels in essential amino acids	------

Predicted results for three treatment groups



Treatment Group

14N urea + bacteria | 15N urea - bacteria | 15N urea + bacteria

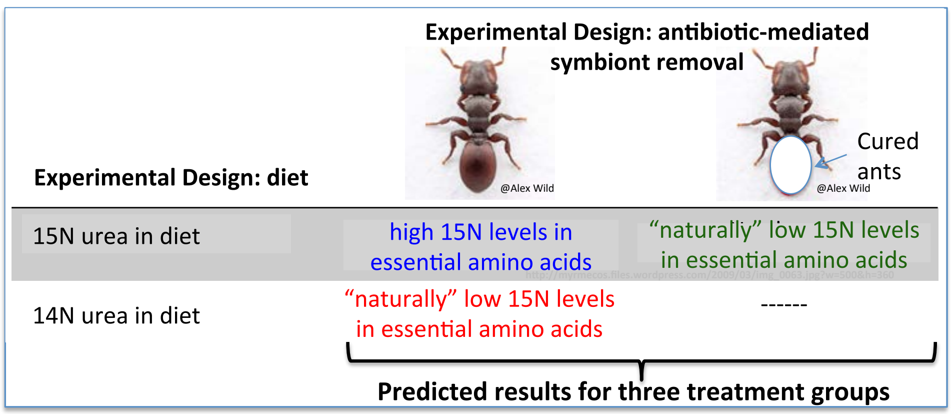
MANOVA $P < 0.05$ for all seven essential amino acids

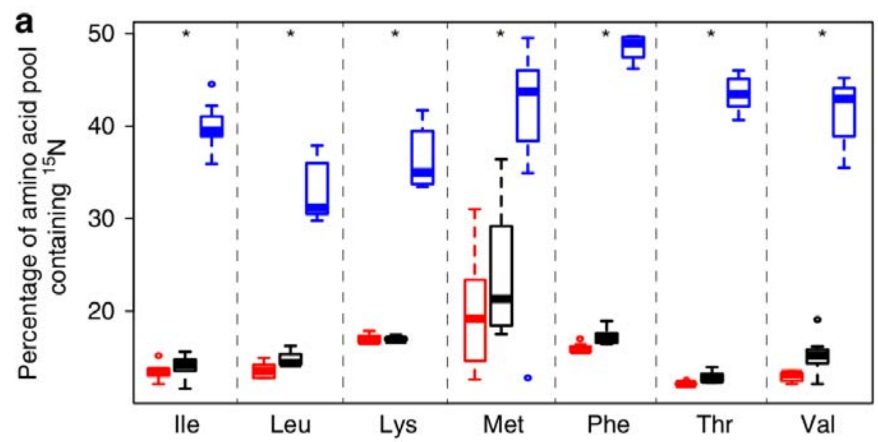
Cured ants = Diet included 1% of Tetracycline, Rifampicin, and Kanamycin each

Hu *et al*. (2018). *Nature Communications* 9: e964.

Are bacteria providing nutrition to ants? Direct evidence

Testing nutrient provisioning capabilities of gut bacteria in *C. varians*





Gut bacteria of *Cephalotes* provide amino acids to ants via N-recycling

Treatment Group

14N urea + bacteria | 15N urea - bacteria | 15N urea + bacteria

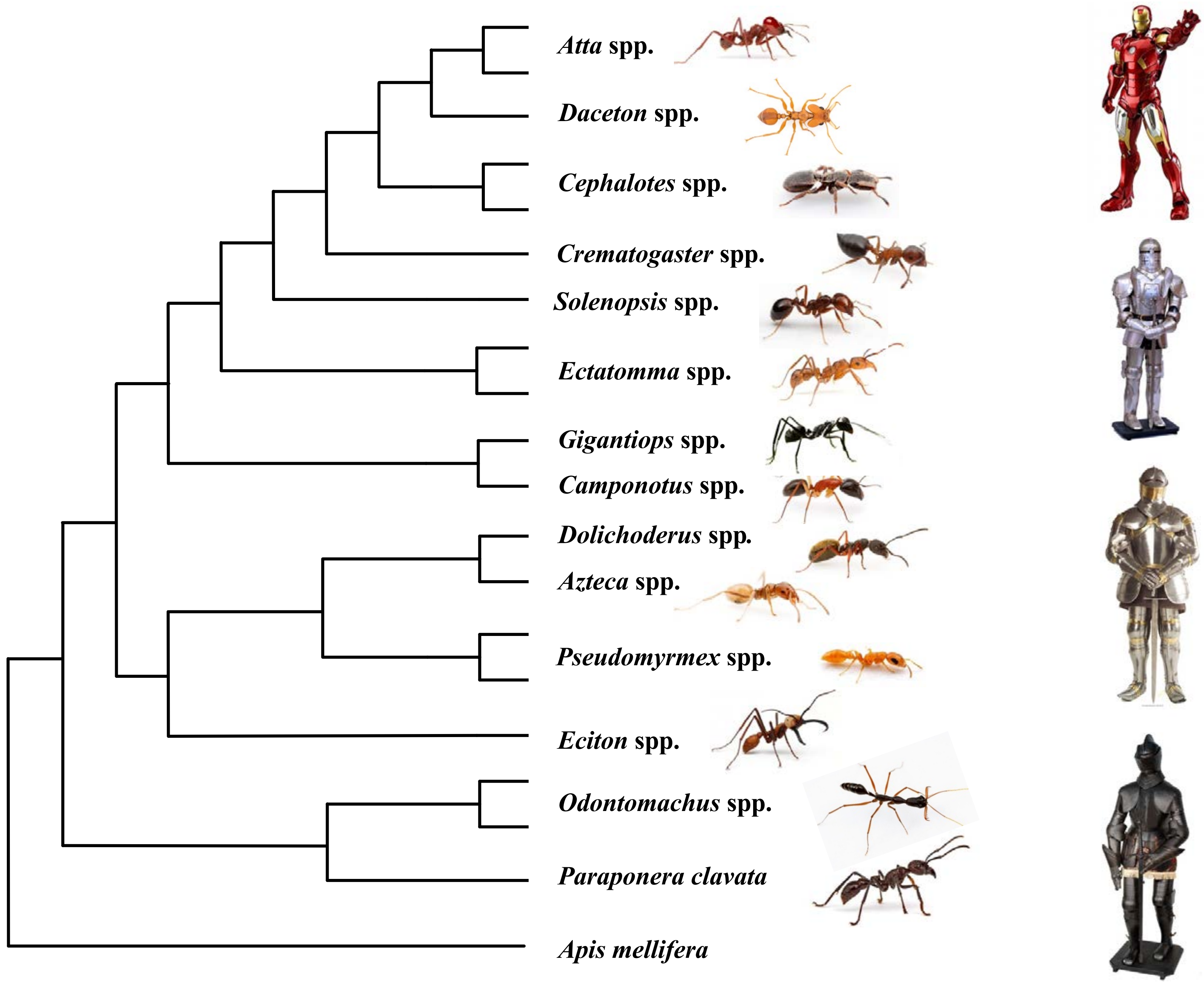
MANOVA $P < 0.05$ for all seven essential amino acids

Cured ants = Diet included 1% of Tetracycline, Rifampicin, and Kanamycin each

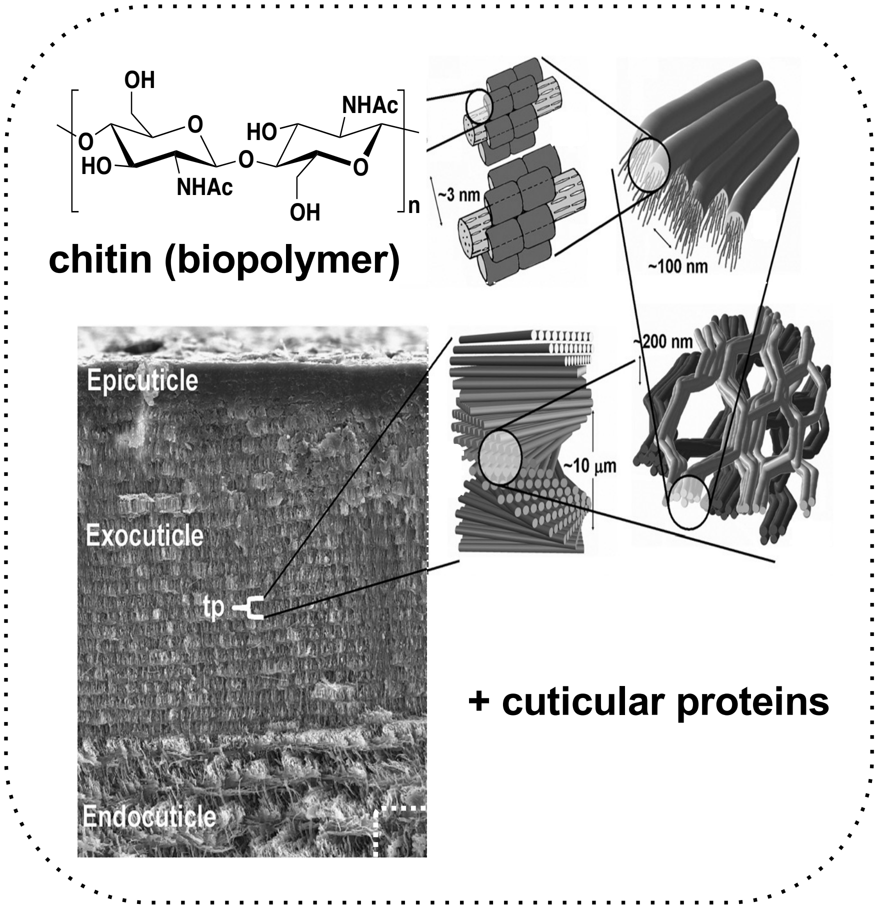
Hu *et al*. (2018). *Nature Communications* 9: e964.

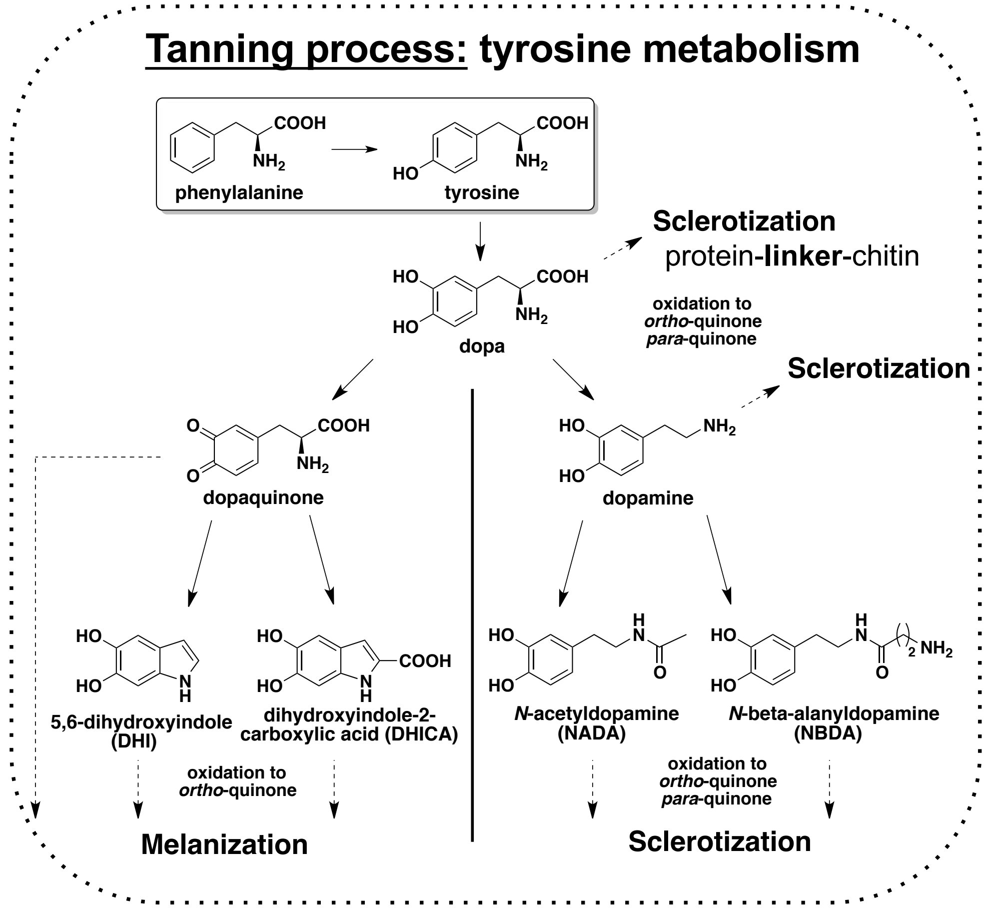
Evidence for the nitrogen contribution of gut symbionts to the cuticle formation in herbivorous turtle ants

Exoskeleton = insect armor


Atta spp.
Daceton spp.
Cephalotes spp.
Crematogaster spp.
Solenopsis spp.
Ectatomma spp.
Gigantiops spp.
Camponotus spp.
Dolichoderus spp.
Azteca spp.
Pseudomyrmex spp.
Eciton spp.
Odontomachus spp.
Paraponera clavata
Apis mellifera

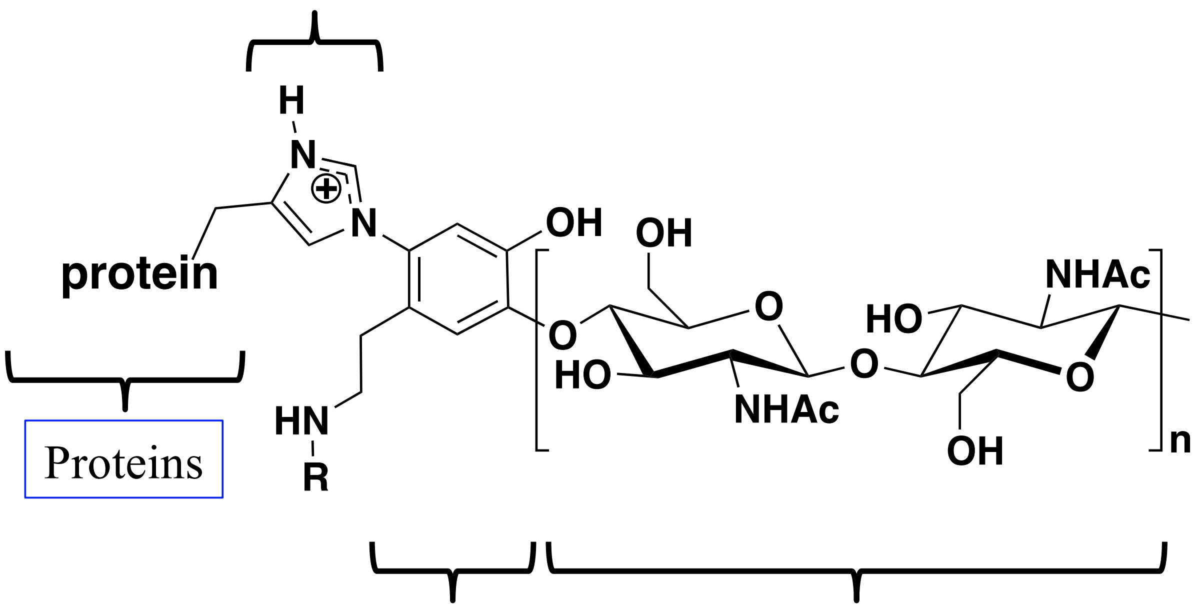
Composition and structure





Insect cuticle

Imidazole of histidine



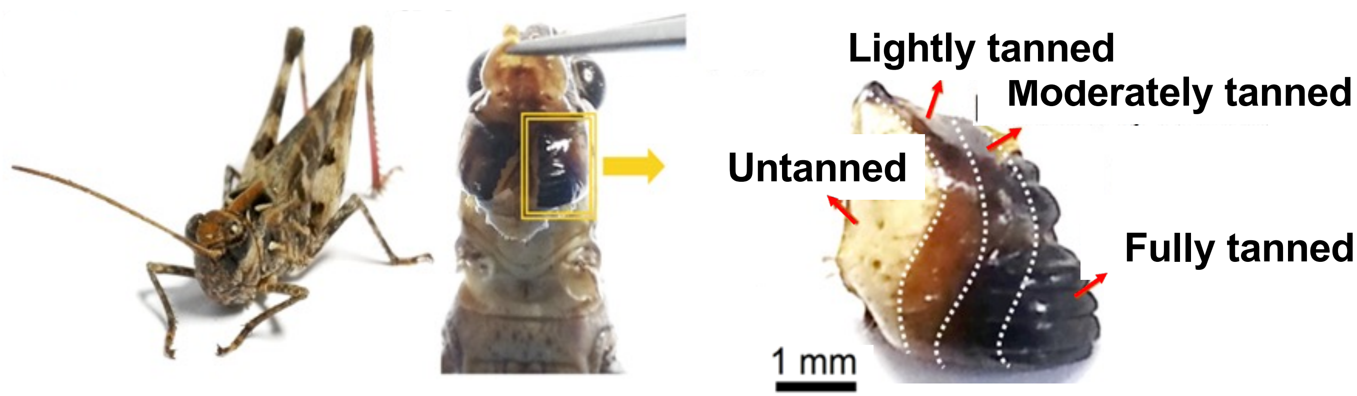
Proteins

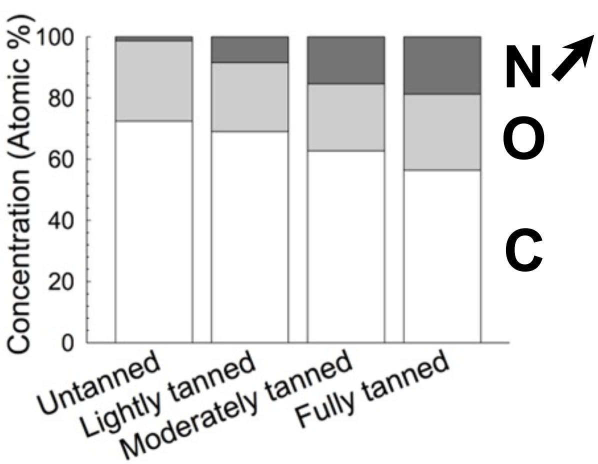
Linker

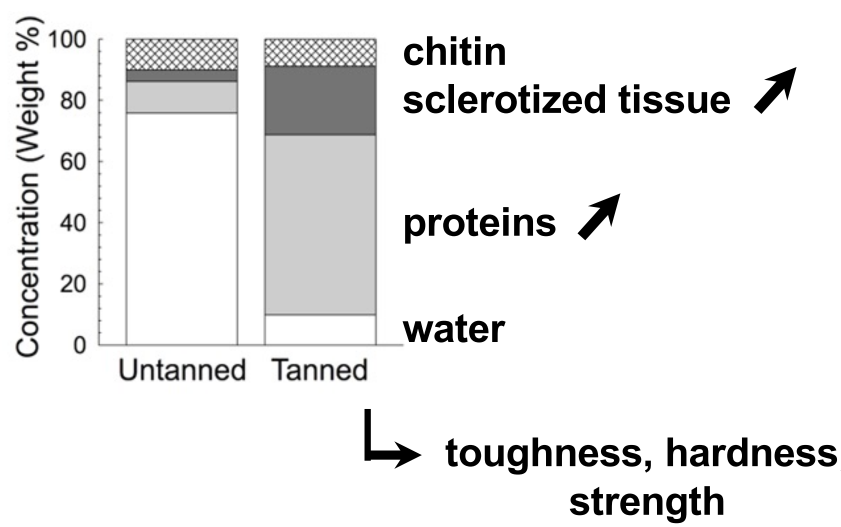
Chitin

+ Other imbedded particles
(i.e. melanin, metals, etc.)

Example: Grasshopper mandibles

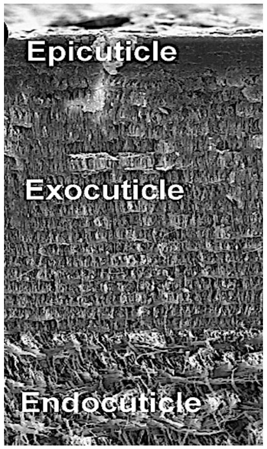






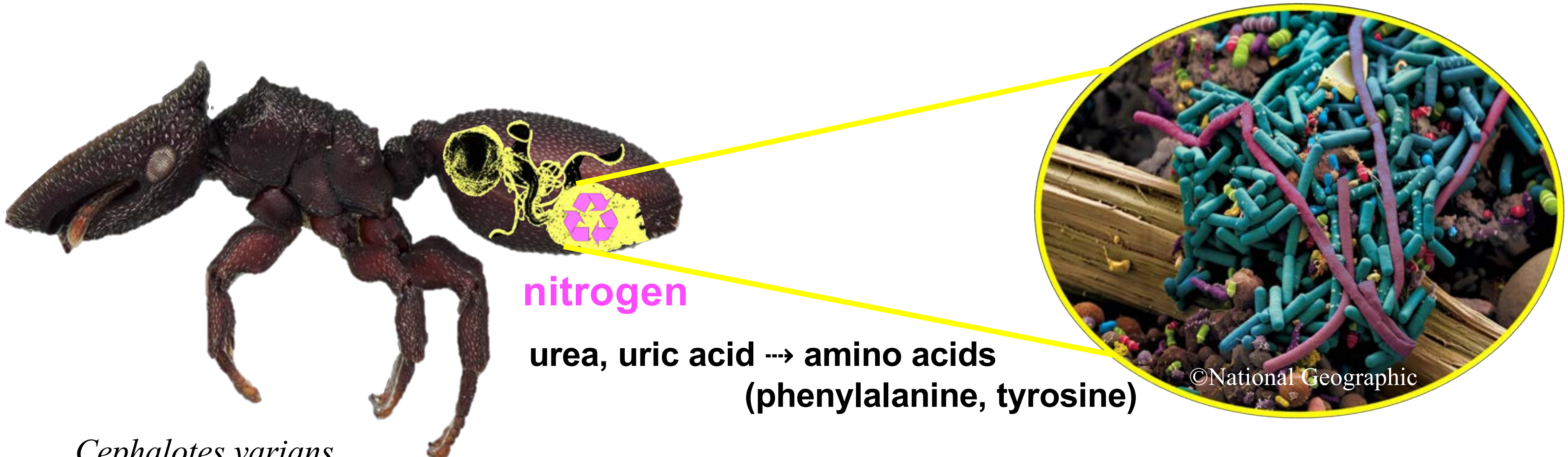
Lee *et al*. (2015) *Chem. Mater*. 27: 6478-6481.

Hypothesis



Contribution?

Burkholderiales, Pseudomonadales, Xanthomonadales, Rhizobiales, and Opitutales



Cephalotes varians

Russell, Moreau, *et al*. (2009) *Proc. Natl. Acad. Sci. USA* 50: 21236-21241.
Hu *et al*. (2018) *Nat. Comm*. 9: e964.

Previous research

PNAS PLUS

Small genome symbiont underlies cuticle hardness in beetles

Hisashi Anbutsu[a,b,1,2], Minoru Moriyama[a,1], Naruo Nikoh[c,1], Takahiro Hosokawa[a,d], Ryo Futahashi[a], Masahiko Tanahashi[a], Xian-Ying Meng[a], Takashi Kuriwada[e,f], Naoki Mori[g], Kenshiro Oshima[h], Masahira Hattori[h,i], Manabu Fujie[j], Noriyuki Satoh[k], Taro Maeda[l], Shuji Shigenobu[l], Ryuichi Koga[a], and Takema Fukatsu[a,m,n,2]

AMERICAN SOCIETY FOR MICROBIOLOGY mBio®

A Novel, Extremely Elongated, and Endocellular Bacterial Symbiont Supports Cuticle Formation of a Grain Pest Beetle

Bin Hirota,[a,b] Genta Okude,[a,b] Hisashi Anbutsu,[a,c] Ryo Futahashi,[a] Minoru Moriyama,[a] Xian-Ying Meng,[a] Naruo Nikoh,[d] Ryuichi Koga,[a] Takema Fukatsu[a,b,e]

Received: 12 September 2017 | Accepted: 22 September 2017
DOI: 10.1111/mec.14418

SPECIAL ISSUE: THE HOST-ASSOCIATED MICROBIOME: PATTERN, PROCESS AND FUNCTION

WILEY MOLECULAR ECOLOGY

Ancient symbiosis confers desiccation resistance to stored grain pest beetles

Tobias Engl[1] | Nadia Eberl[1*] | Carla Gorse[1*] | Theresa Krüger[1*] | Thorsten H. P. Schmidt[2] | Rudy Plarre[3] | Cornel Adler[4] | Martin Kaltenpoth[1]

Current Biology *24*, 2267–2273, October 6, 2014

Insects Recycle Endosymbionts when the Benefit Is Over

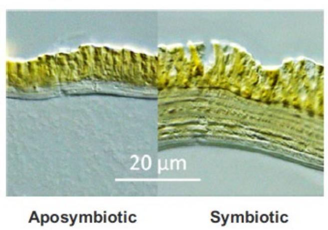
Aurélien Vigneron,[1,2] Florent Masson,[1] Agnès Vallier,[1] Séverine Balmand,[1] Marjolaine Rey,[1] Carole Vincent-Monégat,[1] Emre Aksoy,[1] Etienne Aubailly-Giraud,[1] Anna Zaidman-Rémy,[1] and Abdelaziz Heddi[1,*]
[1]Biologie Fonctionnelle Insectes et Interactions, UMR203 BF2I, INRA, INSA-Lyon, Université de Lyon, 69621 Villeurbanne, France

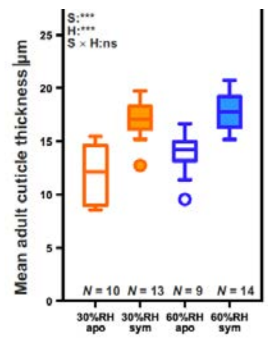
Previous research

Quantifying the impact of symbiont on cuticle formation

- Cuticle coloration

- Cuticle thickness





- One identified biosynthetic pathway from the symbiont

Erythrose 4-phosphate + Phosphoenol pyruvate $\xrightarrow{\text{AroF}}$ $\xrightarrow{\text{AroB}}$ $\xrightarrow{\text{AroD}}$ $\xrightarrow{\text{AroE}}$ $\xrightarrow{\text{AroK}}$ $\xrightarrow{\text{AroA}}$ $\xrightarrow{\text{AroC}}$ $\xrightarrow{\text{TyrA}}$ 4-hydroxy phenyl pyruvate (HPP) $\xrightarrow{\cancel{\text{TyrB}}}$ Tyrosine

Previous research

Small genome symbiont underlies cuticle hardness in beetles

Hisashi Anbutsu[a,b,1,2], M
Masahiko Tanahashi[a], X
Manabu Fujie[j], Noriyuki

Received: 12 September 2017 | Accepted
DOI: 10.1111/mec.14418

SPECIAL ISSUE: THE HO
PATTERN, PROCESS AN

Ancient symbiosi
grain pest beetle

Tobias Engl[1] | Nadia Eberl[1*] | Carla Gorse[1*] | Theresa Krüger[1*] |
Thorsten H. P. Schmidt[2] | Rudy Plarre[3] | Cornel Adler[4] | Martin Kaltenpoth[1]

AMERICAN SOCIETY FOR MICROBIOLOGY mBio

A Novel, Extremely Elongated, and
...nt
...a Grain

...ionts

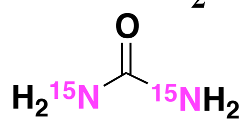
Carole Vincent-Monégat,[1] Emre Aksoy,[1]
Etienne Aubailly-Giraud,[1] Anna Zaidman-Rémy,[1]
and Abdelaziz Heddi[1,*]
[1]Biologie Fonctionnelle Insectes et Interactions,
UMR203 BF2I, INRA, INSA-Lyon, Université de Lyon,
69621 Villeurbanne, France

What is still left to learn?

- **Which cuticular components are impacted? (protein, linker, chitin, melanin)**
- **Are other pathways involved in the process?**
- **Are these mechanisms conserved across many species?**

Experimental design and methods

Diet with labelled urea-$^{15}N_2$



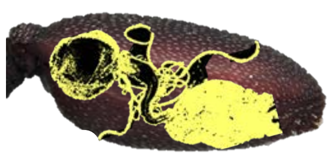
nitrogen

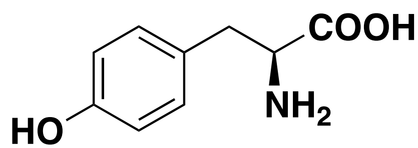
Colony n° 1:
No treatment

Colony n° 2:
Antibiotics treatment

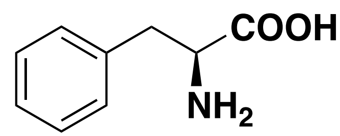
1) Two months experiment ⇢ new adults emerge
2) Gut dissection ⇢ solvent extraction ⇢ liquid-state ^{1}**H** NMR spectroscopy 
3) Cuticle ⇢ solid-state ^{13}**C** and ^{15}**N** NMR spectroscopy 
4) Cuticle ⇢ scanning electron microscopy (SEM) 

Liquid-state ^{1}H NMR of gut chemical extracts





tyrosine



phenylalanine

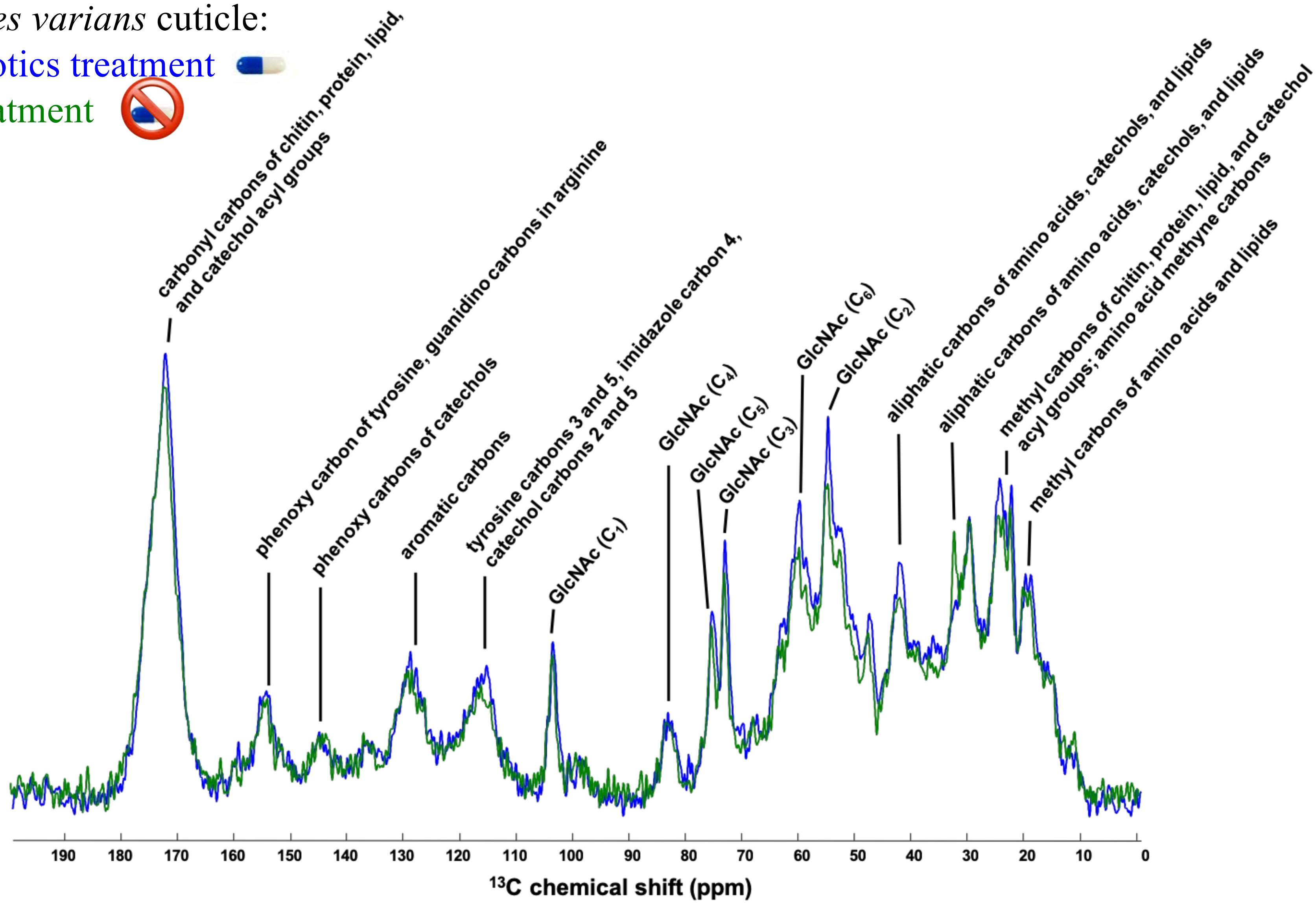
C. varians samples	Tyrosine concentration in µM (SD)	Phenylalanine concentration in µM (SD)
Untreated	**11.3 (0.1)**	**11.2 (0.3)**
Antibiotic treated	4.9 (0.3)	4.7 (0.3)

Duplais, C., Sarou-Kanian, V., Massiot, D., Hassan, A., Perrone, B., Estevez, Y., Wertz, J. T., Martineau, E., Farjon, J., Giraudeau, P. & Moreau, C. S. (2021) *Nature Communications* 12: e676.

Solid-state ^{13}C NMR of cuticle

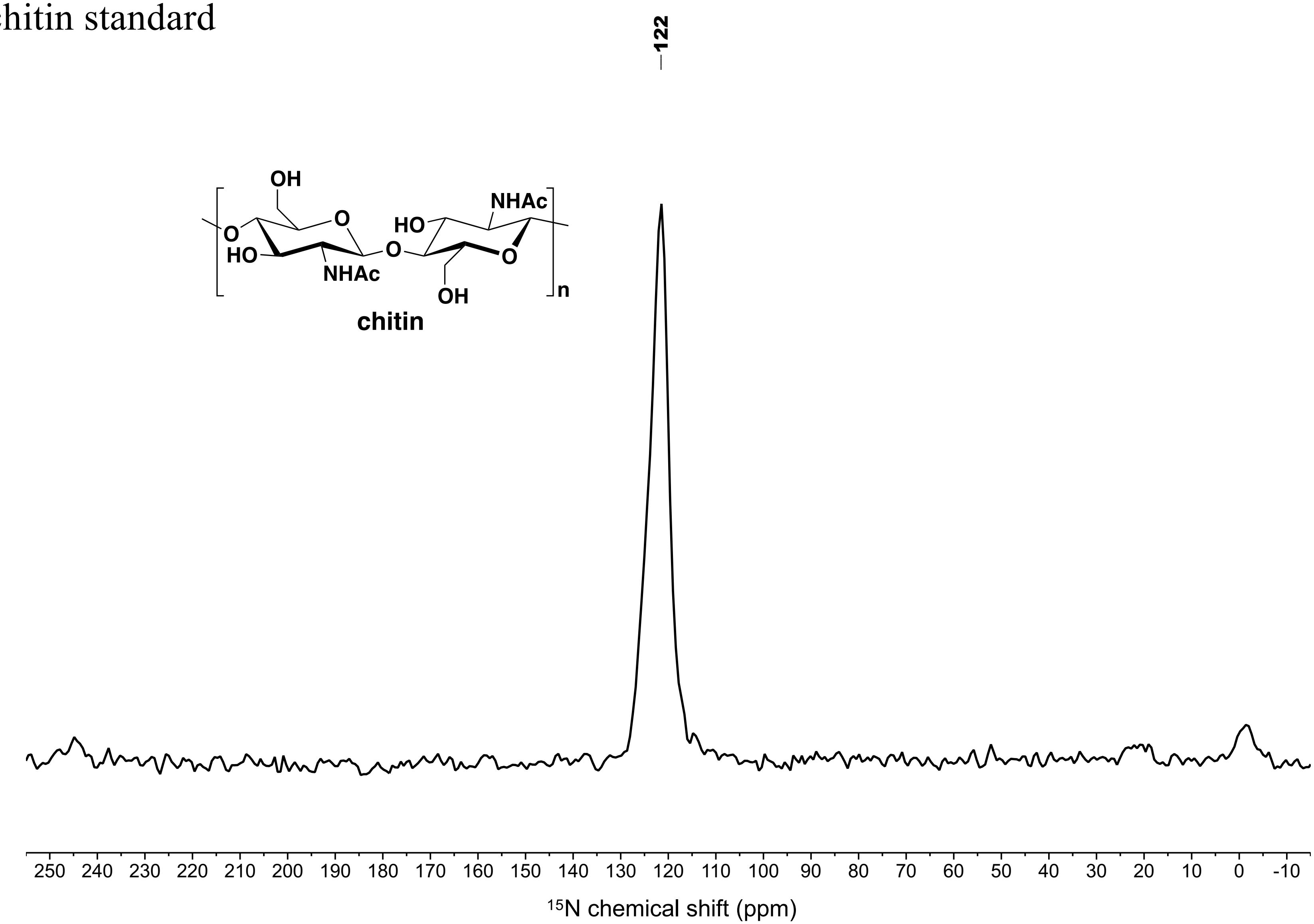
Cephalotes varians cuticle:

- antibiotics treatment
- no treatment



Solid-state ^{15}N NMR of cuticle

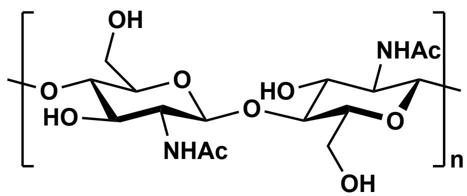
Pure chitin standard



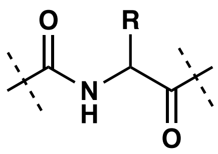
Solid-state ^{15}N NMR of cuticle

Cephalotes ant cuticle: no labelled urea

^{15}N natural abundance = 0.4 %

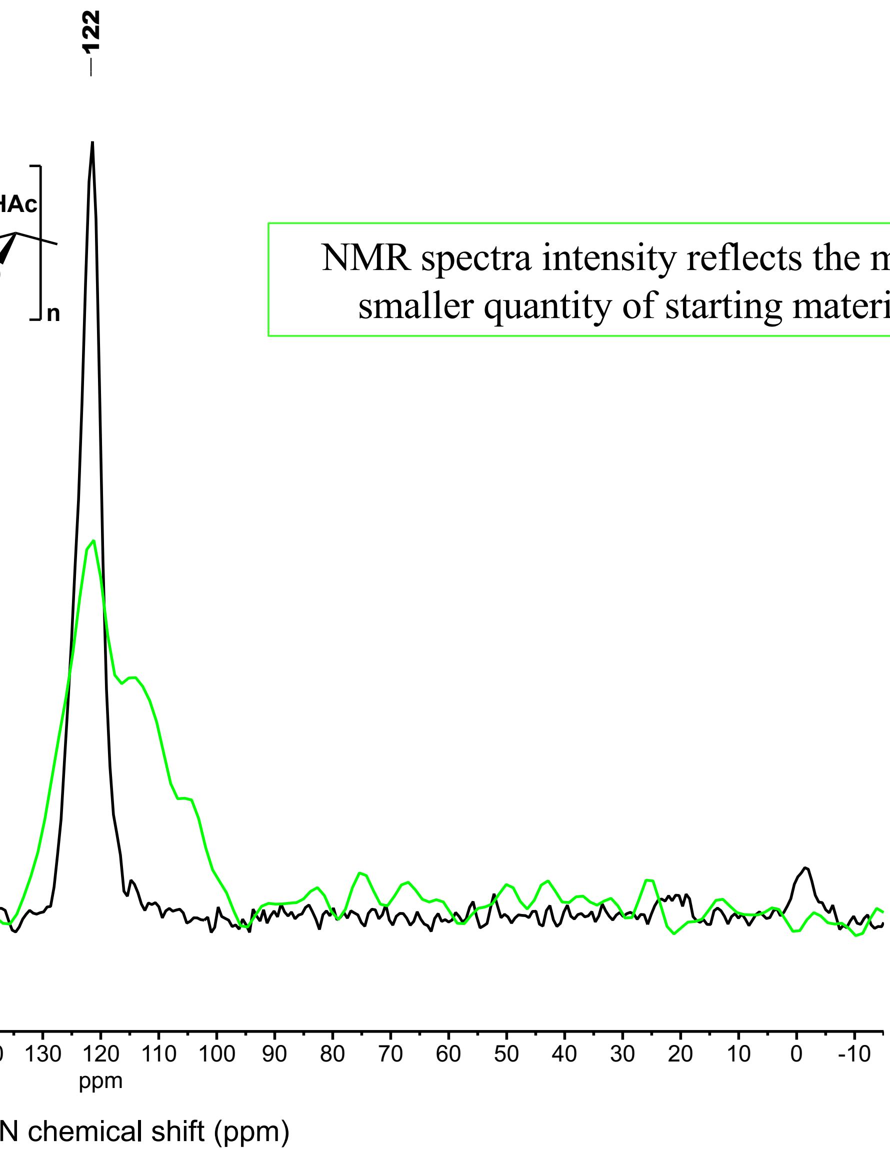


chitin



proteins

NMR spectra intensity reflects the much smaller quantity of starting material

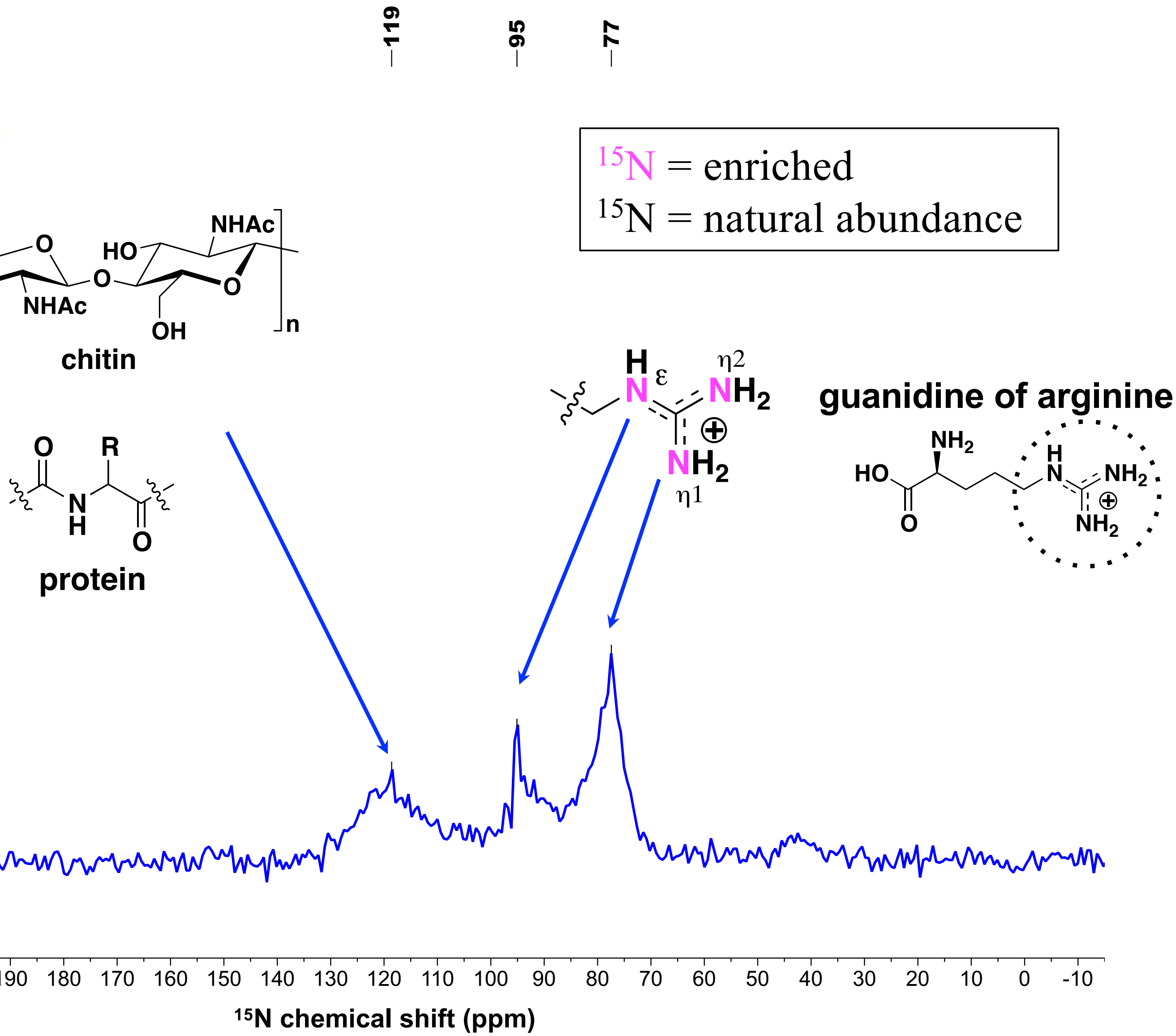


Solid-state ^{15}N NMR of cuticle

C. varians cuticle:

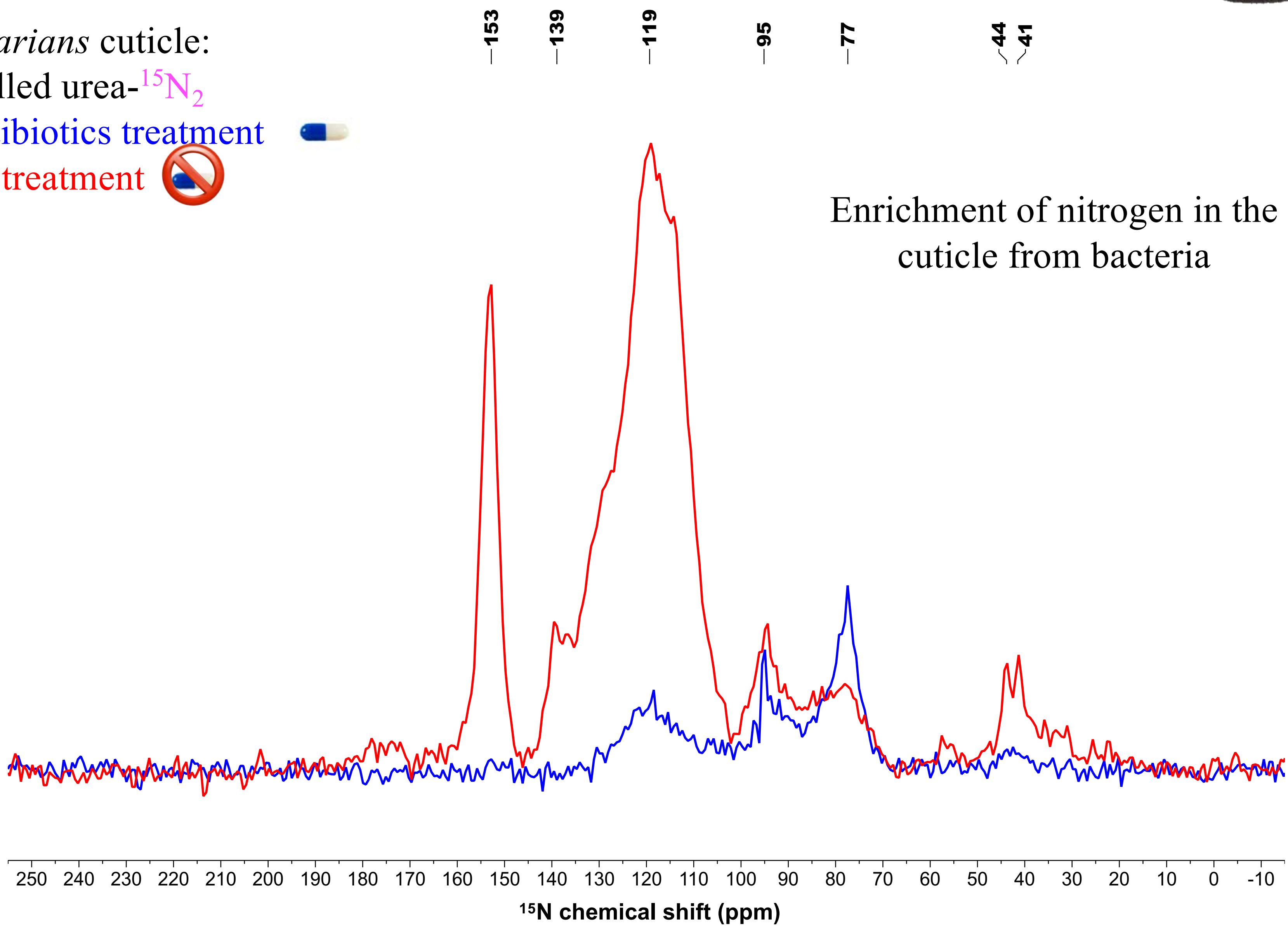
labelled urea-$^{15}N_2$

- antibiotics treatment 



Solid-state ^{15}N NMR of cuticle

C. varians cuticle:
labelled urea-$^{15}N_2$
- antibiotics treatment
- no treatment



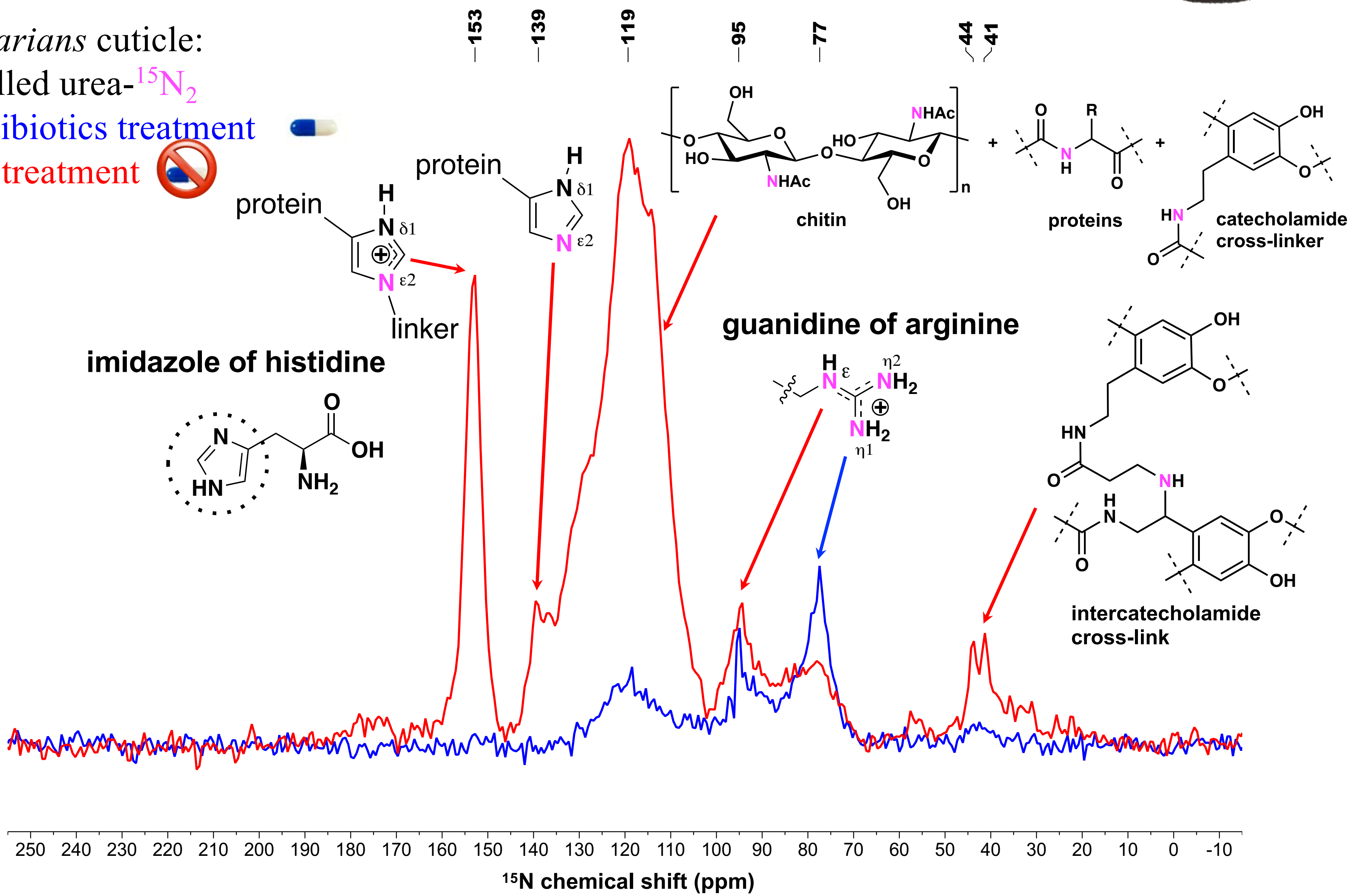
Enrichment of nitrogen in the cuticle from bacteria

Solid-state ^{15}N NMR of cuticle

C. varians cuticle:

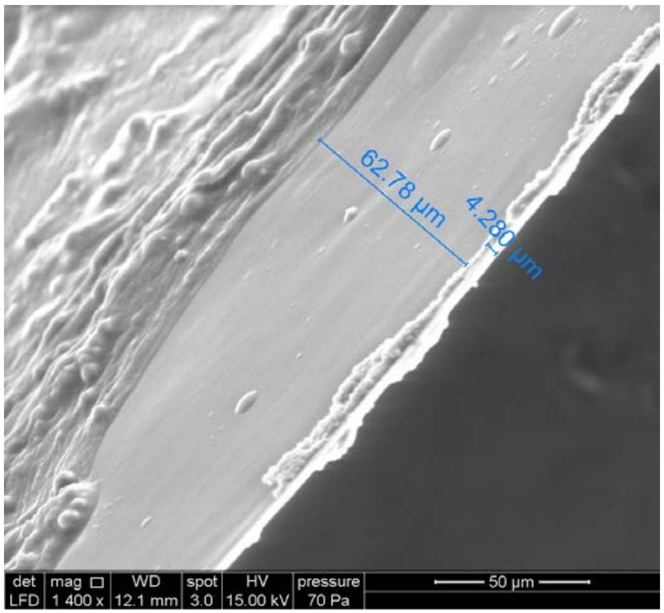
labelled urea-$^{15}N_2$

- antibiotics treatment
- no treatment

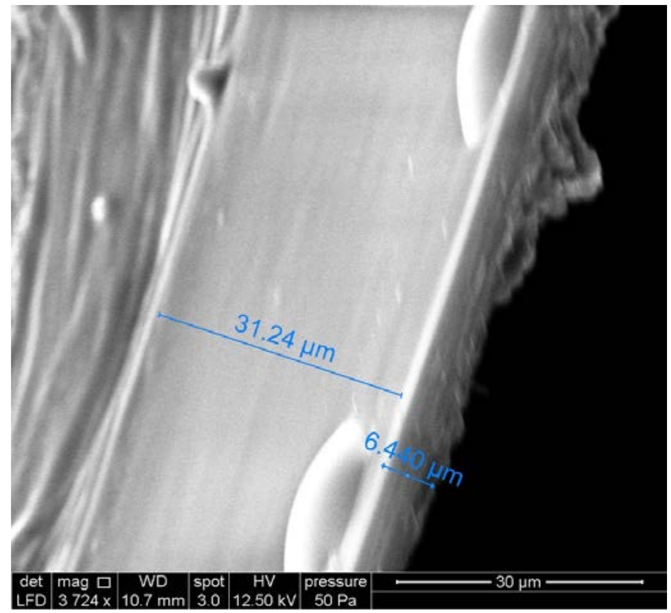


Electronic microscopy

Colony n° 1:
No treatment



Colony n° 2:
Antibiotics treatment

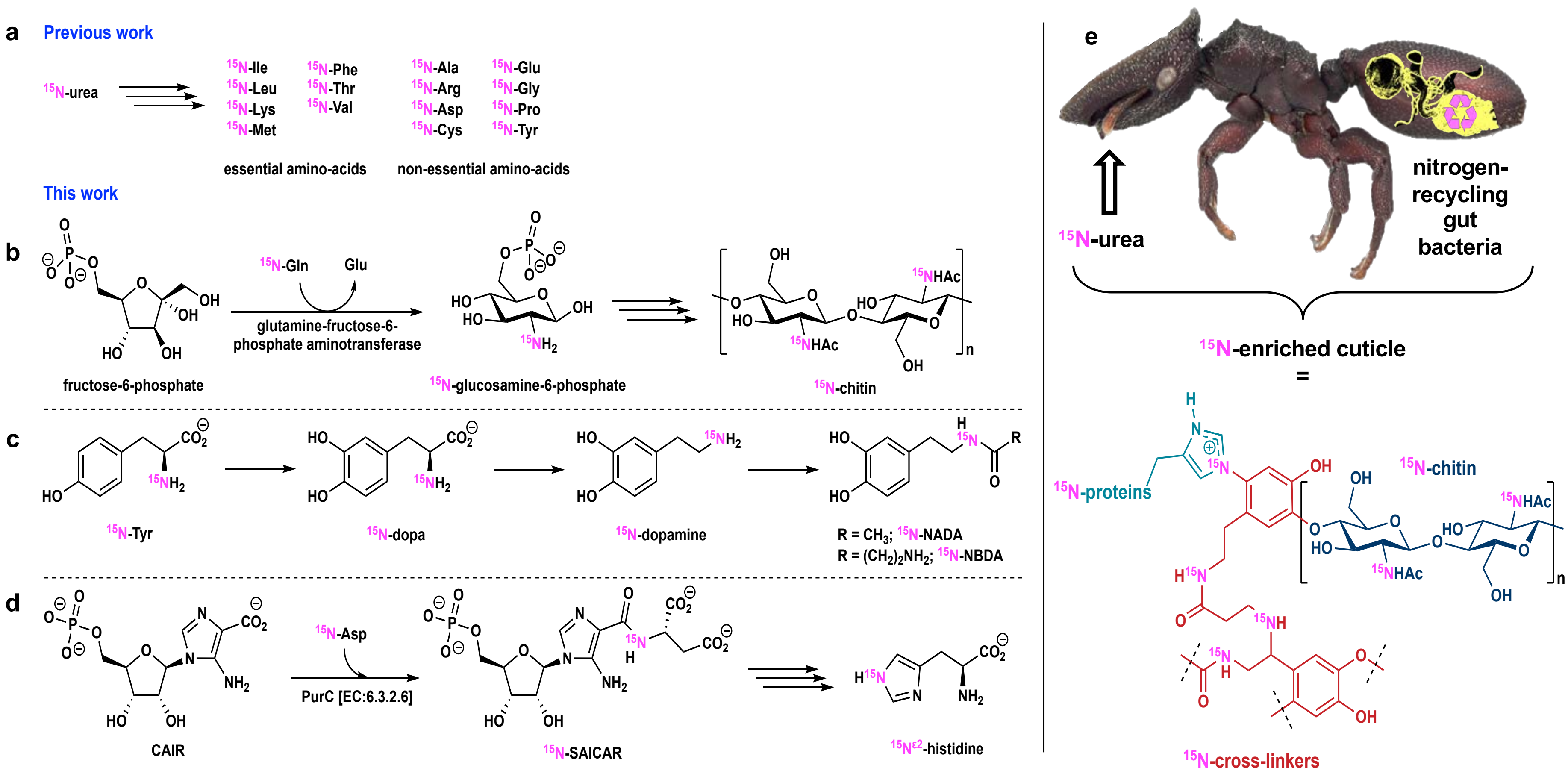


Cuticle thickness reduce by 50% in antibiotic treatment

Antibiotics treated individuals are fully black with no sign of depigmentation

Duplais, C., Sarou-Kanian, V., Massiot, D., Hassan, A., Perrone, B., Estevez, Y., Wertz, J. T., Martineau, E., Farjon, J., Giraudeau, P. & Moreau, C. S. (2021) *Nature Communications* 12: e676.

Gut bacteria contribute to most cuticular components: protein, linker, & chitin ✅ melanin ❌



Duplais, C., Sarou-Kanian, V., Massiot, D., Hassan, A., Perrone, B., Estevez, Y., Wertz, J. T., Martineau, E., Farjon, J., Giraudeau, P. & Moreau, C. S. (2021) *Nature Communications* 12: e676.

Integrative approach to study symbiosis as a driver of animal ecology and evolution

- Ant-plant interactions evolved through increasing interdependence
- Convergent patterns in genome evolution driven by life history strategy
- Symbiotic bacteria facilitated the ants to be able to shift to a herbivorous diet and expand into the canopy
- Host-associated bacteria contribute to cuticle formation

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