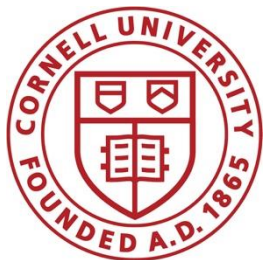


Macroevolution to Microbiomes: Piecing Together the Puzzle to Understand the Evolution of the Ants



Image from www.imaginetoy.com



Corrie S. Moreau (she/her)

Cornell University
www.moreaulab.org

 @CorrieMoreau

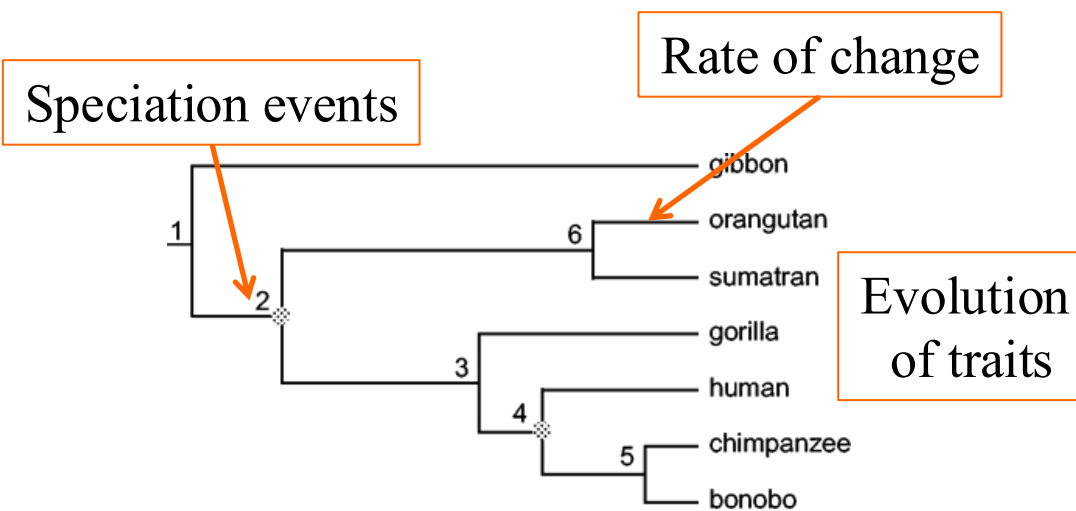


What processes generate biodiversity?

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

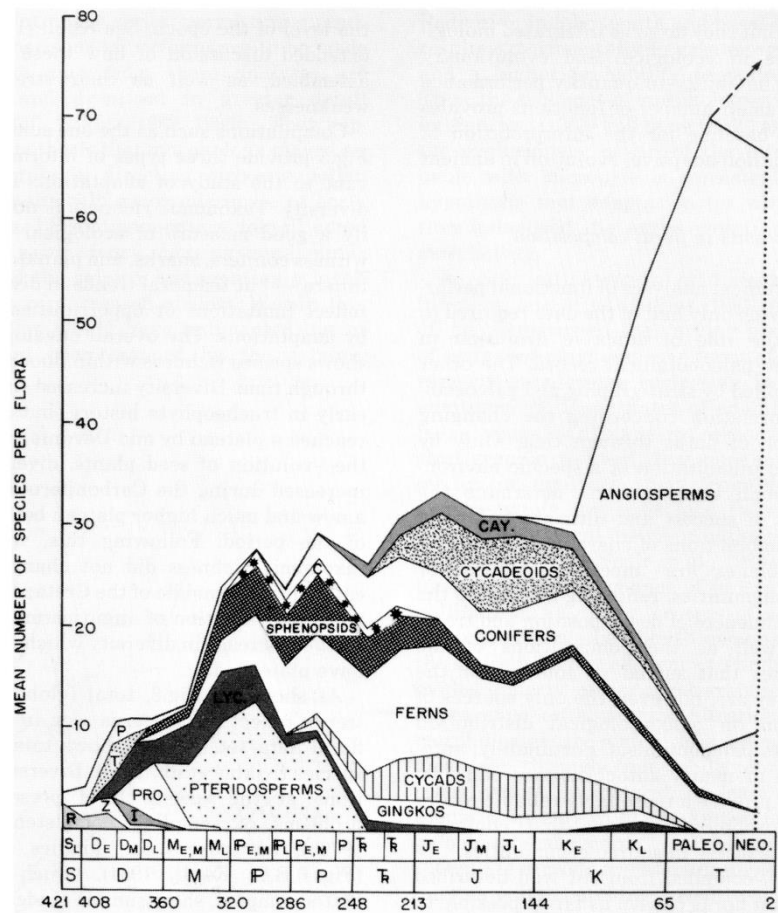


Image from: Niklas et al. (1980) *Evol Biol* 12: 1-89.



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



Beetles photograph Mark Wilson;
Staghorn fern photograph <http://wikimedia.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*



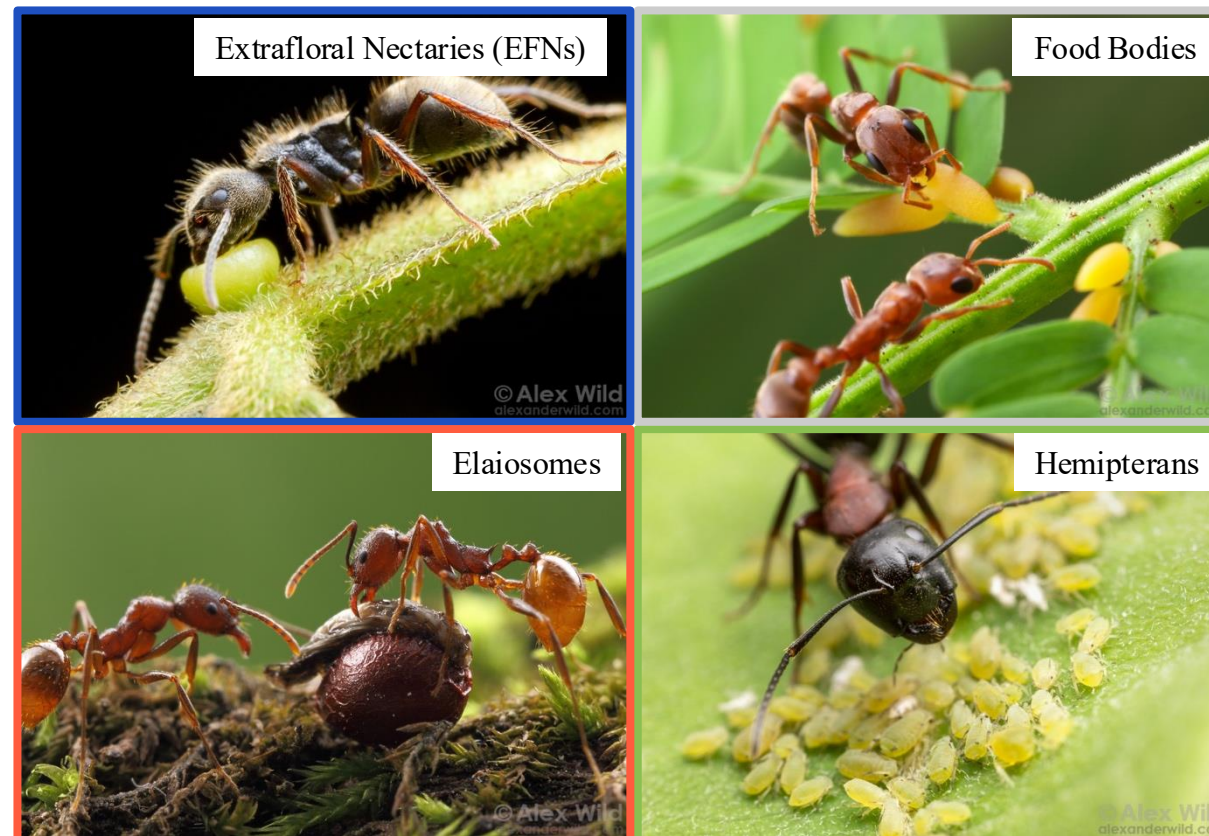
Numerous clades linked to angiosperm-forests

- 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*



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?



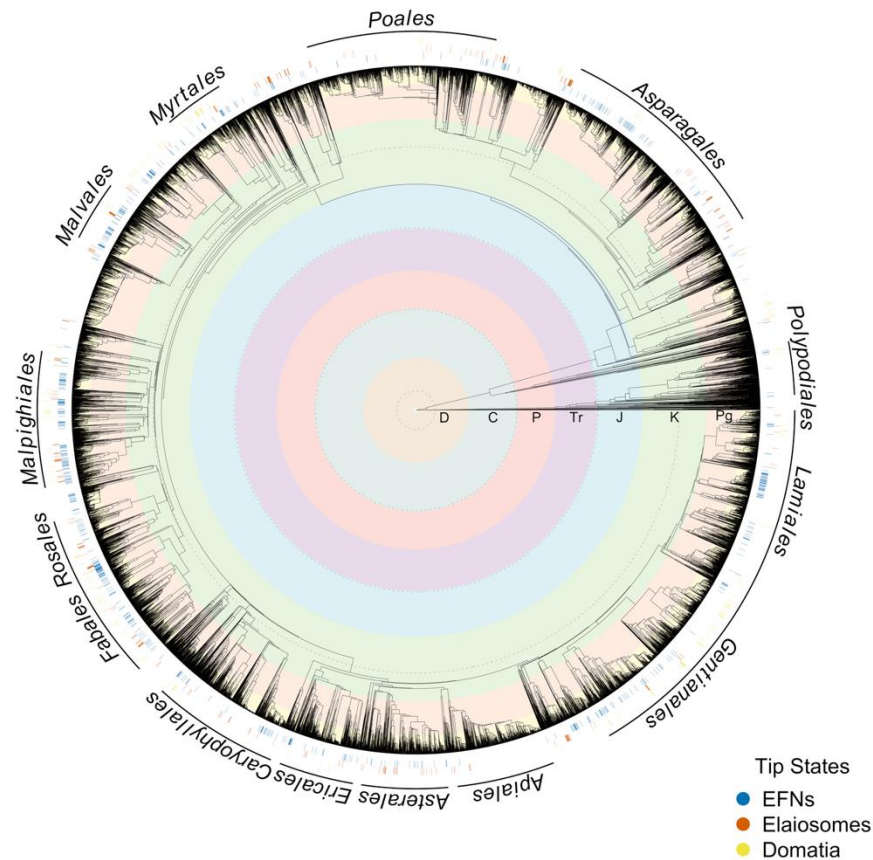
Plant evolution

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

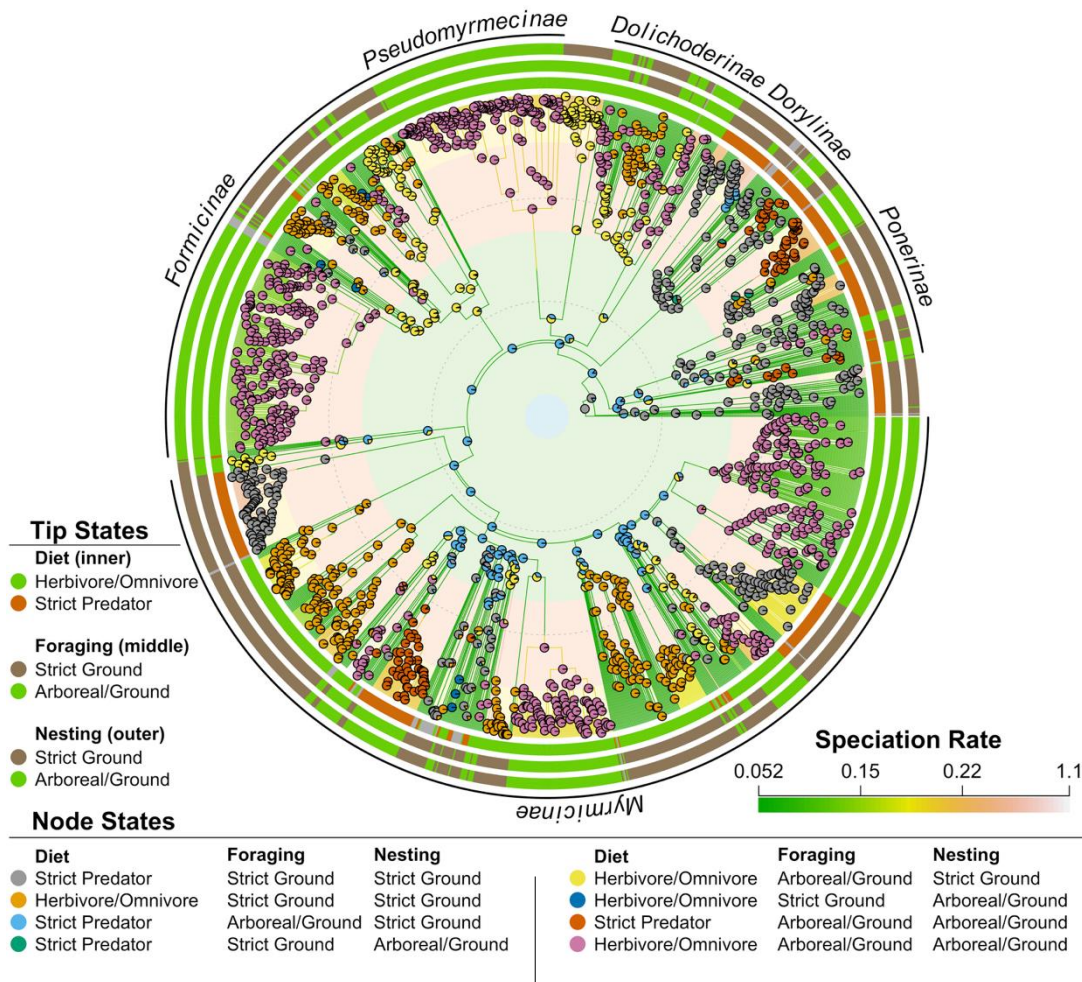


Matt Nelsen



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

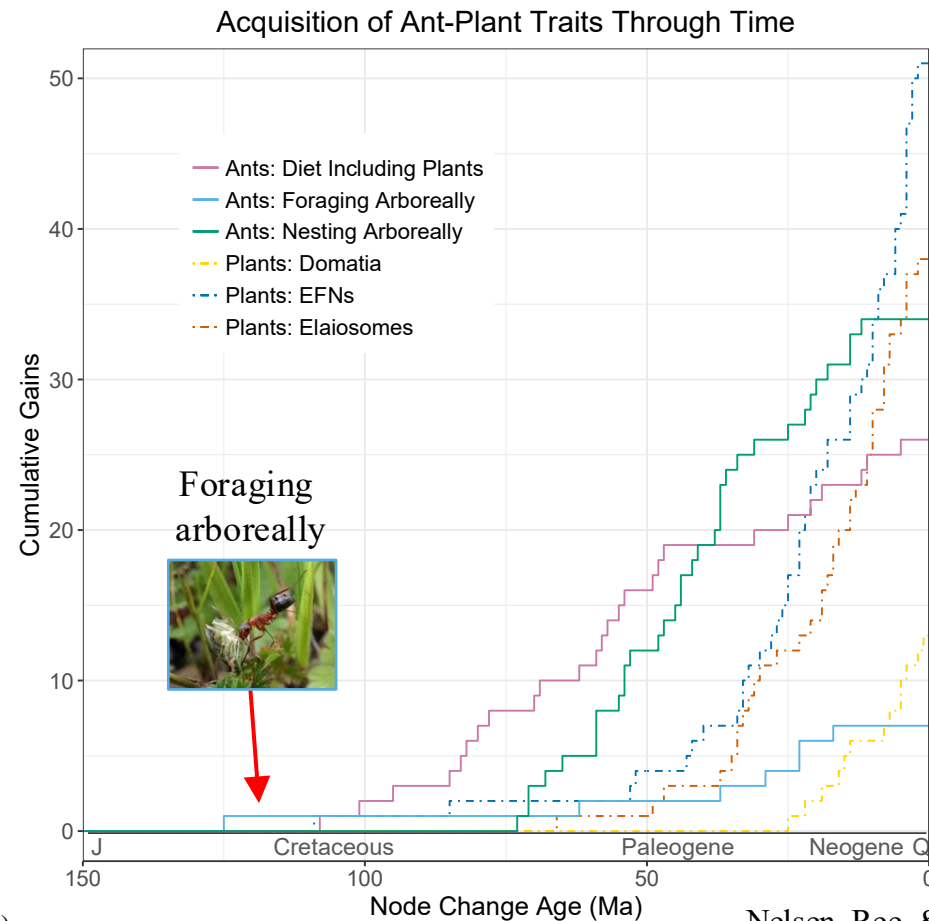
Matt Nelsen



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



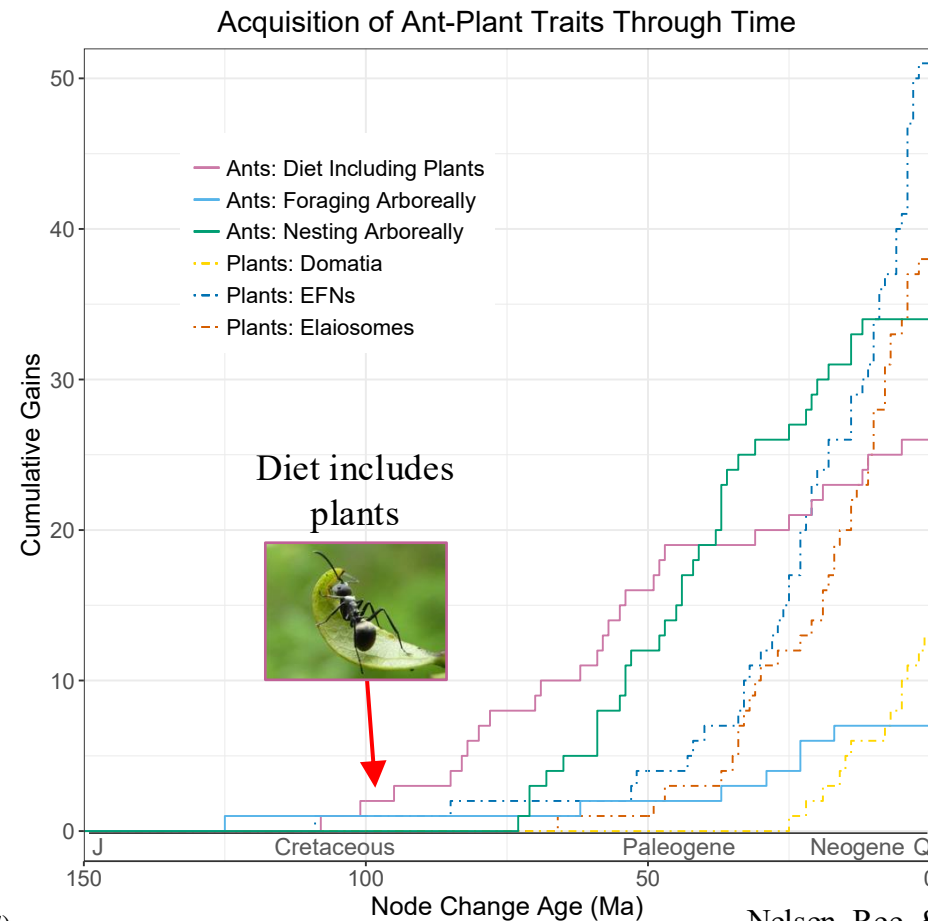
Matt Nelsen



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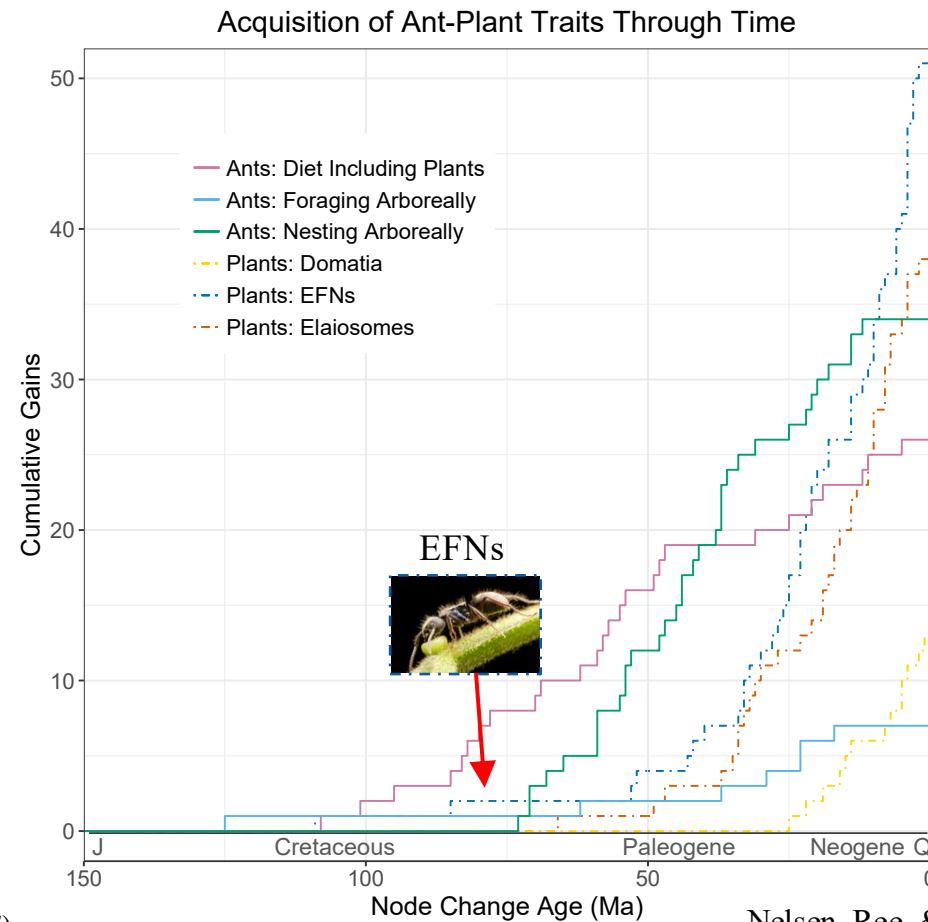
Matt Nelsen



Are plant and ant evolution linked?

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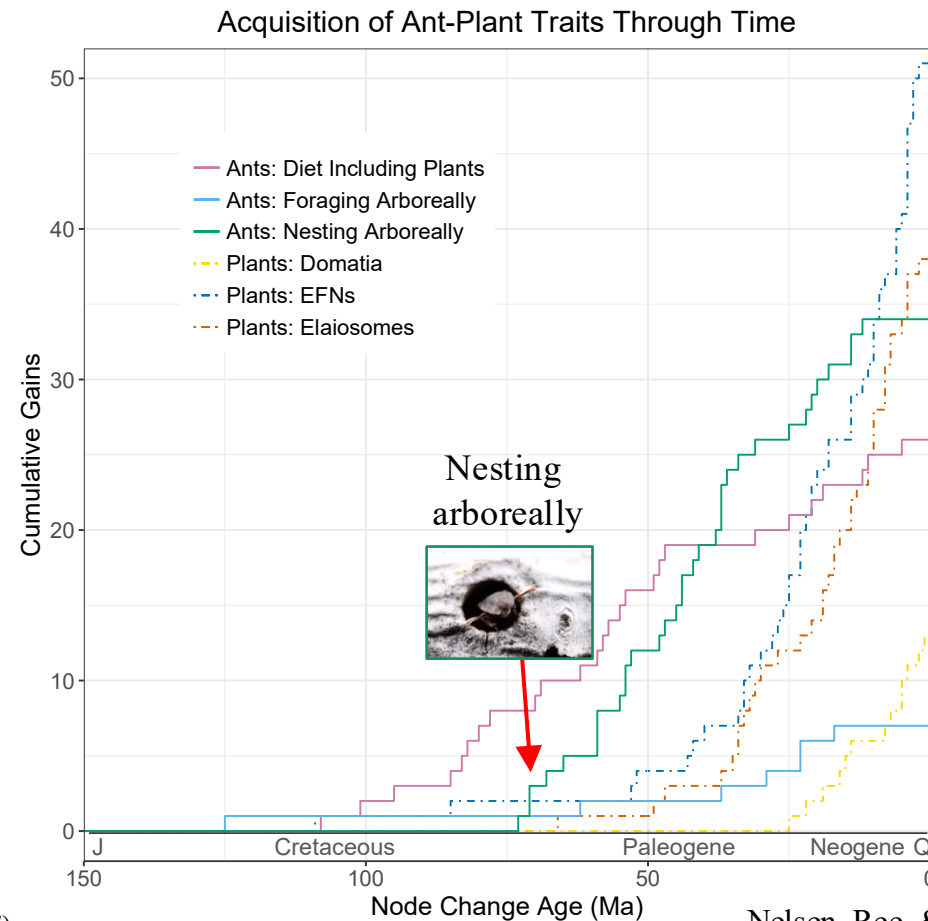
Matt Nelsen



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



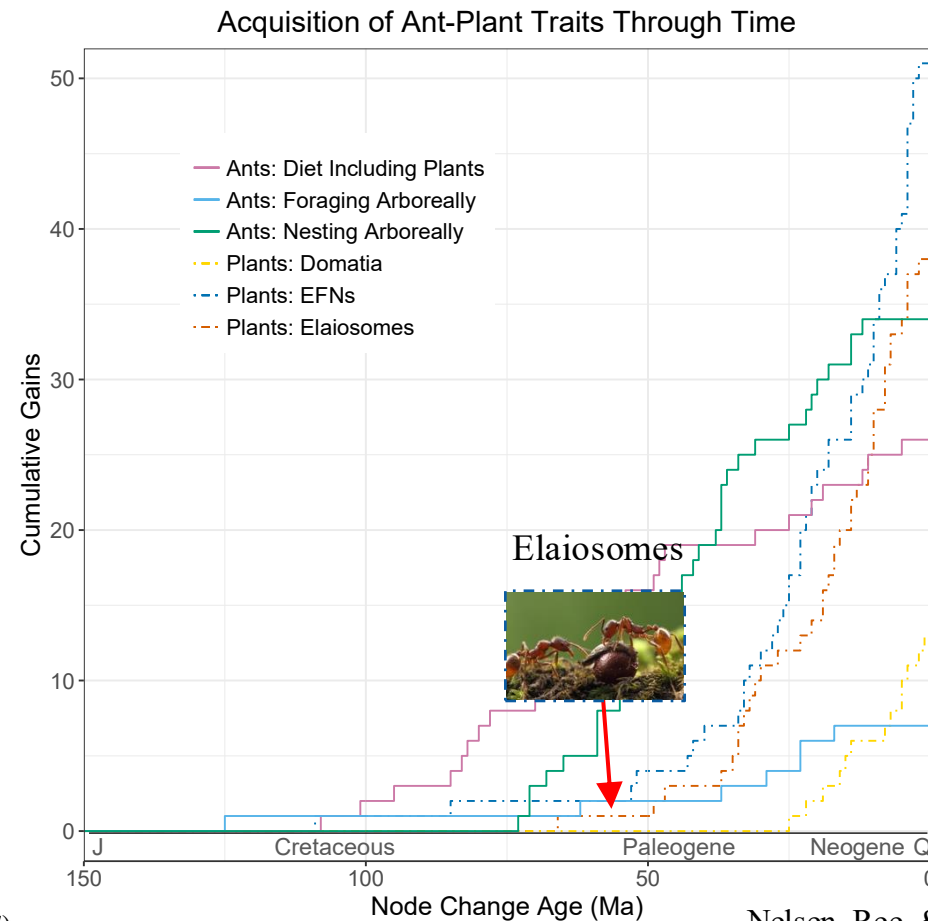
Matt Nelsen



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



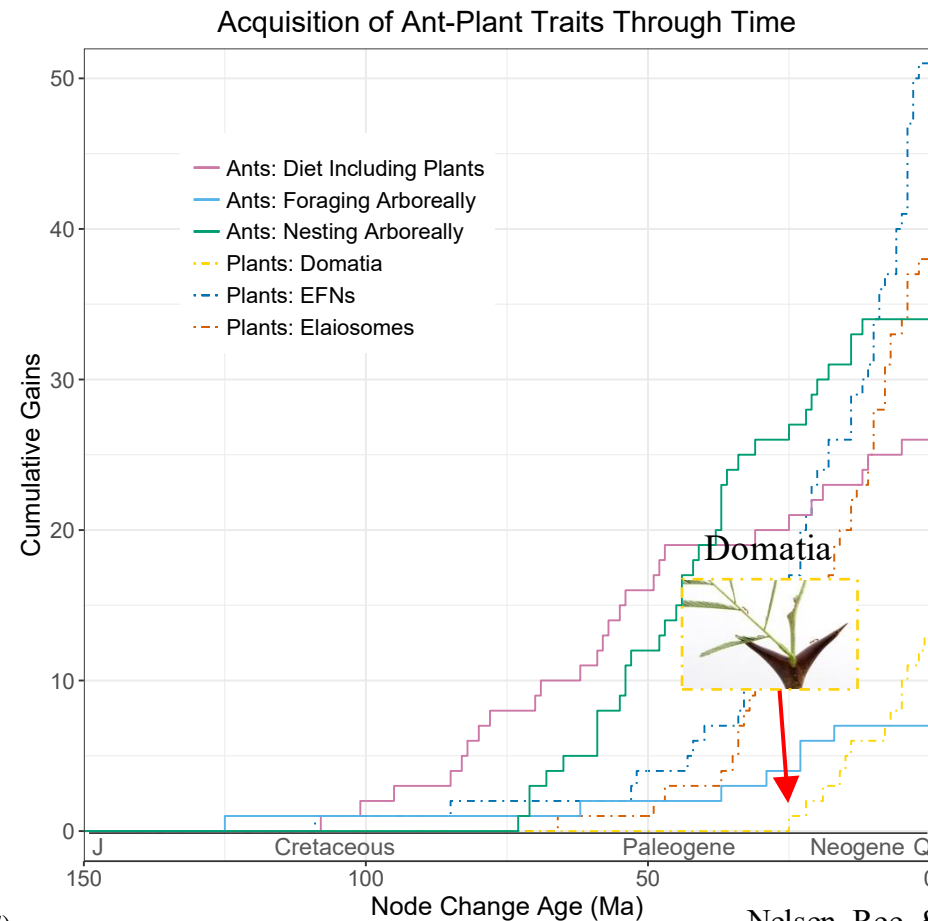
Matt Nelsen



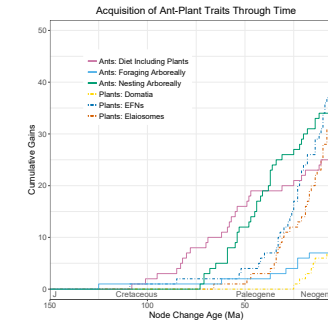
Are plant and ant evolution linked?

How did associations between ants and plants evolve?

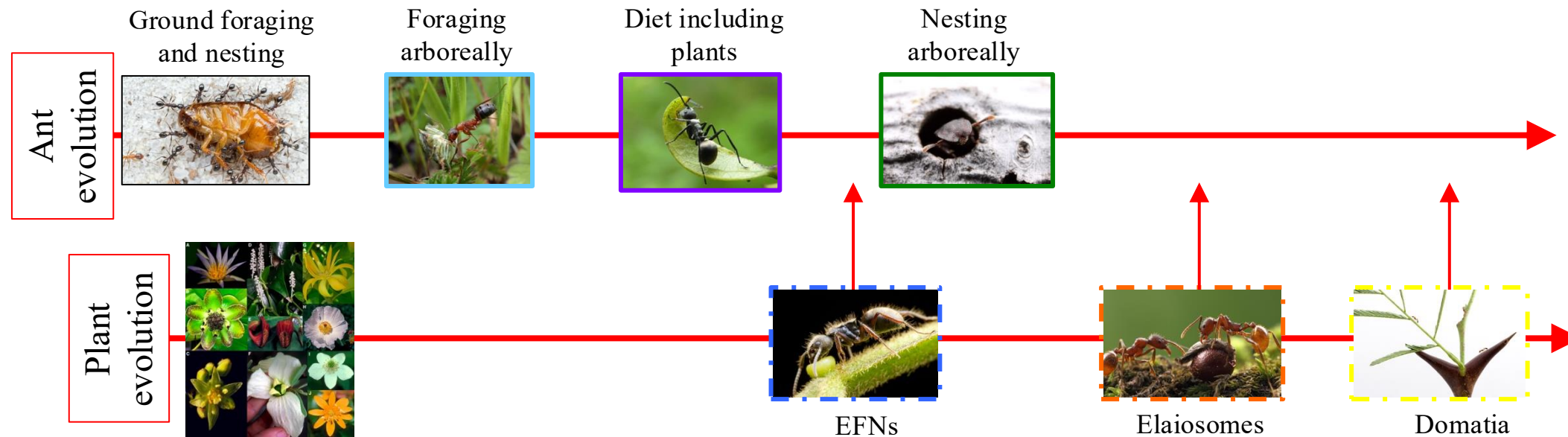
- Ages of ant-associated plant structures vs. ant utilization of plants for food, foraging, nesting



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



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



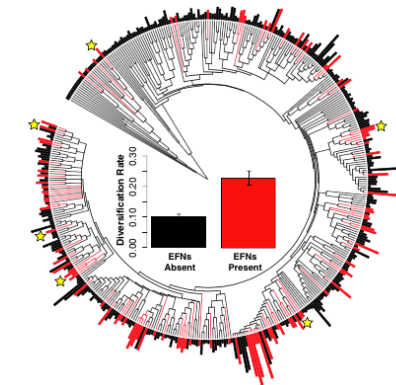
Plants that participate in ant-plant mutualisms (and other defensive mutualisms) have twofold higher rates of diversification



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

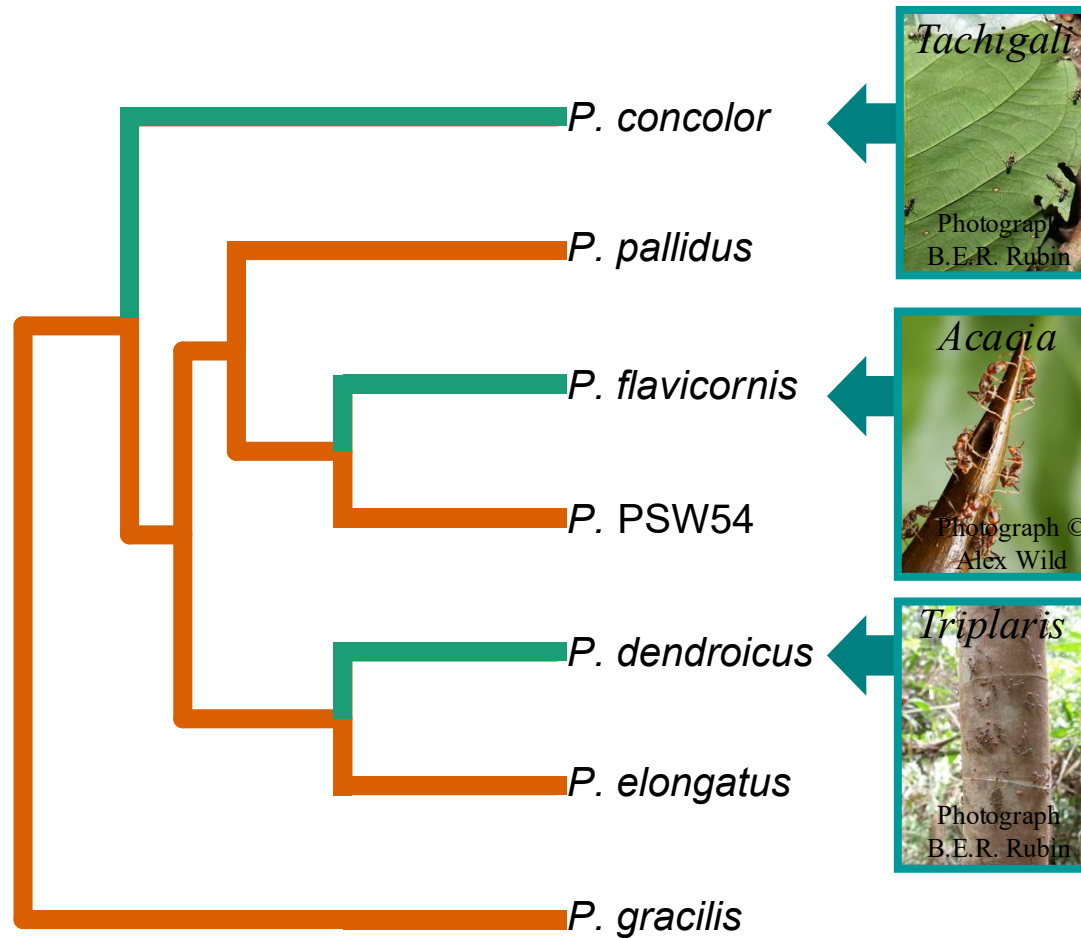
Non-aggressive defensive behavior

~260 species

Benjamin Rubin



Convergent evolution of mutualism



■ = mutualist ■ = generalist

Natural evolutionary experiment to investigate the role of mutualisms on genomes

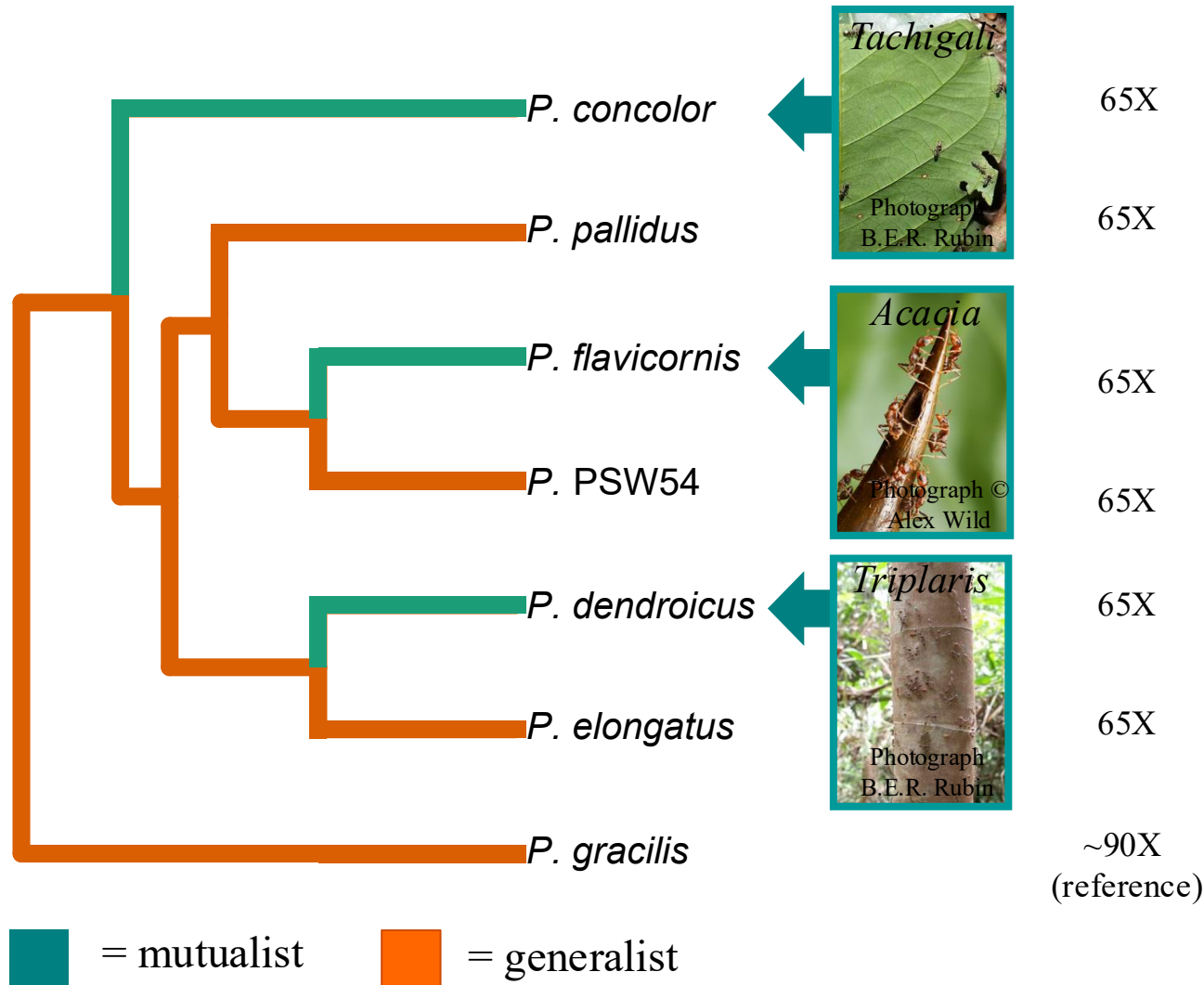
Although there are were 8 ant genomes sequenced the closest relative to *Pseudomyrmex* is very distantly related

Benjamin Rubin



Genome evolution of ant-plant mutualisms

Genome sequencing coverage completed



Summary of findings:

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

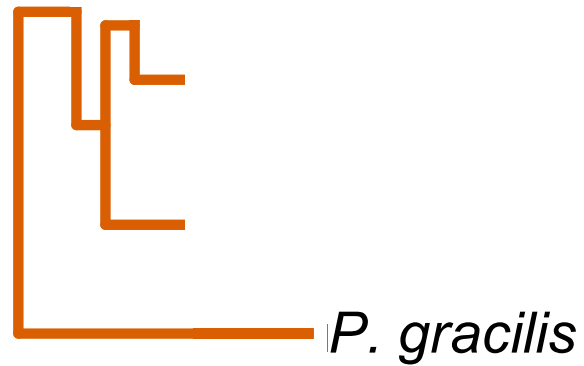
Reference genome: *P. gracilis*

- 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

Benjamin Rubin

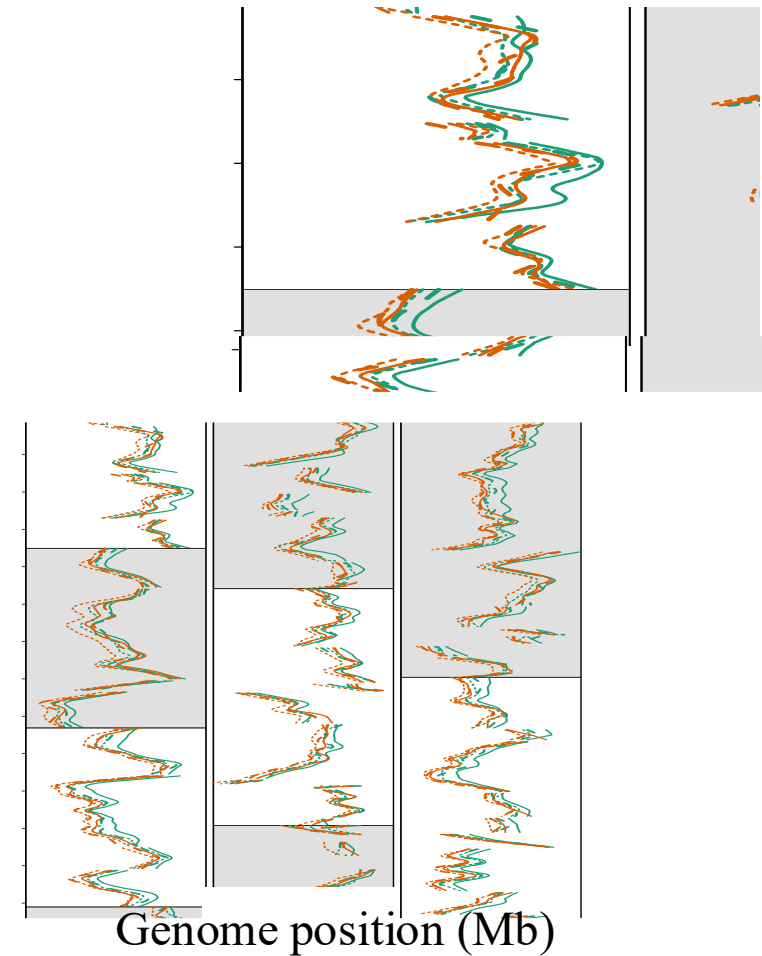


Genome evolution of ant-plant mutualisms



Genetic distance from *P. gracilis*

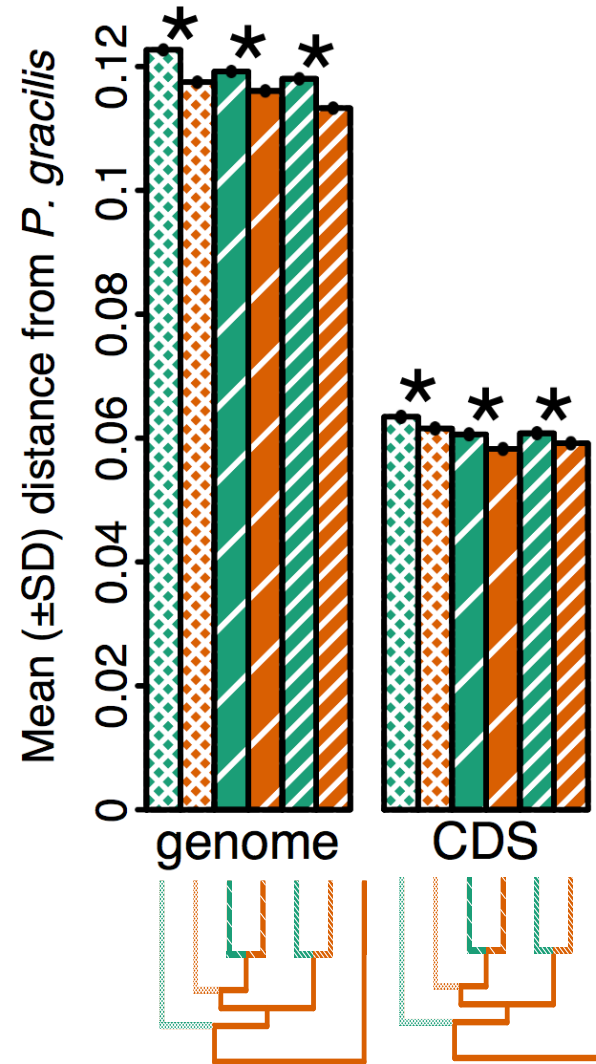
Genetic distance from *Pseudomyrmex gracilis*



Benjamin Rubin



Genome evolution of ant-plant mutualisms

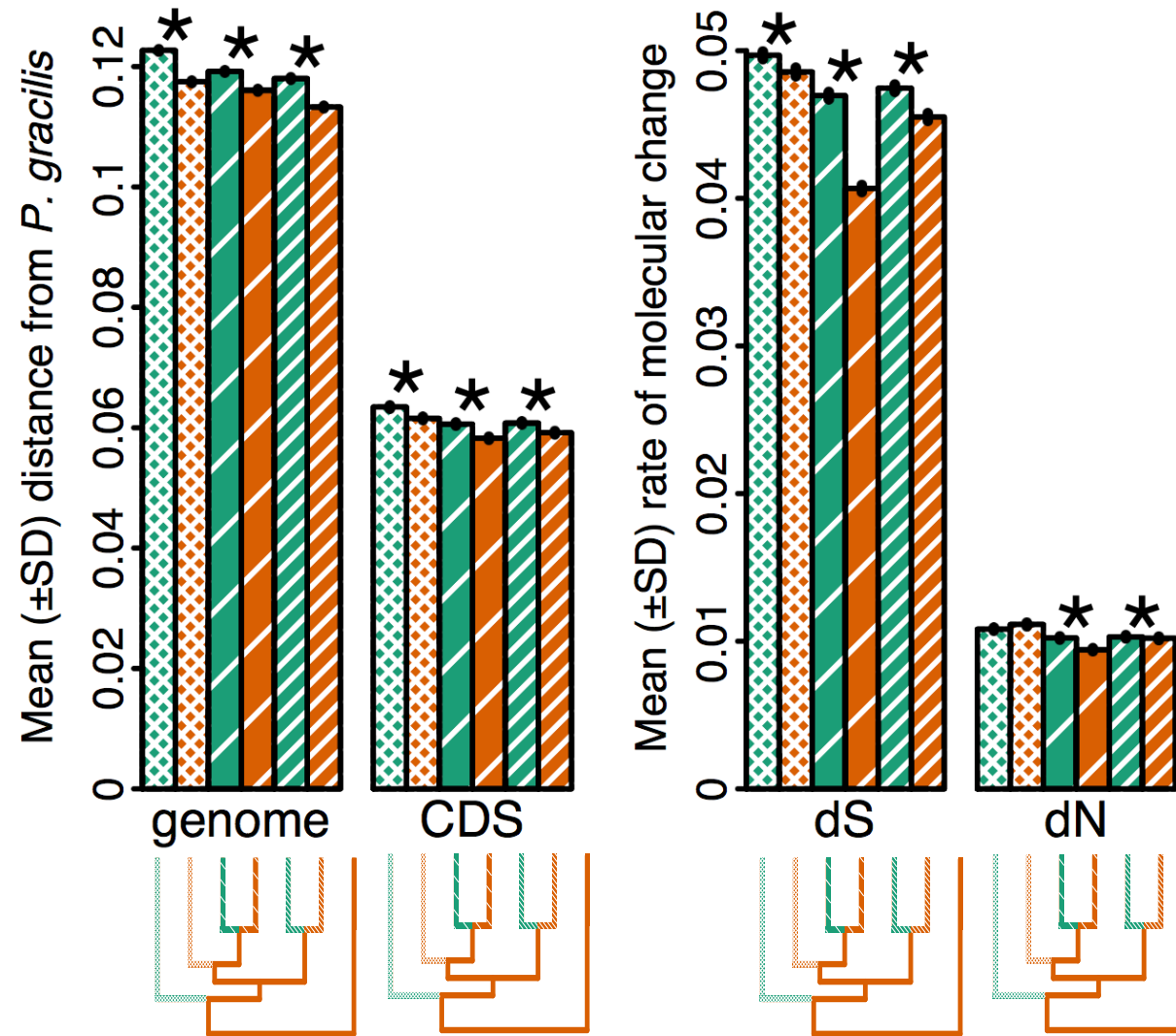


(Significance determined with bonferroni corrected paired t-tests of 25kb sliding windows.)

Benjamin Rubin



Genome evolution of ant-plant mutualisms



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

Benjamin Rubin



Difference in rates not due to differences in generation time

1. Generation time

Prediction: Mutualists have consistently shorter generation times



Benjamin Rubin



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)



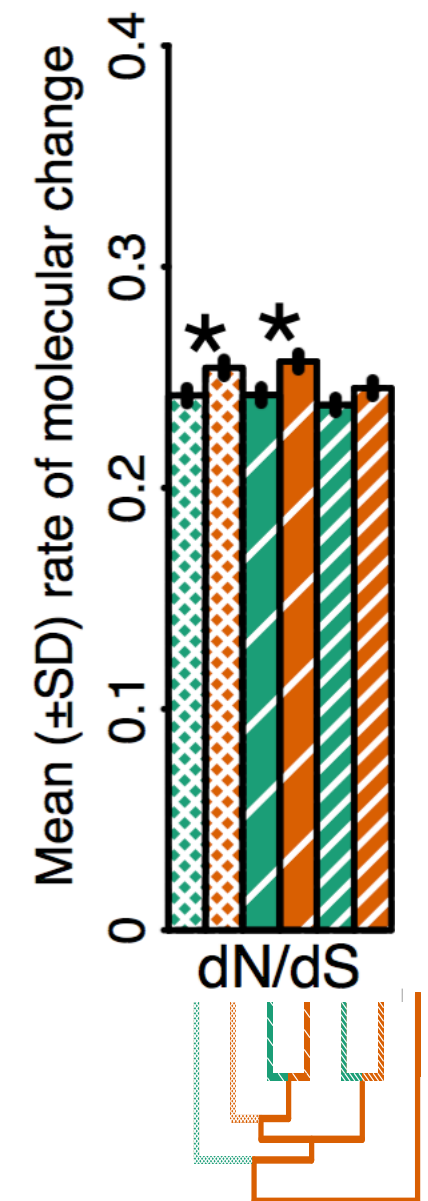
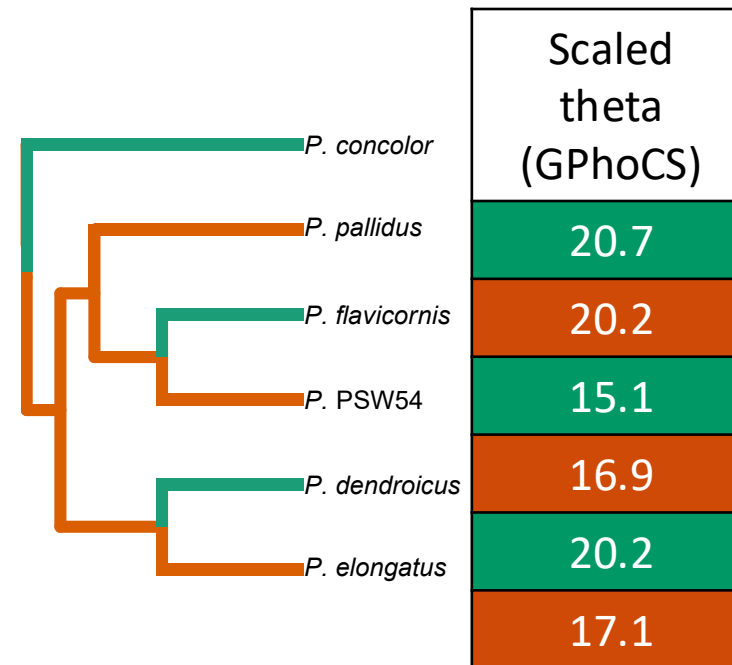
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Difference in rates not due to differences in population size

- 1. ~~Generation time~~
- 2. Population size

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



Benjamin Rubin

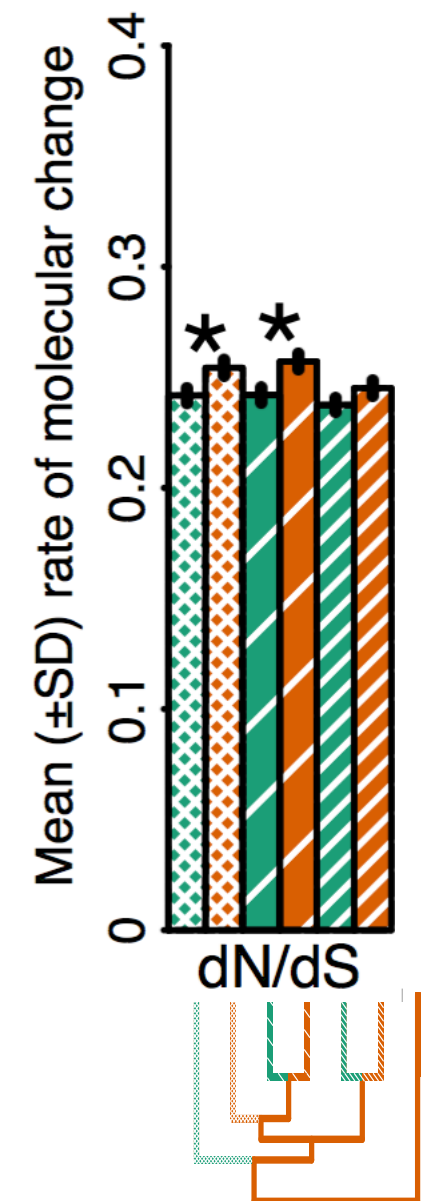
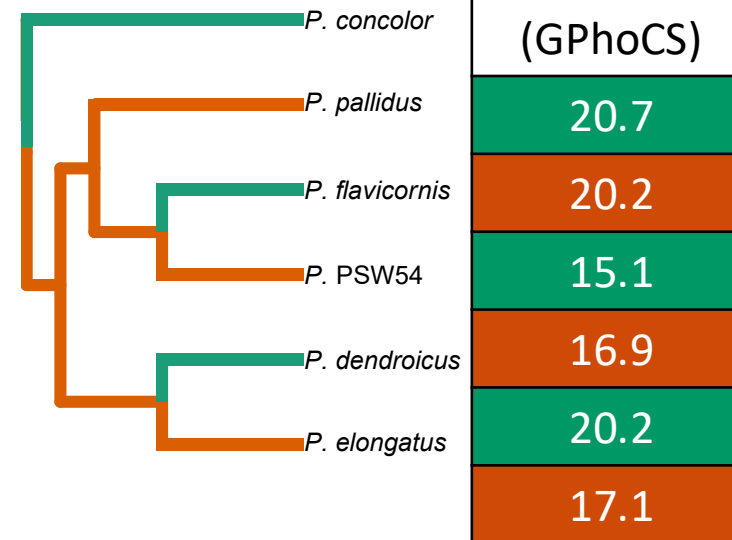


Difference in rates not due to differences in population size

- 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



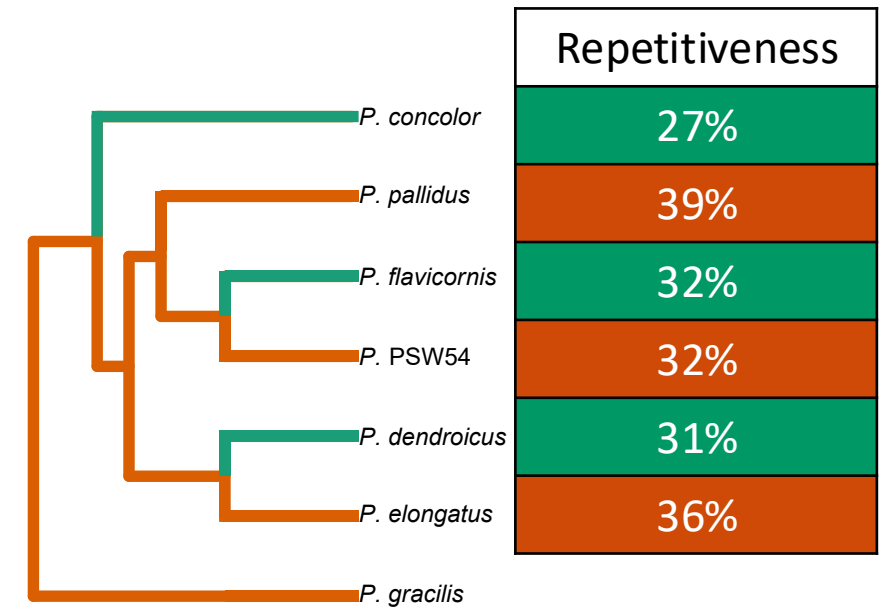
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Difference in rates may be due to relaxed selection

1. ~~Generation time~~
2. ~~Population size~~
3. Relaxed selection

Prediction: Transposable elements increase in frequency in mutualists



Benjamin Rubin

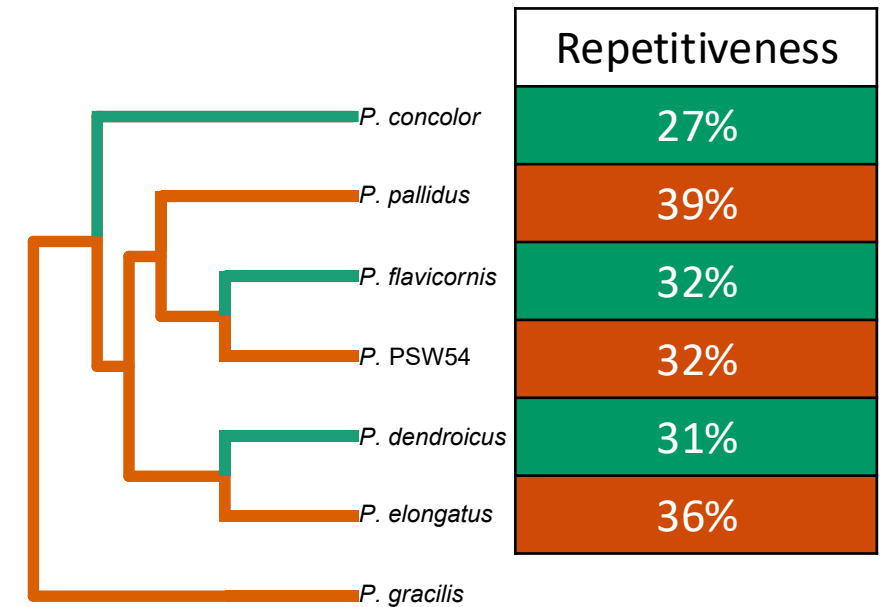


Difference in rates may be due to relaxed selection

1. ~~Generation time~~
2. ~~Population size~~
3. Relaxed selection

Prediction: Transposable elements increase in frequency in mutualists

Result: Repeat content not consistently different



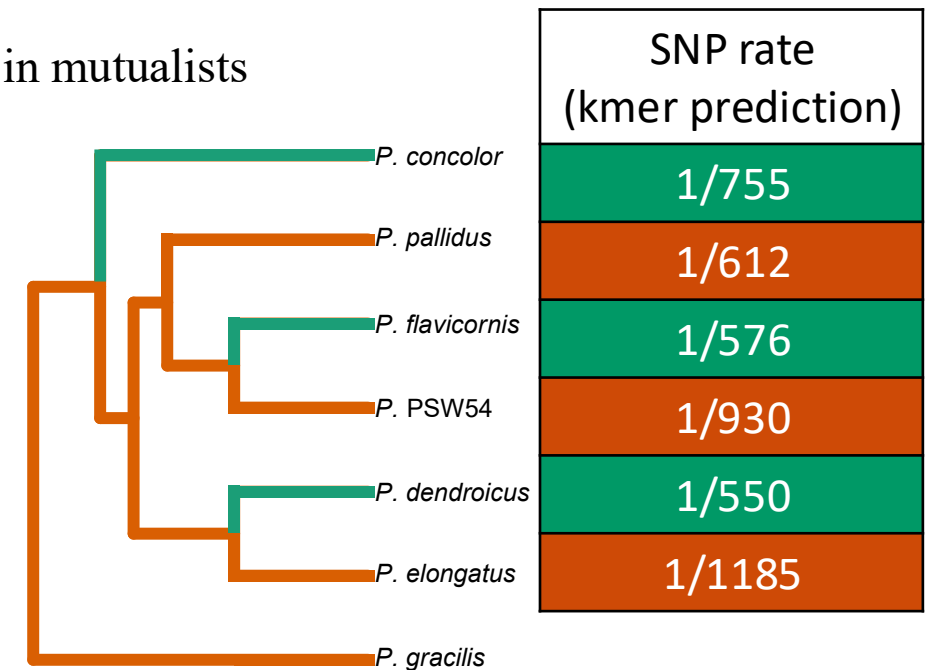
Benjamin Rubin



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



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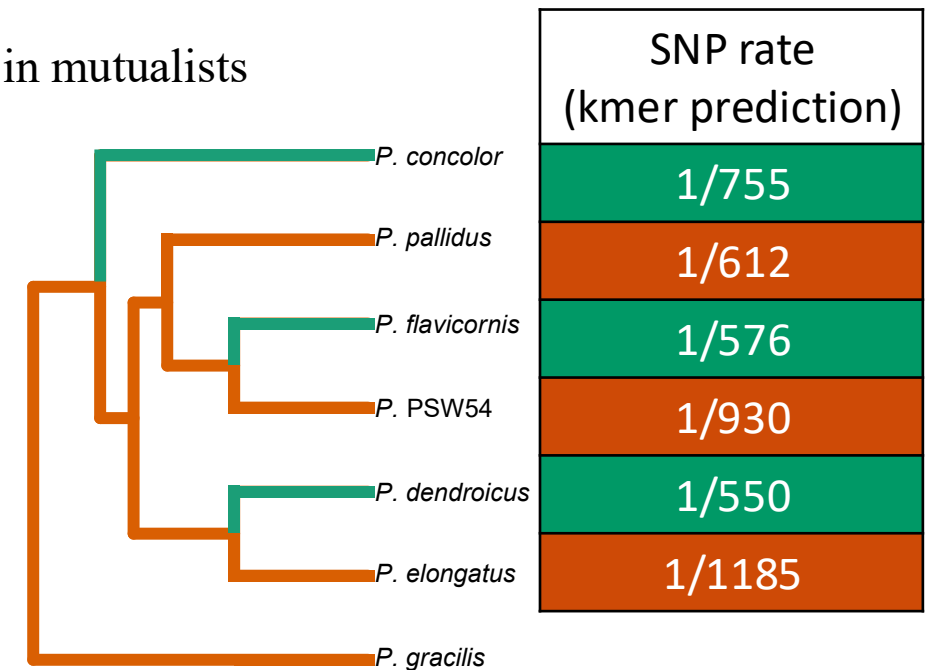


Difference in rates may be due to higher mutation rates

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



Benjamin Rubin

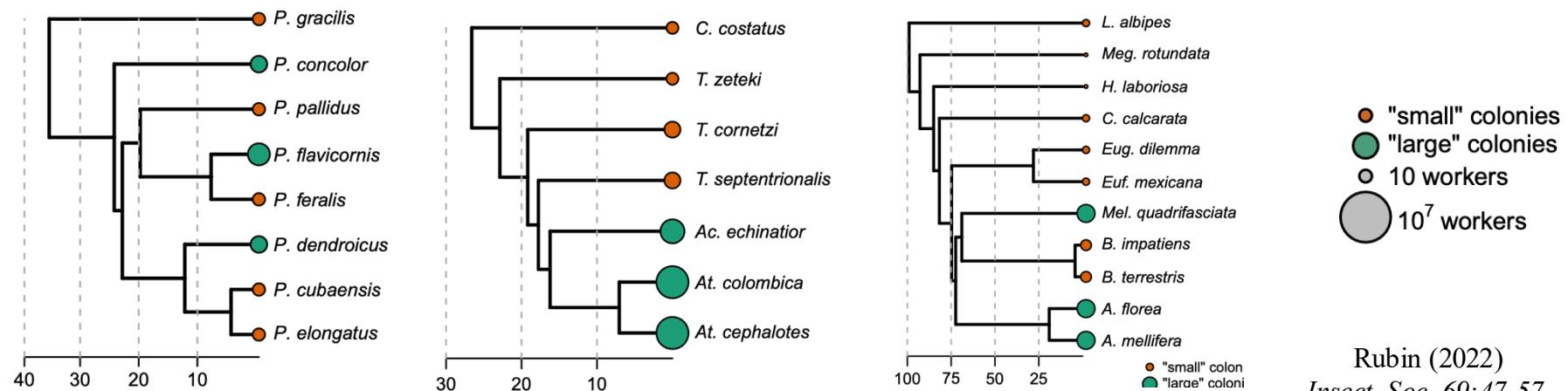


Difference in rates may be due to higher mutation rates

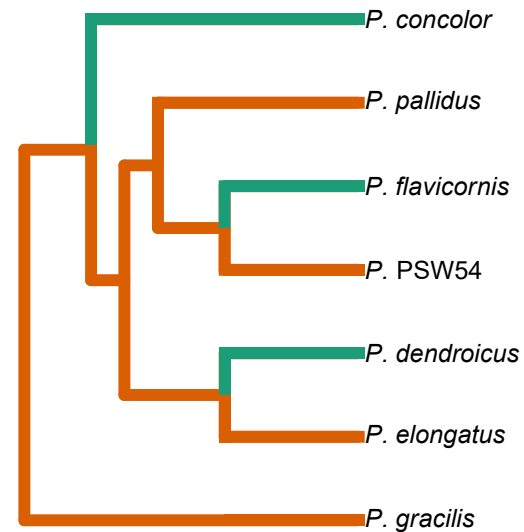
1. ~~Generation time~~
2. ~~Population size~~
3. Relaxed selection?
4. Elevated mutation rate?
5. Colony size (comparing whole genomes across multiple ant and bee lineages)

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



Convergent genome evolution due to life history

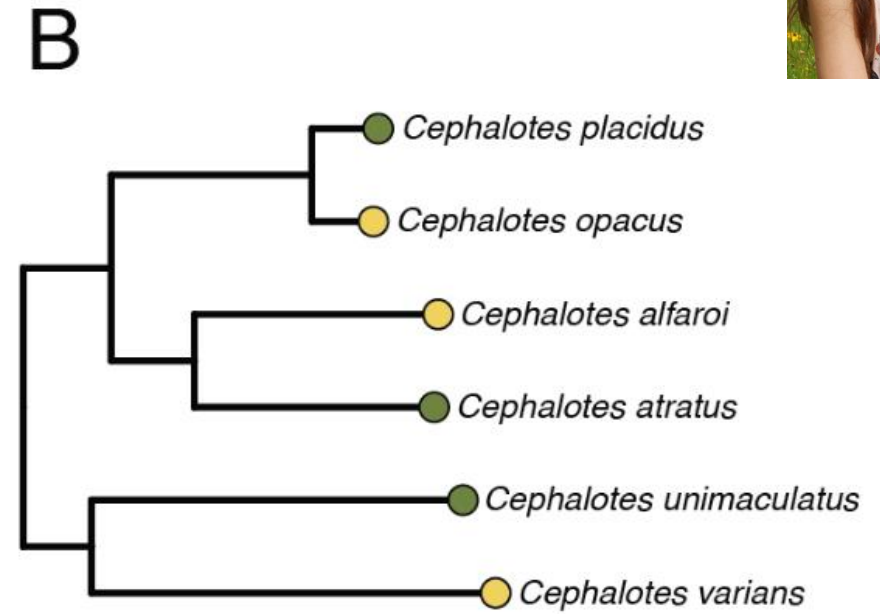
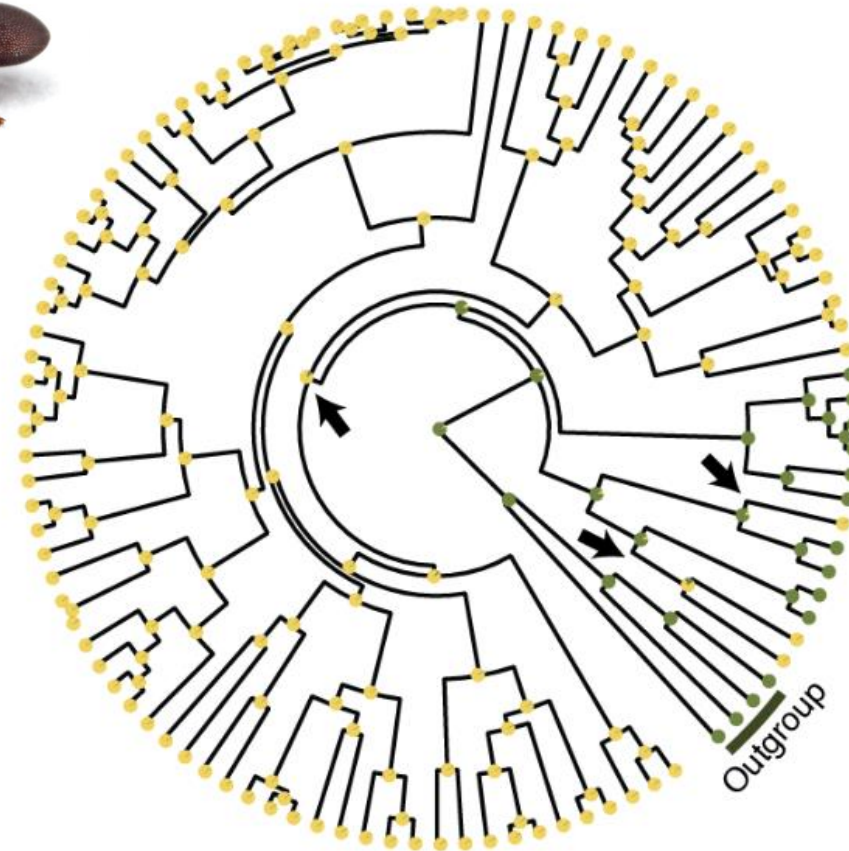


There are many factors that can impact genome evolution in social organisms and we need more comparative studies to understand how genomes shape sociality, but also how sociality shapes genomes evolution.

How does phenotypic plasticity evolve in soldier ants?

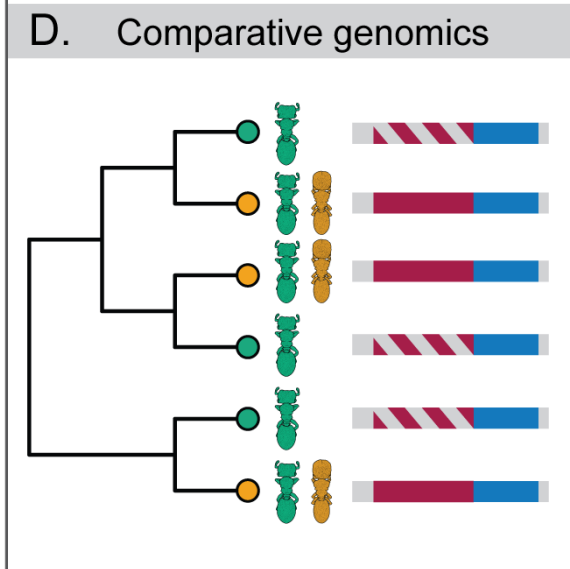
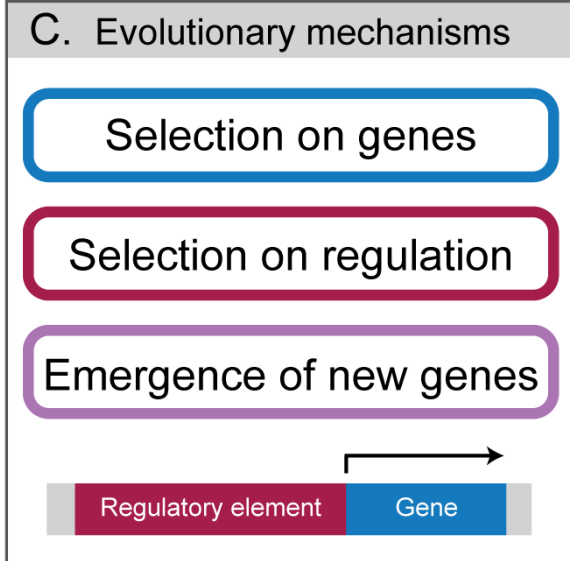
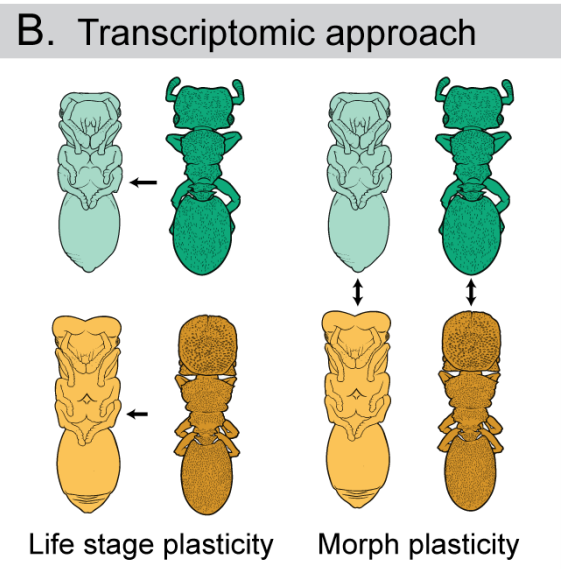
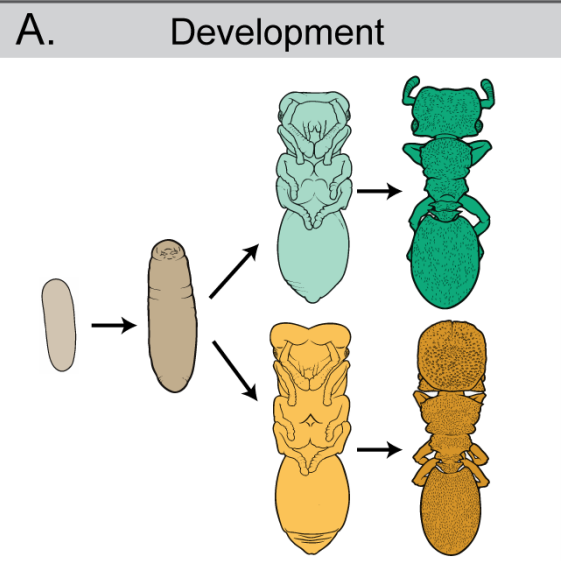
Turtle ants have evolved soldiers multiple times

Megan Barkdull



Evolutionary gain of a soldier ↑ Does the species produce a soldier? ● Yes ● No

How do you make distinct morphs from the same genome?



Megan Barkdull

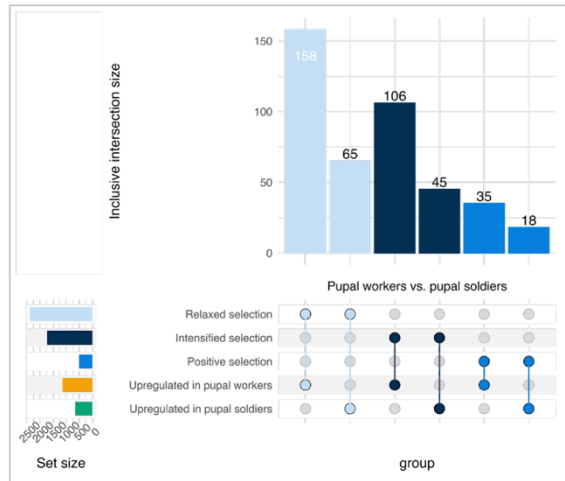


Gene regulation and not existing genes or new genes lead to the evolution turtle ant soldiers

Megan Barkdull



Differential expressed genes are associated with the presence of a soldier morph



Selection on genes

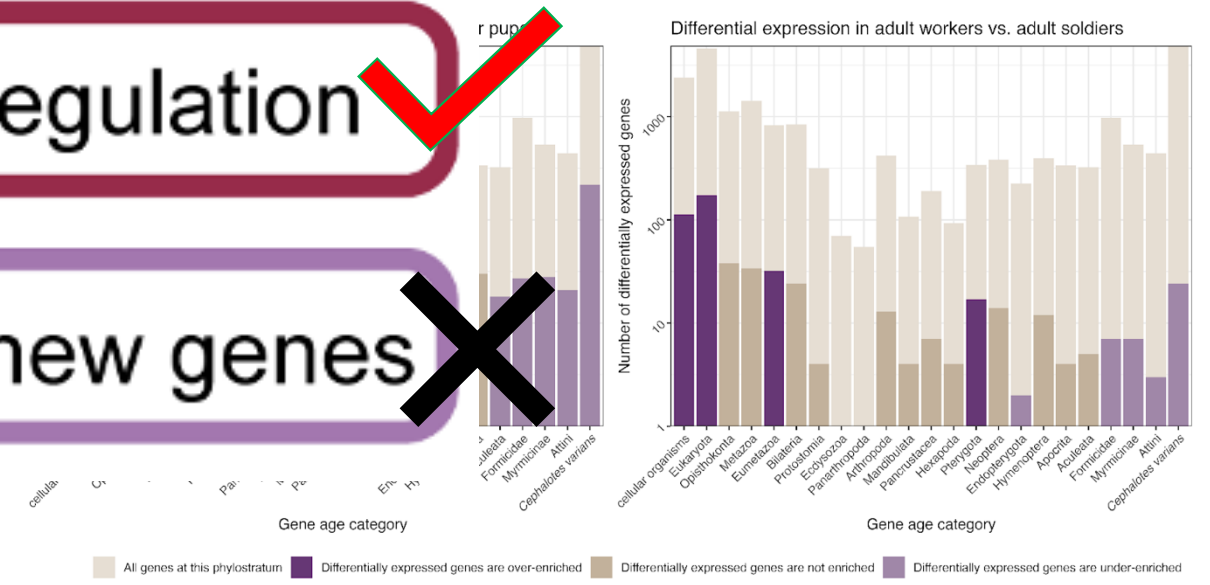


Genes in morph differentiation are disproportionately ancient

Selection on regulation

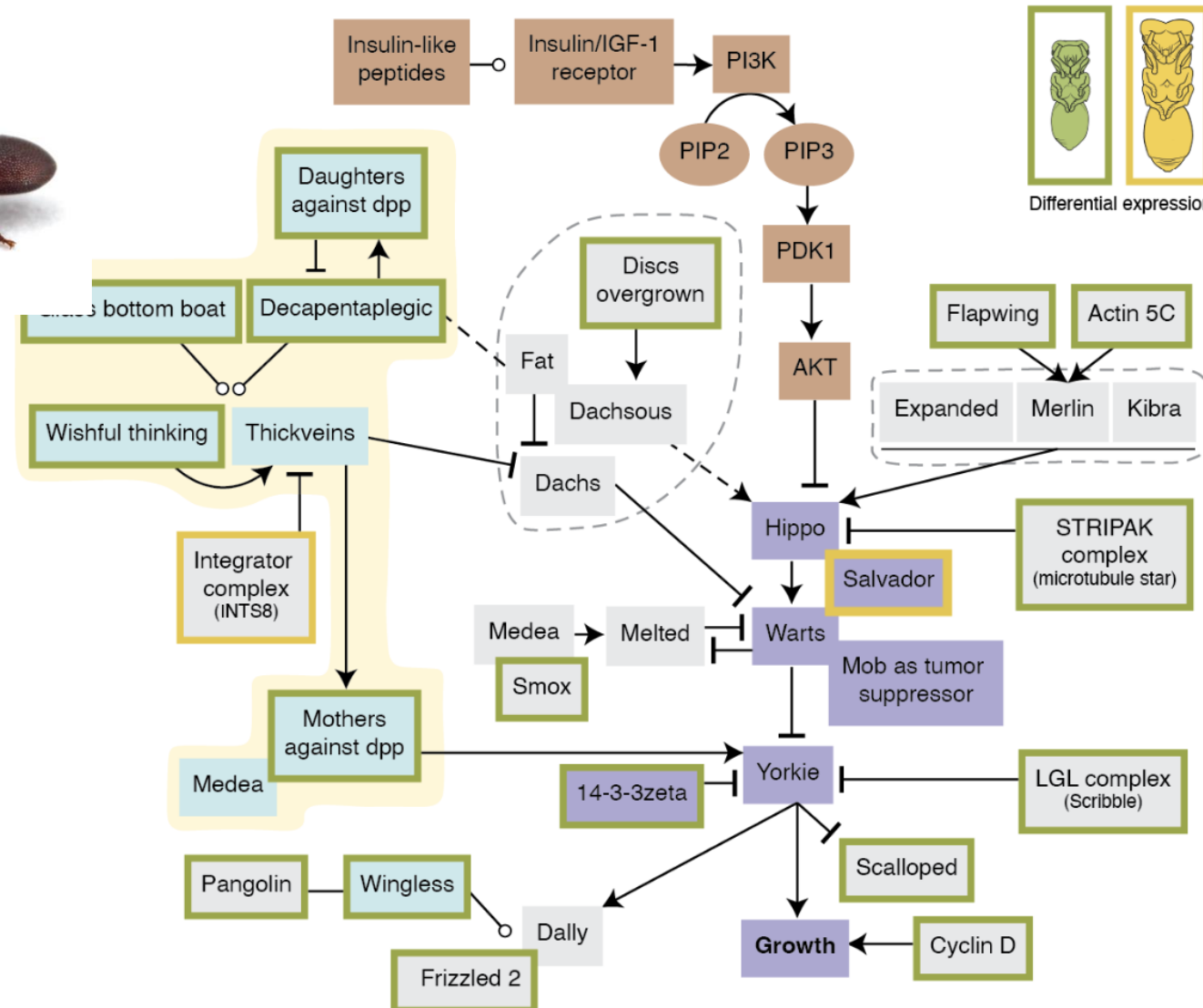


Emergence of new genes



Novel signaling pathways underlie the evolution of soldiers in turtle ants

Megan Barkdull



Symbiotic bacteria across the ants and the
evolution of herbivory




A background image of several ants, including a large brown ant in the top left and a cluster of smaller brown and white ants in the bottom right.

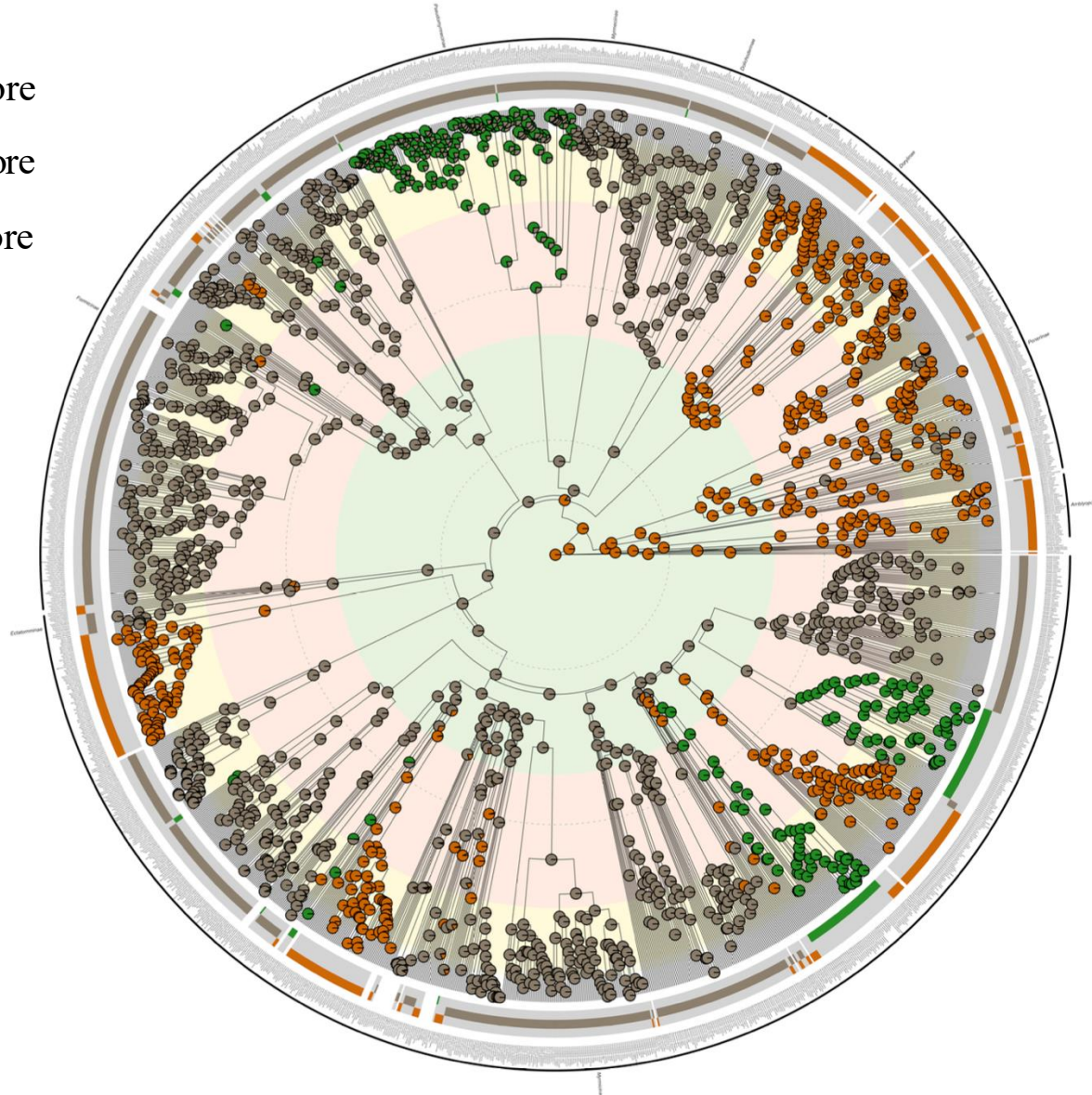
Why gut microbes of ants?

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

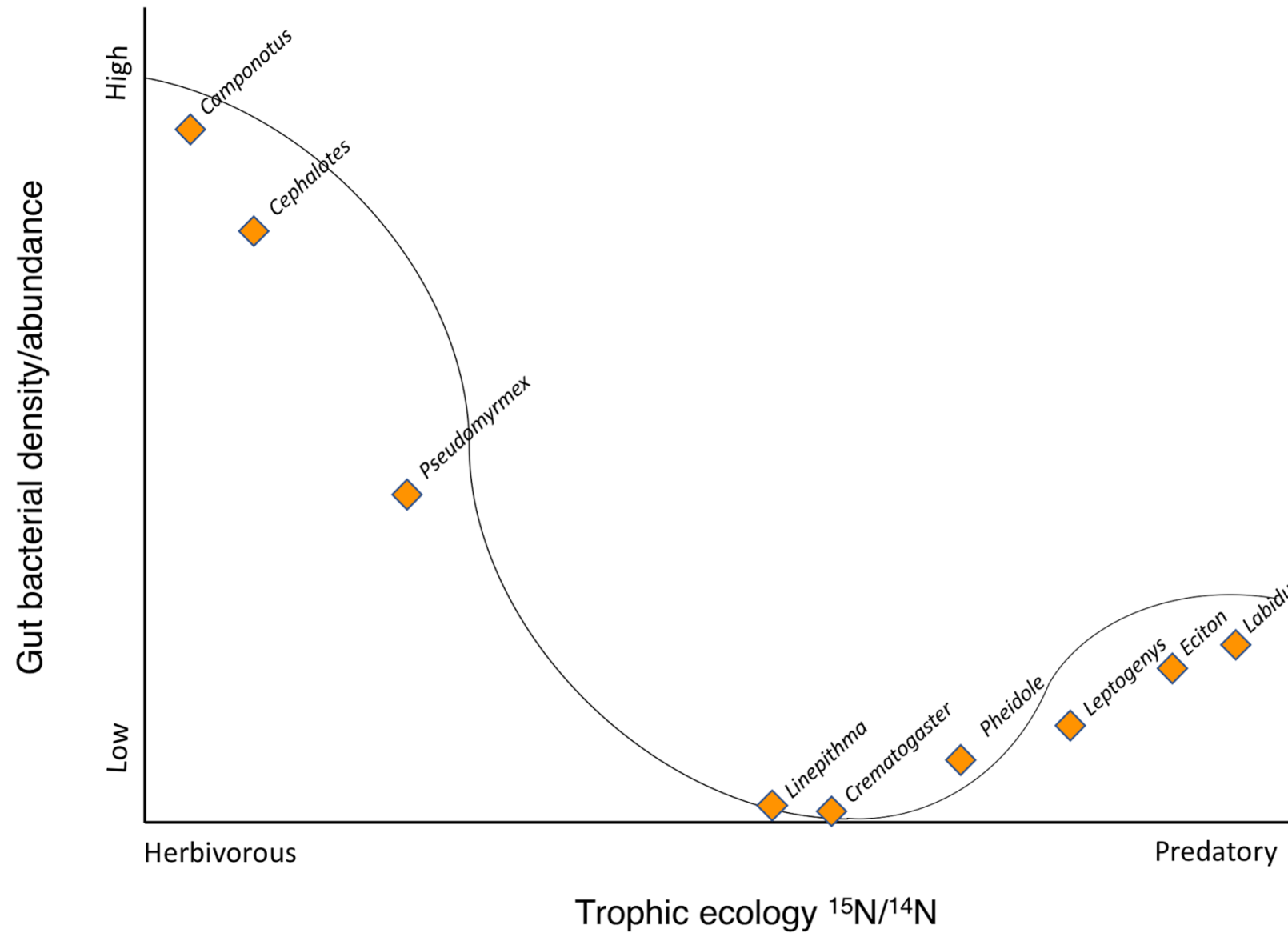
- **Diet & Nutrition:** How does diet influences associations?
- **Transmission:** How are microbes transmitted among individuals and through time?
- **Host-symbiont coevolution:** What is the role of shared evolutionary history and/or convergence?
- **Geography:** Are microbes acquired from the environment?

Herbivory has evolved at least 11 times independently across the ants

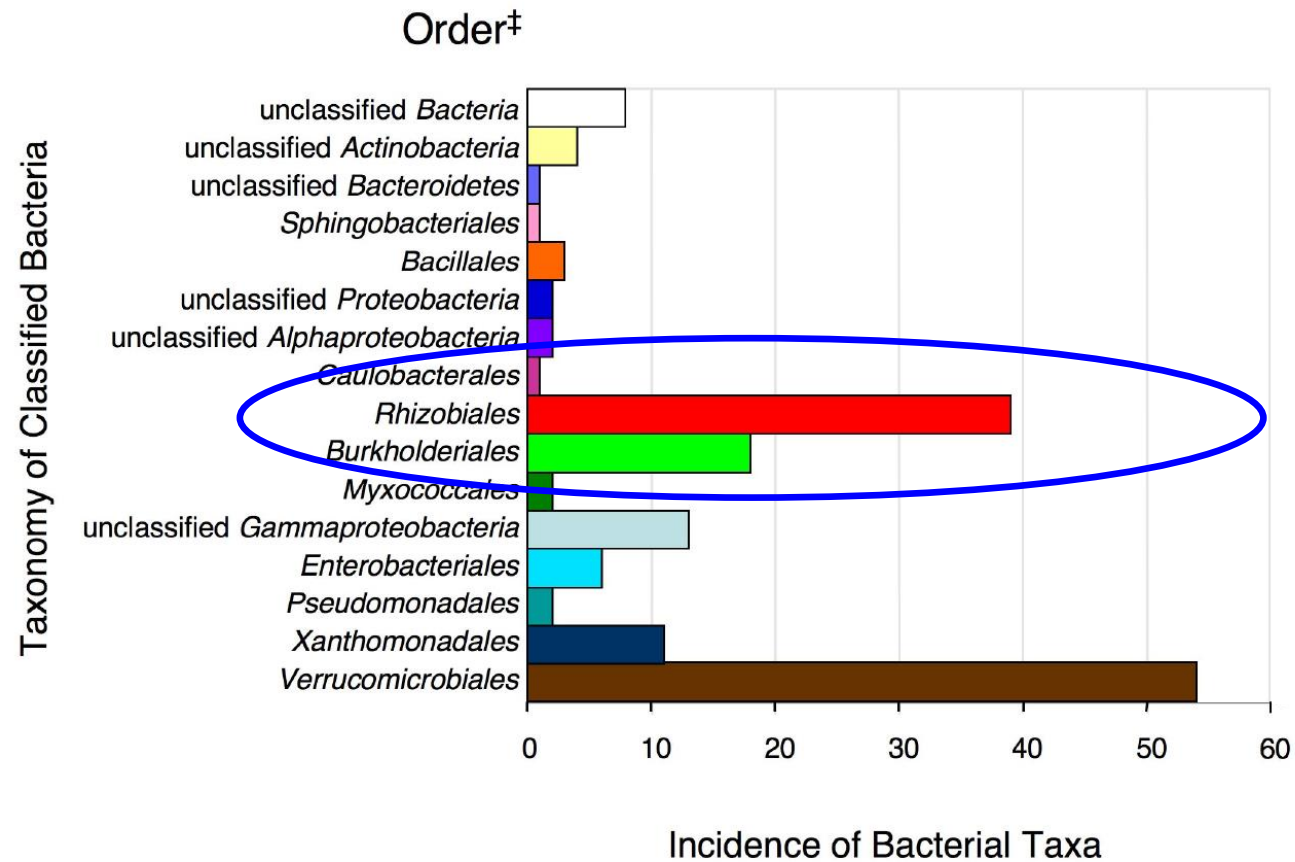
-  = Herbivore
-  = Omnivore
-  = Carnivore



Gut bacteria are associated with ants with specialized diets



Ants have diverse bacterial communities

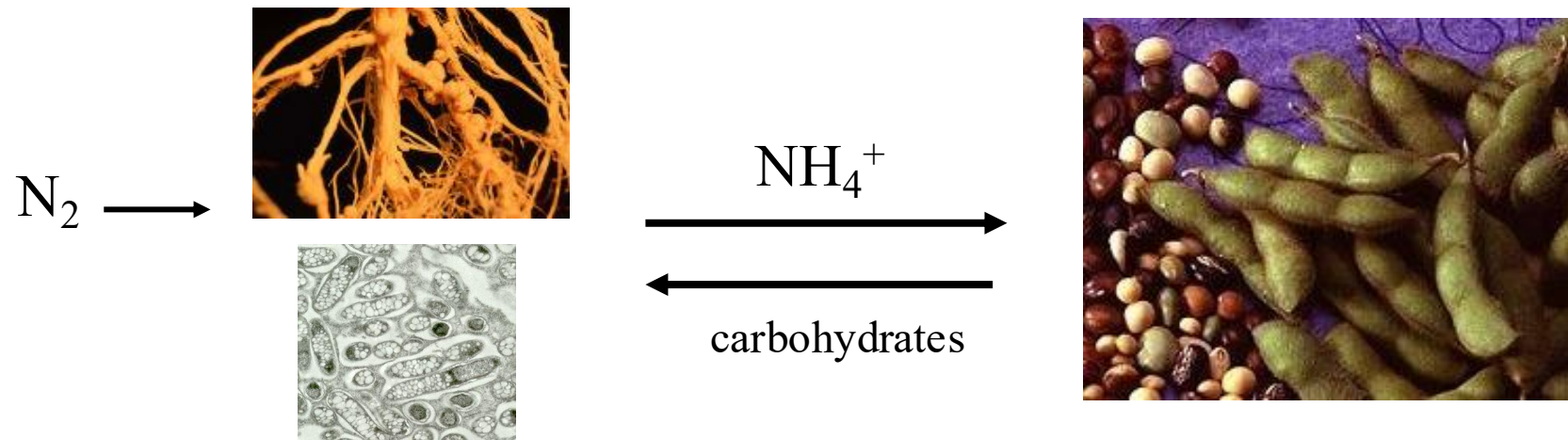


Results of using general and targeted bacterial sequencing across 398 individual ants from 148 genera.

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?



Images from <http://MicrobeWiki.kenyon.edu>

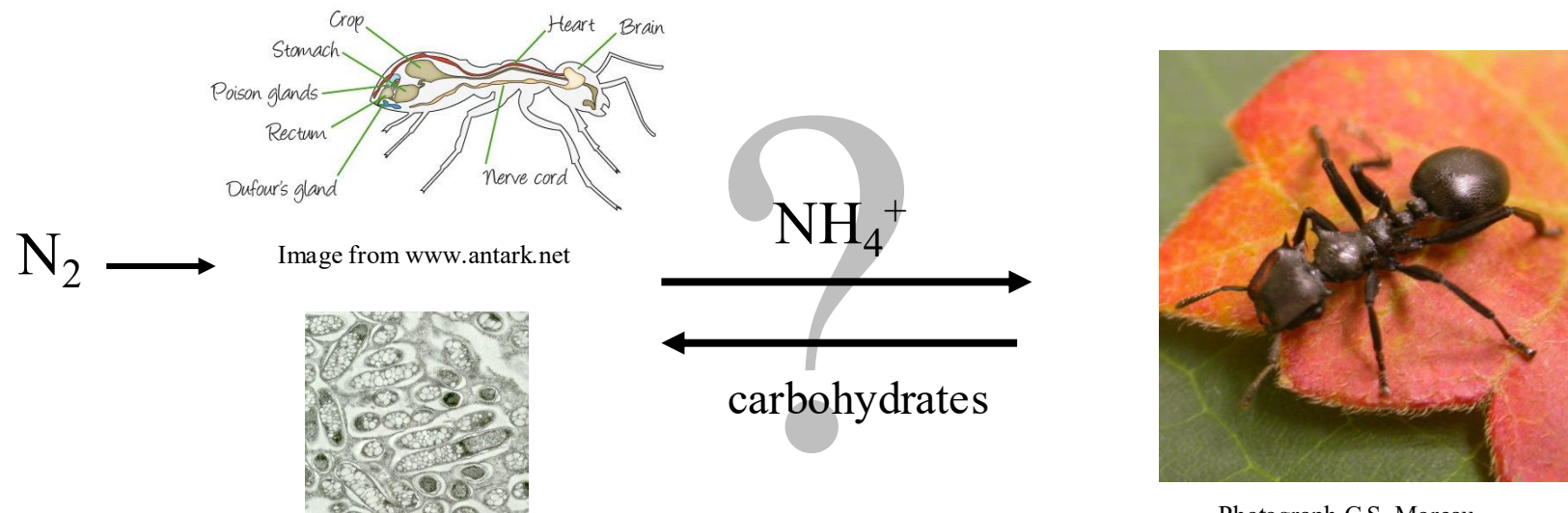
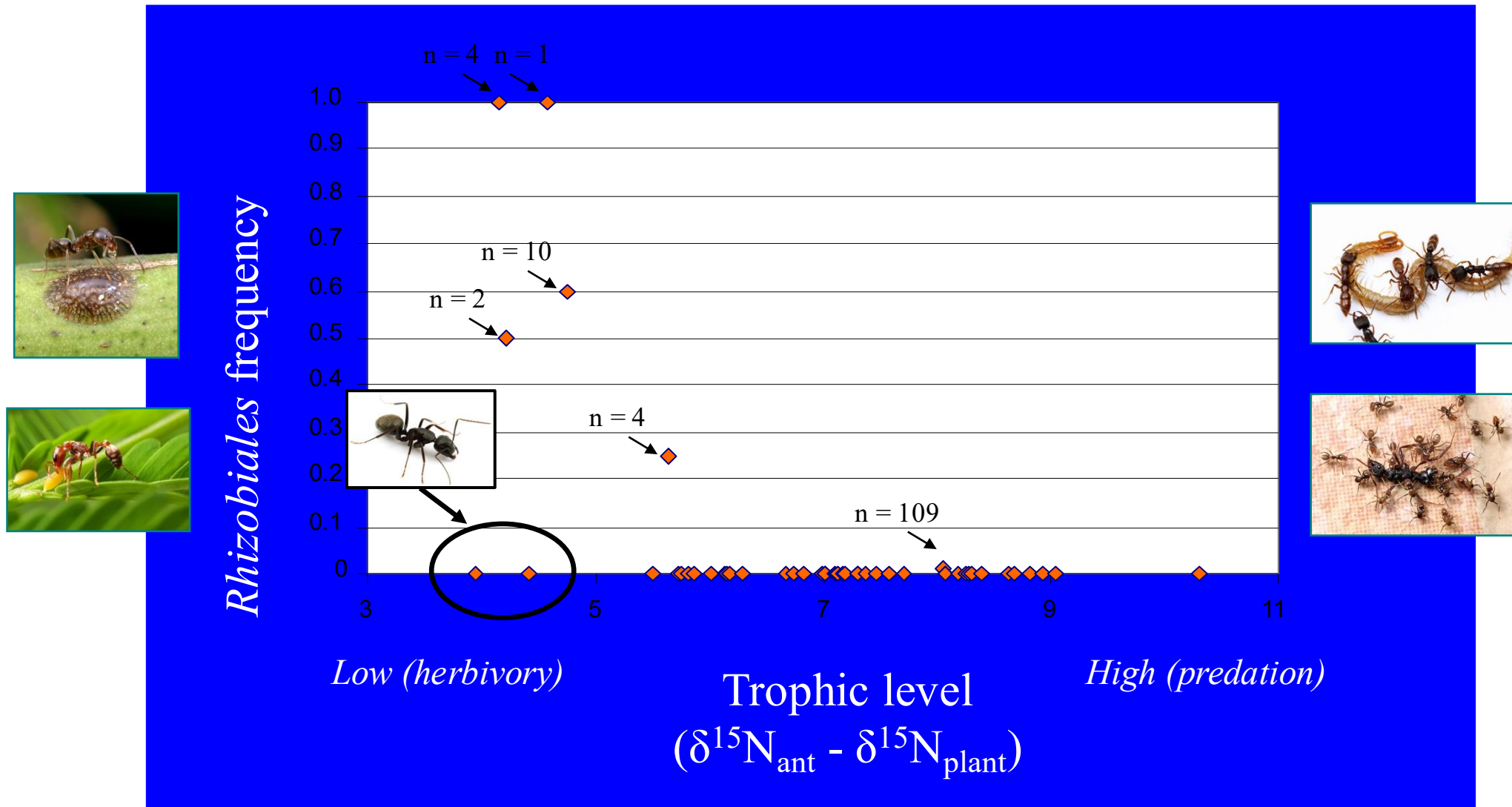


Image from <http://MicrobeWiki.kenyon.edu>

Photograph C.S. Moreau

Rhizobiales bacteria are common at low trophic levels

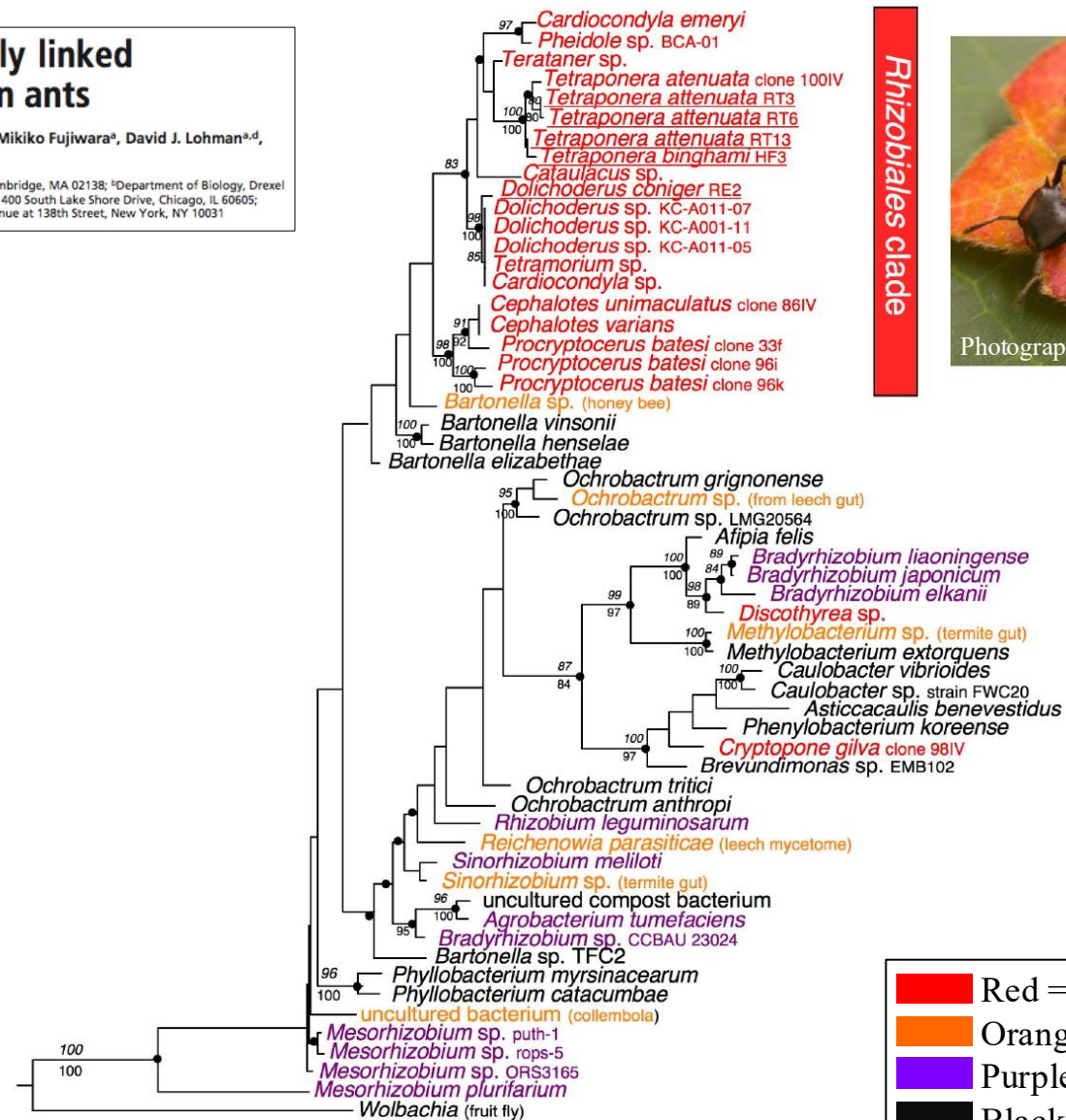


Bacteria phylogeny:
16S rRNA phylogeny of ant-associated *Rhizobiales* bacteria and their GenBank relatives

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
^aDepartment of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138; ^bDepartment of Biology, Drexel University, Philadelphia, PA 19104; ^cDepartment of Zoology, Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605; and ^dDepartment of Biology, The City College of The City University of New York, Convent Avenue at 138th Street, New York, NY 10031



Rhizobiales clade



Red = ant associates
 Orange = animal associates
 Purple = plant associates
 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	<i>Rhizobiales</i>	$\delta^{15}N_{ant} - \delta^{15}N_{plant}$	Ant subfamily
Platythyrea	0/2	4.314	Ponerinae
Pachycondyla	0/4	4.619	
Diacamma	0/3	5.330	
Odontoponera	0/3	5.922	
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	
Technomyrmex	0/4	3.687	
Dolichoderus	1/10	2.005	Dolichoderinae
Azteca	0/1	3.118	
Anonychomyrma	0/1	3.328	
Linepithema	0/1	5.402	
Leptomyrme	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	1/4	1.154	
Apterostigma	0/1	5.649	
Strumigenys	0/1	5.033	
Cyphomyrmex	0/1	3.997	
Atta	0/1	2.873	
Cataulacus	1/4	2.316	Myrmicinae
Crematogaster	0/4	3.154	
Tetramorium	1/4	3.196	
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	



Photograph C.S. Moreau

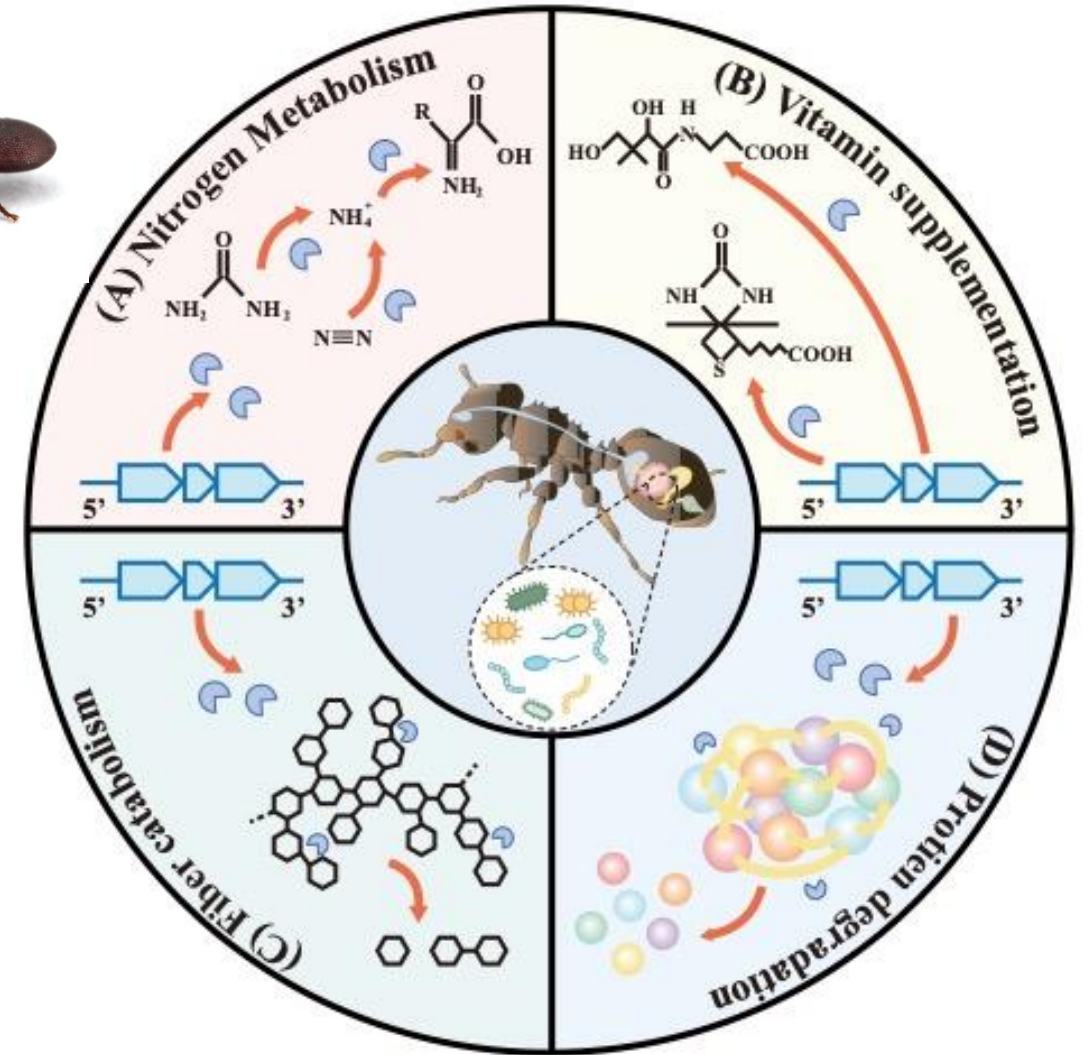
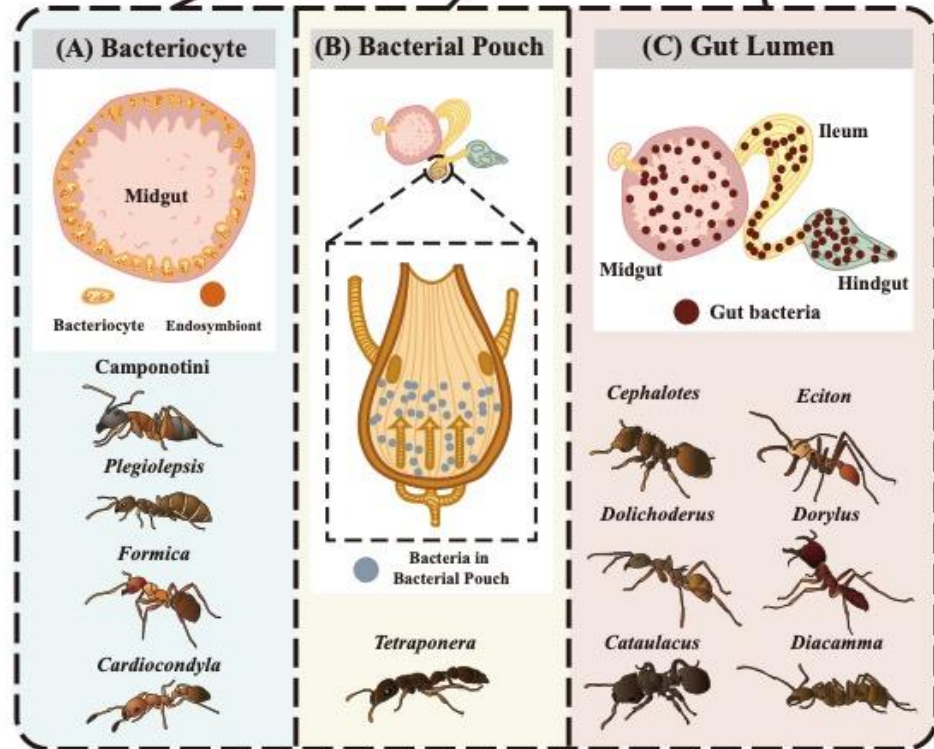
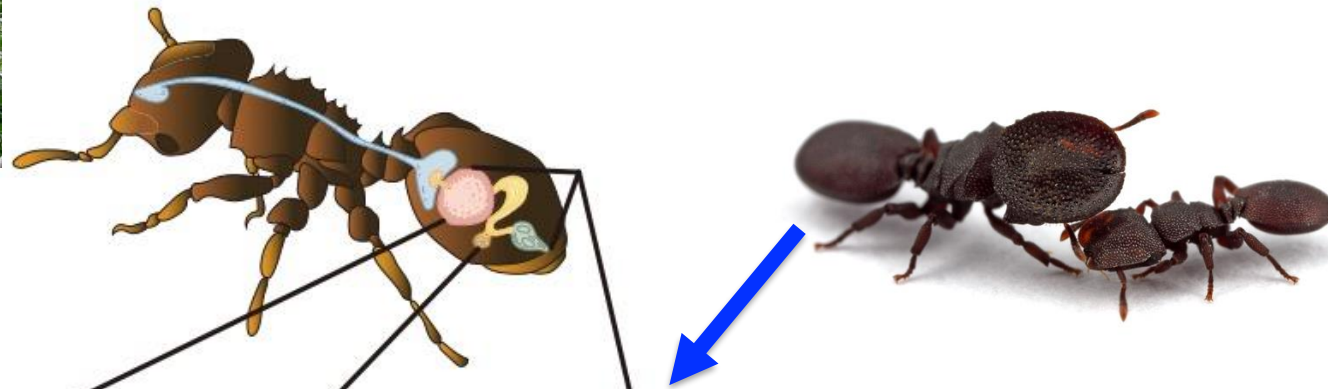
Russell, Moreau *et al.* (2009)
PNAS 106(50): 21236-21241.

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

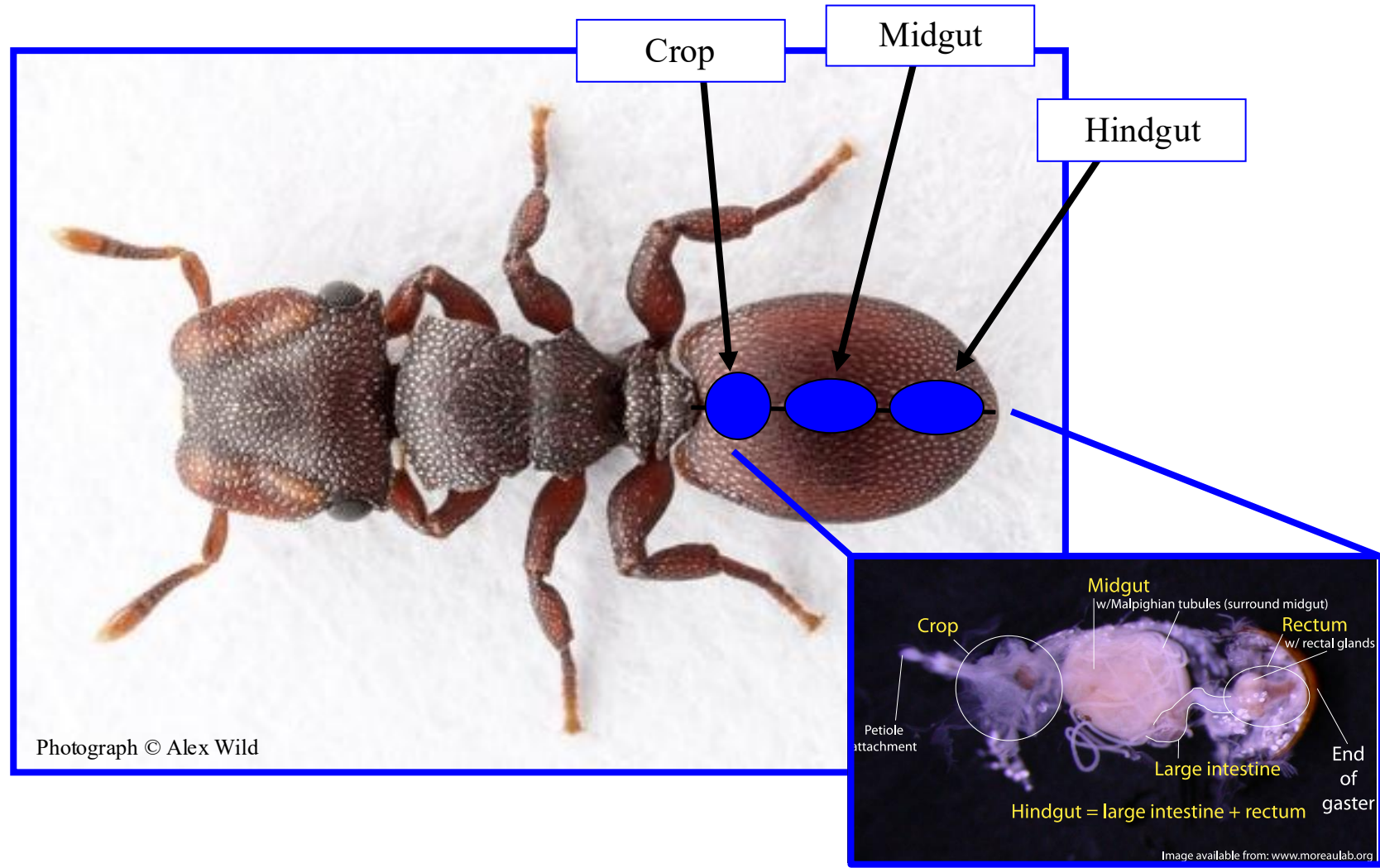
Concentrated changes test (Maddison 1990) provided strong support for the correlated evolution between the presence of *Rhizobiales* symbionts and herbivorous diets across the ant phylogeny (P = 0.001)



Gut-associated bacteria in ants

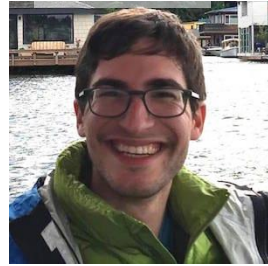


Tissue specificity: Bacterial screening of individual body parts



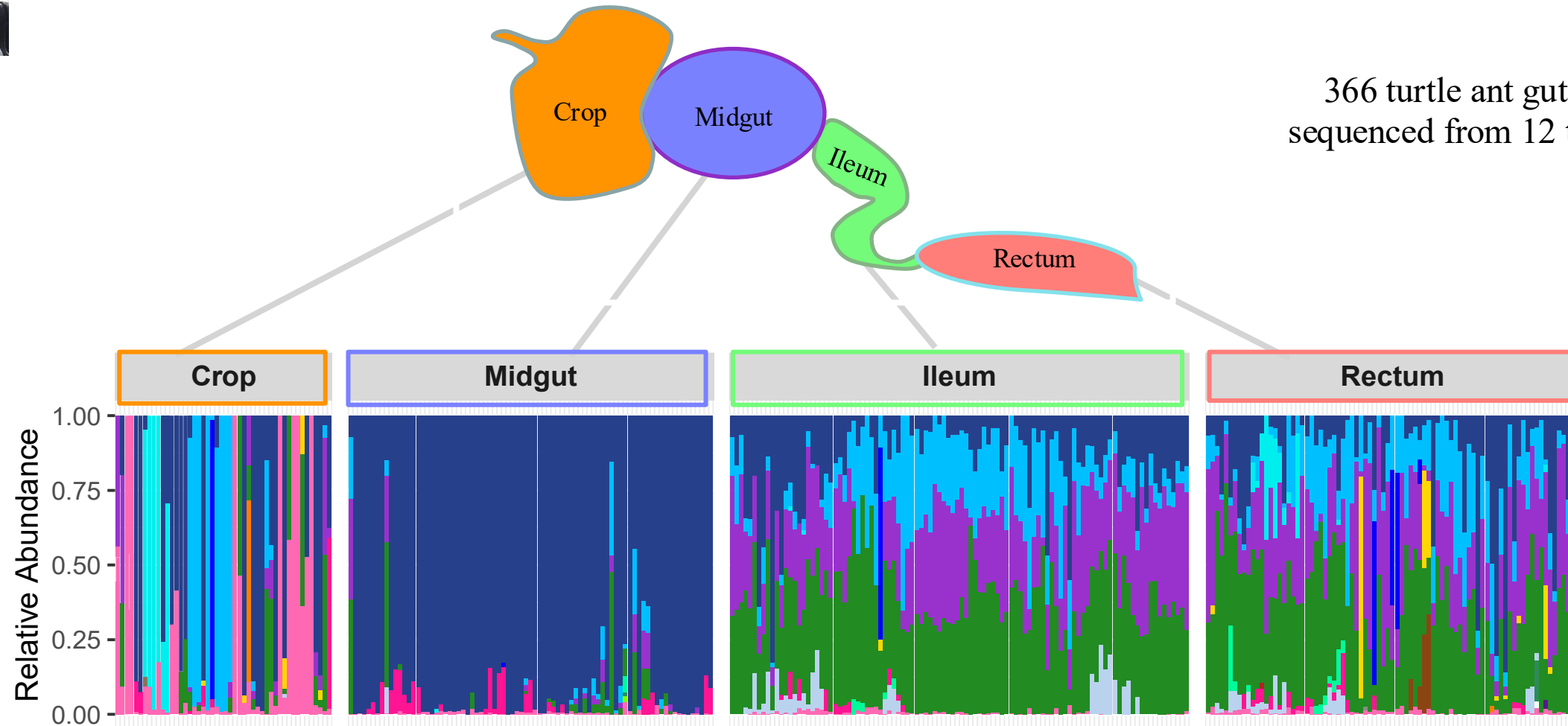
Photograph by C.S. Moreau

Peter Flynn



Bacteria are structured along the digestive tract in turtle ants

366 turtle ant gut samples were sequenced from 12 turtle ant species

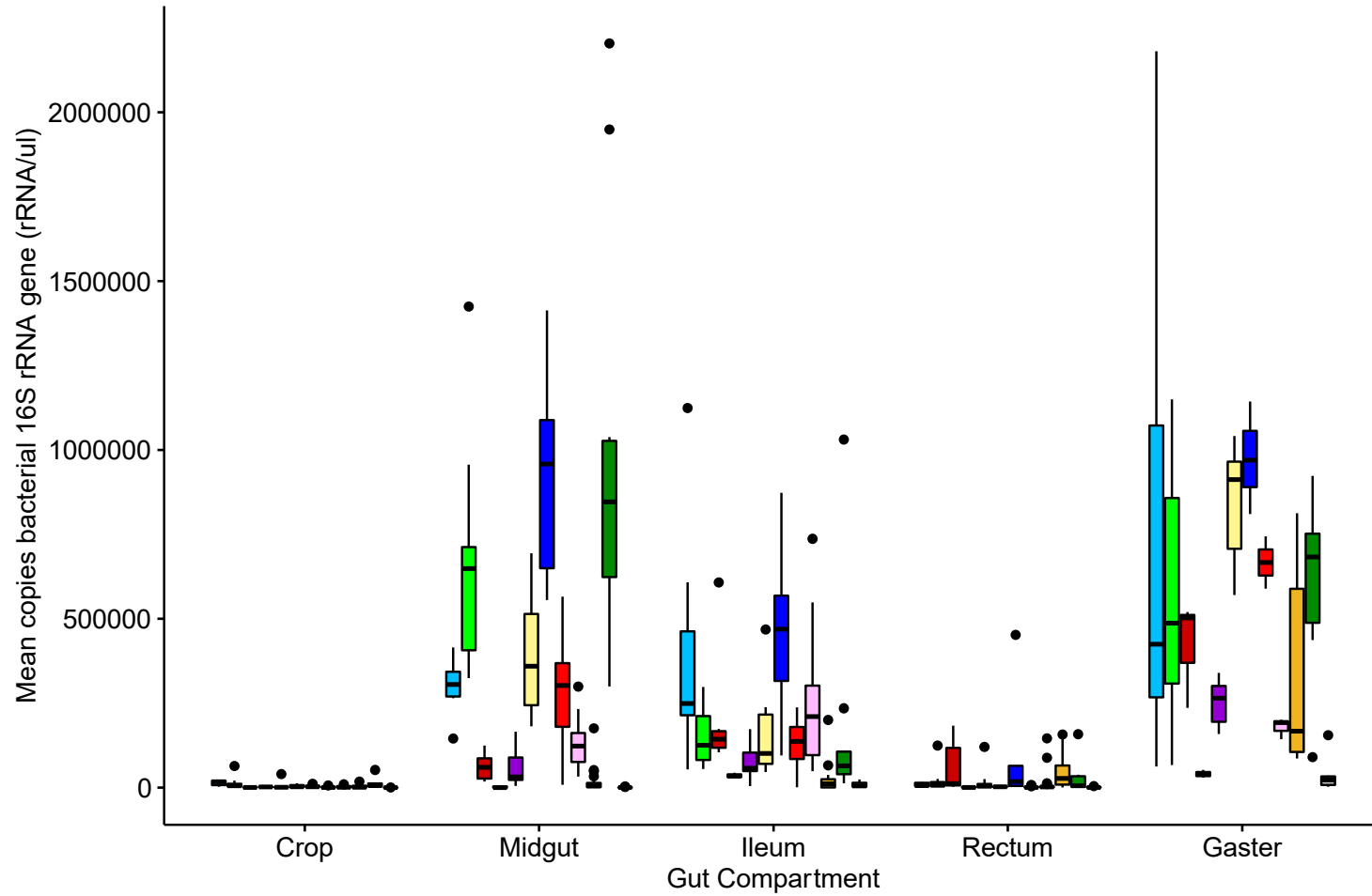


- Opitutales
- Rhizobiales
- Enterobacteriales
- Rickettsiales
- Xanthomonadales
- Pseudomonadales
- Betaproteobacteriales
- Bacillales
- Micrococcales
- Lactobacillales
- Sphingobacteriales
- Campylobacteriales
- Flavobacteriales
- Corynebacteriales
- Chloroplast
- Clostridiales
- Bacteroidales
- Bdellovibrionales
- < 1% abund.

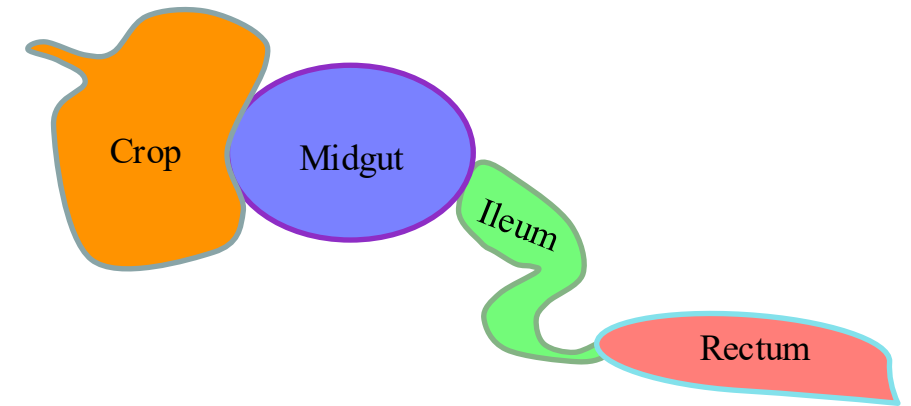
Flynn, D' Amelio, Sanders, Russell, & Moreau (2021) *Appl. Environ. Microbiol* 87(8): e02803-20.

Bacterial density varies across the digestive tract

qPCR values across gut compartment and species



- Species
- atratus
 - grandinosus
 - placidus
 - texanus
 - auricomus
 - minutus
 - setulifer
 - unimaculatus
 - cordatus
 - multispinosus
 - simillimus
 - Procryptocerus



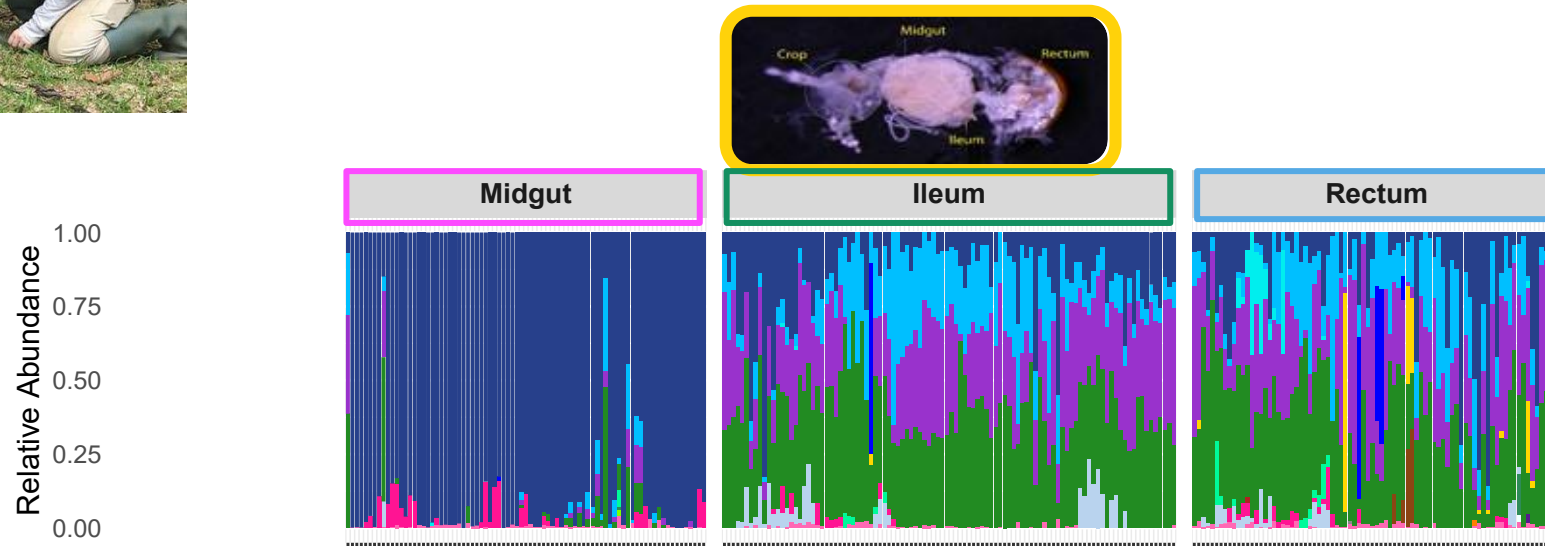
Average amount of bacteria:

>5,000	280,000	120,000	24,000
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Anais Chanson



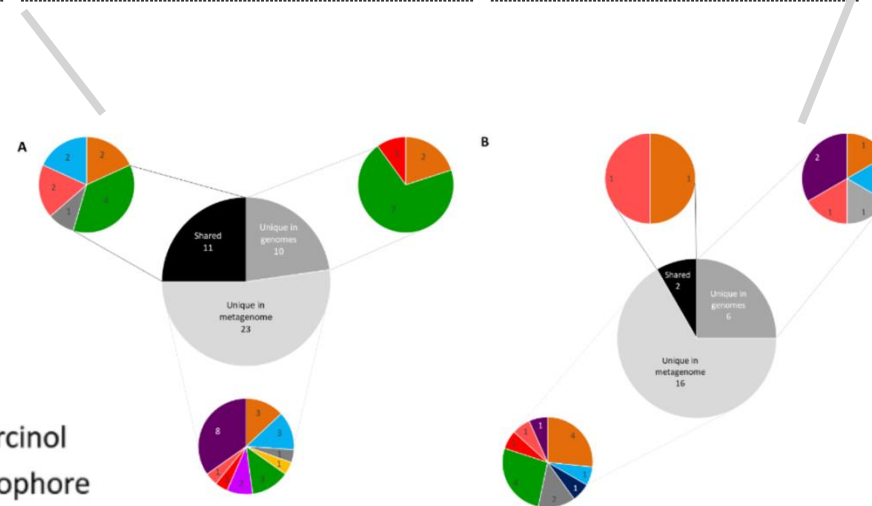
Bacteria are also interacting within the gut



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

Anais Chanson¹, Corrie S. Moreau² and Christophe Duplais^{3*}

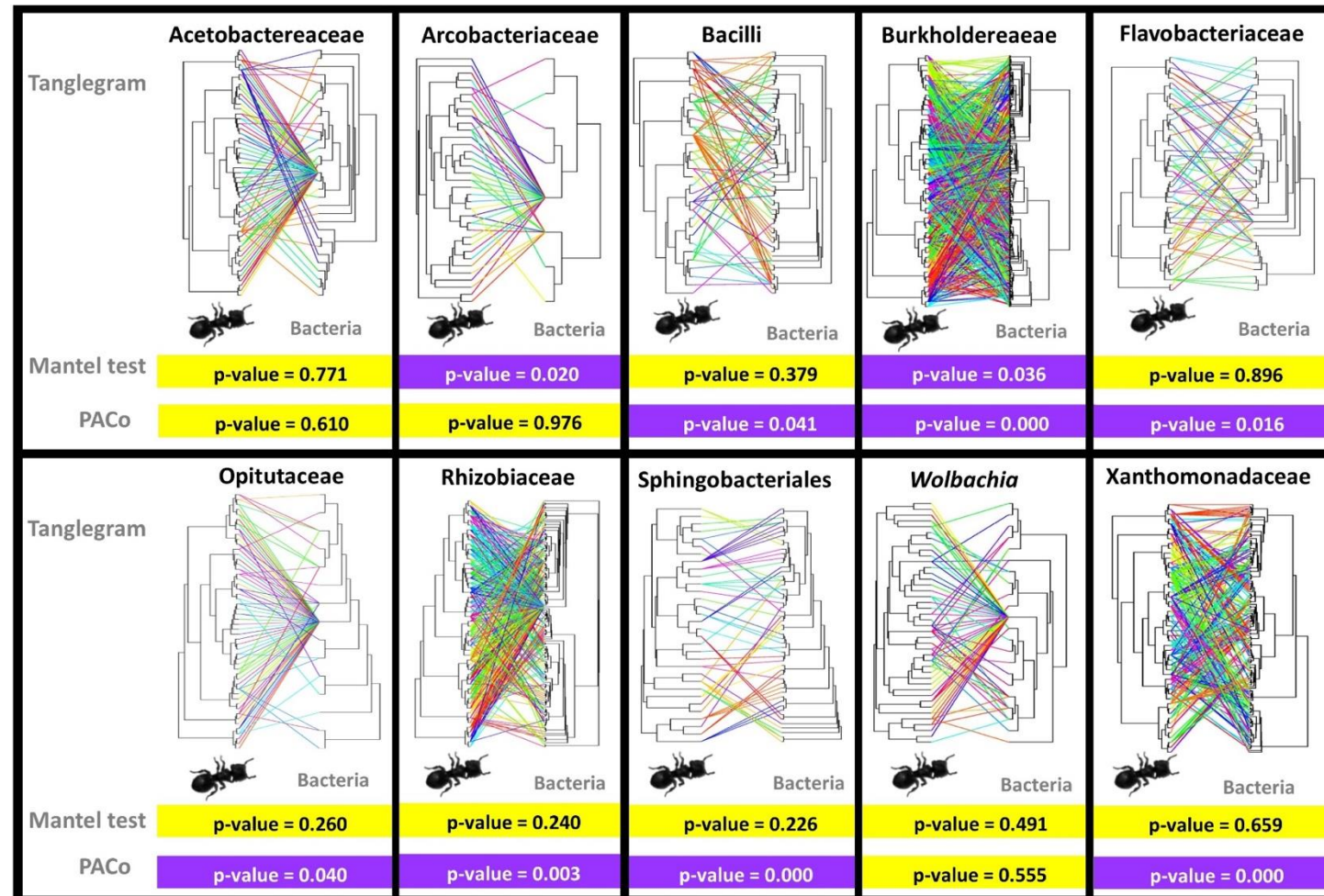
- Arylpolyene
- NRP
- Betalactone
- Resorcinol
- Butyrolactone
- Siderophore
- Ladderane
- T1PK
- Lanthipeptide
- Terpene



Manuela Ramalho



Some gut bacteria codiversify with their hosts



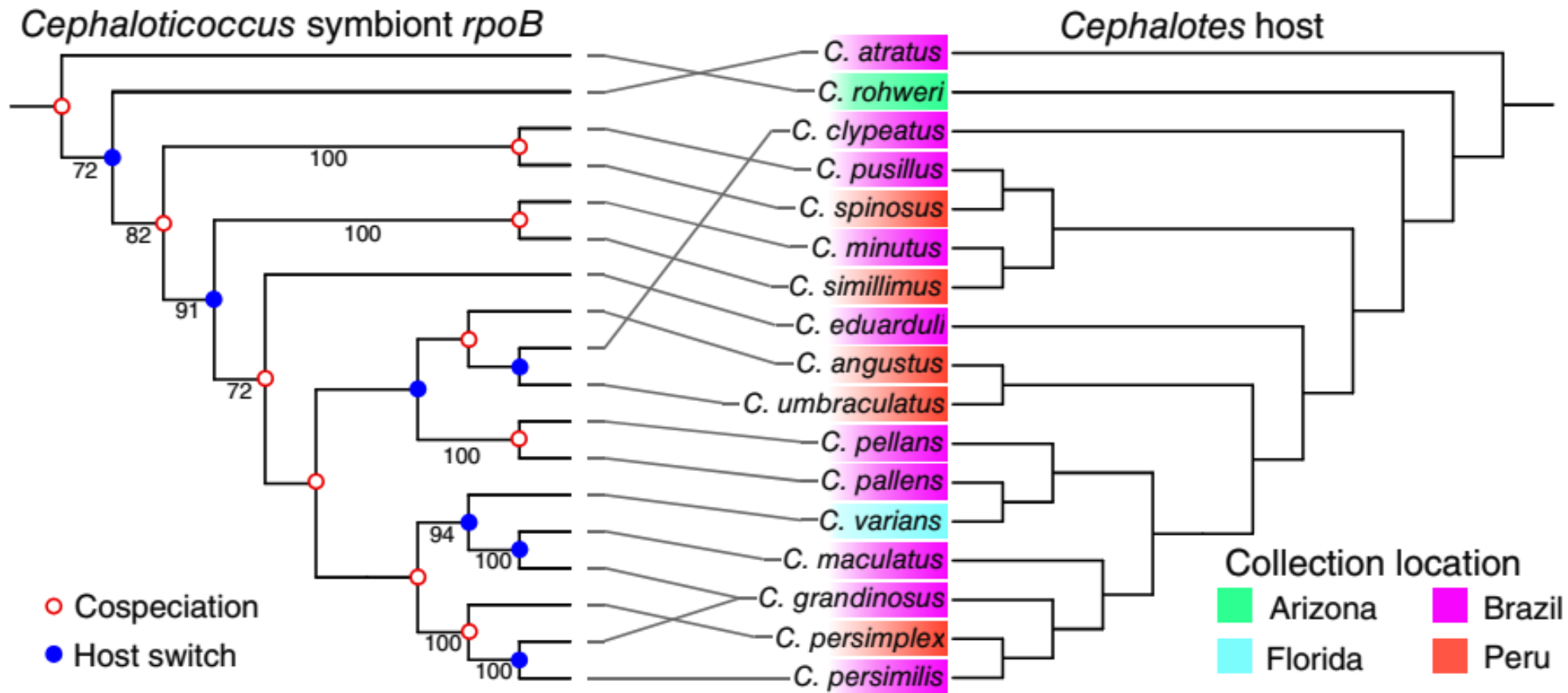
= codiversification
 = no codiversification

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



Are bacteria providing nutrition to ants? Indirect evidence

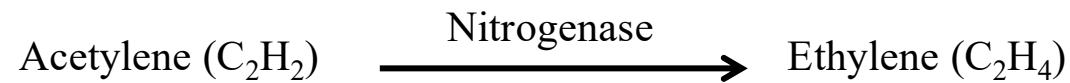
We have sequenced *nifH* genes from the following ants with *Rhizobiales*:

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



Photograph © Alex Wild

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.



Sampling time (h)	YH001		YH026		CSM2266		control	
	C ₂ H ₂ (ppm)	C ₂ H ₄ (ppm)	C ₂ H ₂ (ppm)	C ₂ H ₄ (ppm)	C ₂ H ₂ (ppm)	C ₂ H ₄ (ppm)	C ₂ H ₂ (ppm)	C ₂ H 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.

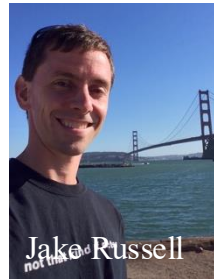
Are bacteria providing nutrition to ants? Indirect evidence

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

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

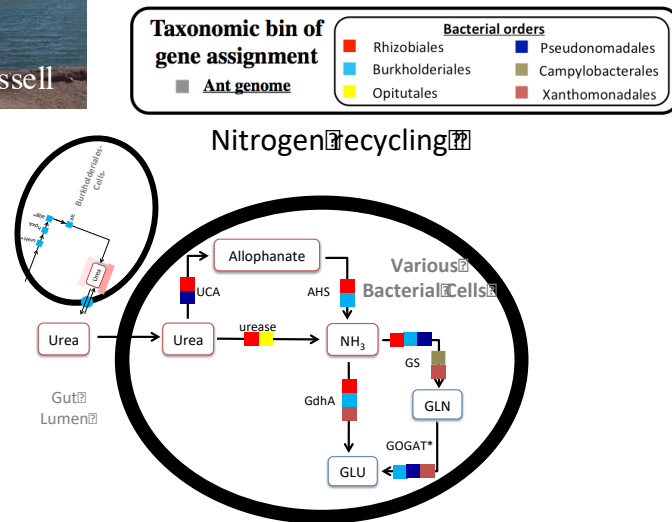


Fluorescent in situ hybridization with labeled probes targeting bacterial specific 16S rRNA

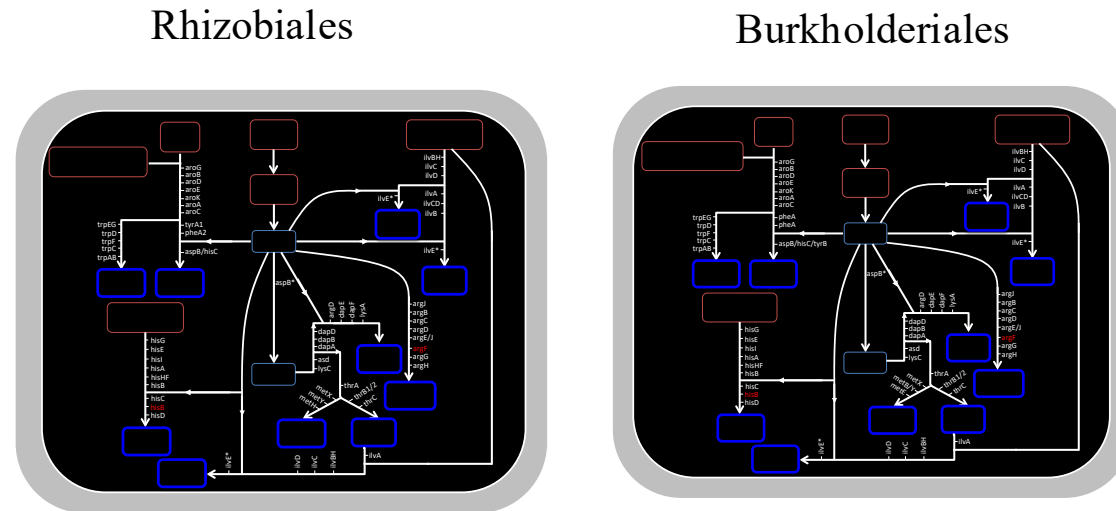


Are bacteria providing nutrition to ants? Direct evidence

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



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

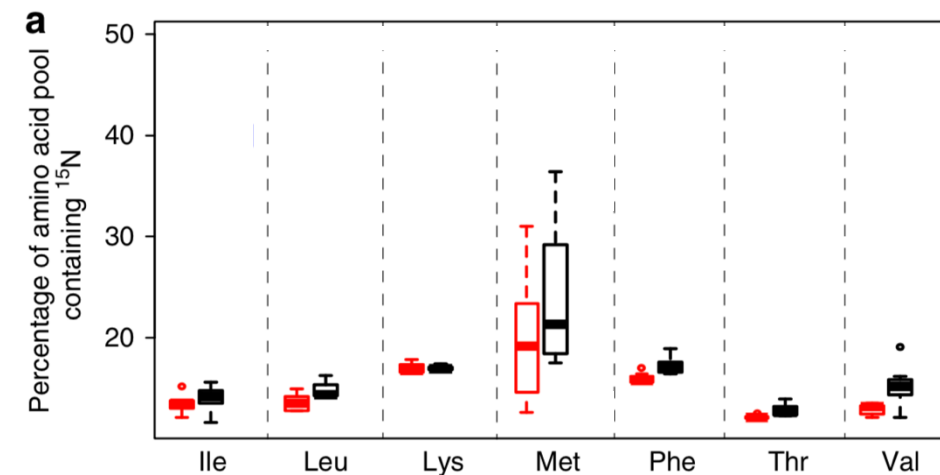
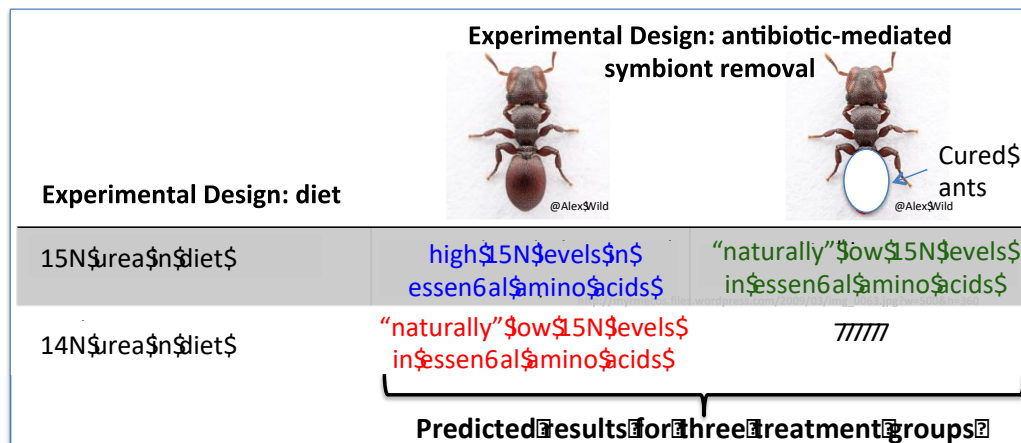
Taxonomic conservation across 18 *Cephalotes* metagenomes demonstrating N-recycling by the core symbionts across 46 million years



Are bacteria providing nutrition to ants?
Direct evidence



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



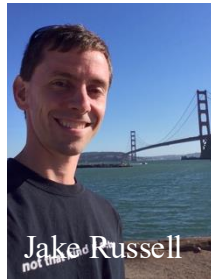
Treatment Group

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

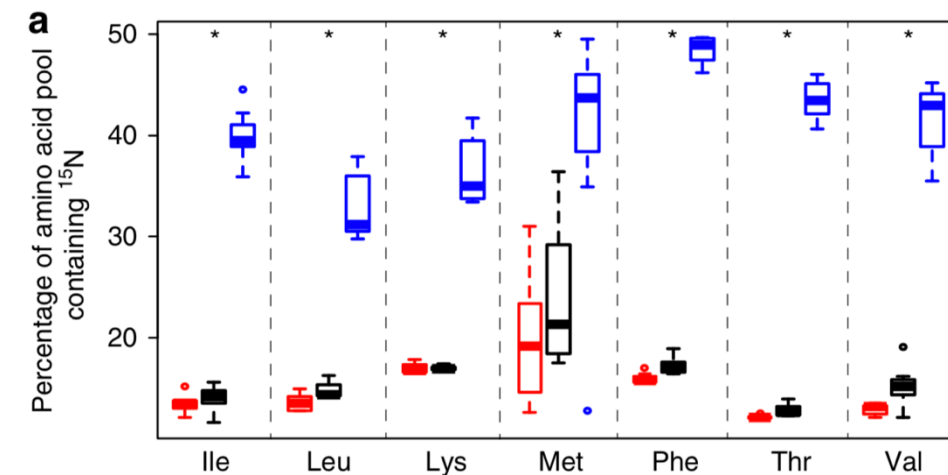
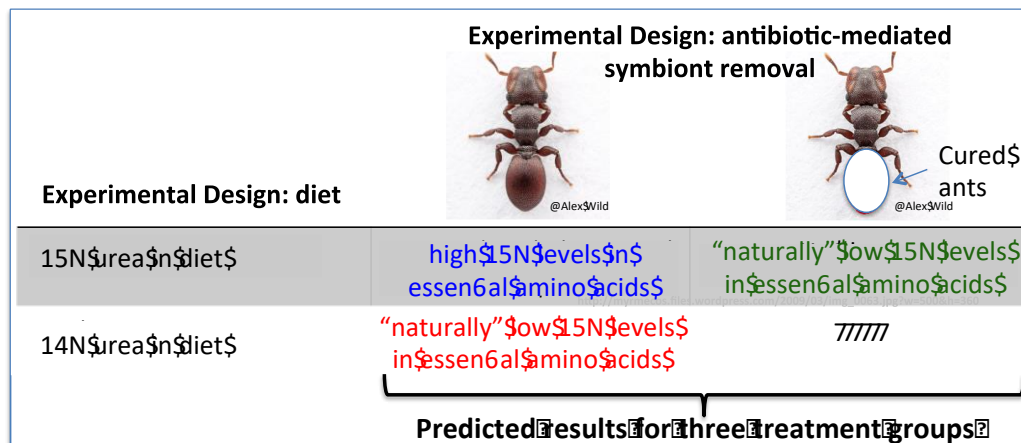
Hu *et al.* (2018).
Nature Communications 9: e964.



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.

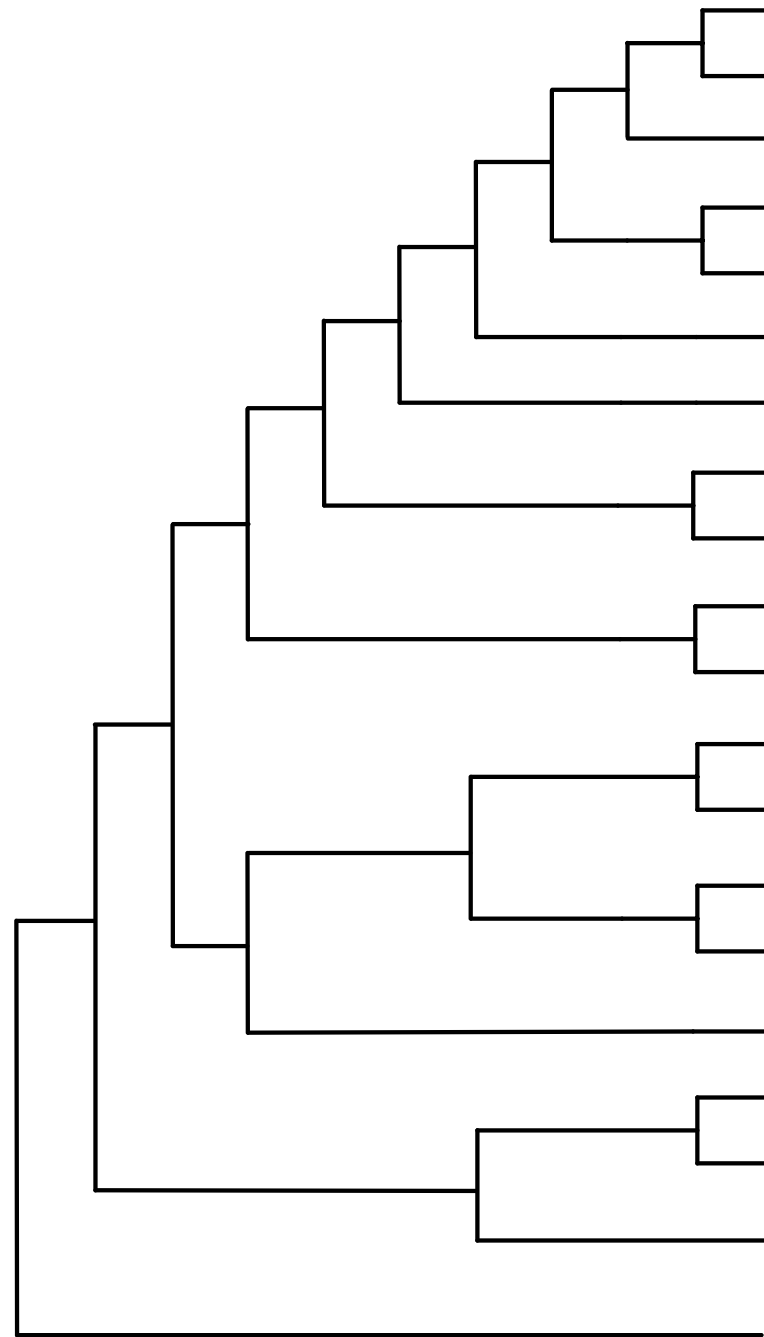
Evidence for the nitrogen contribution of gut symbionts
to the cuticle formation in herbivorous turtle ants

Exoskeleton = insect armor



©Alex Wild





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.



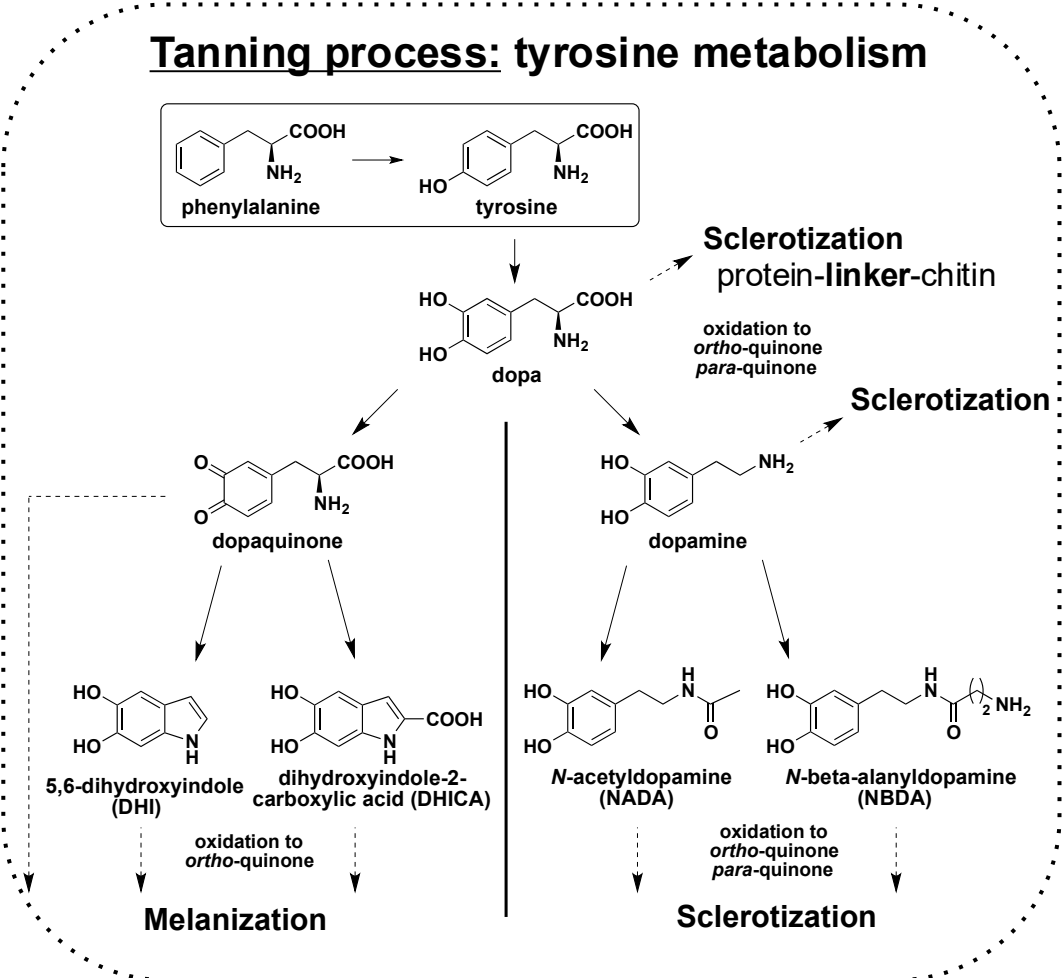
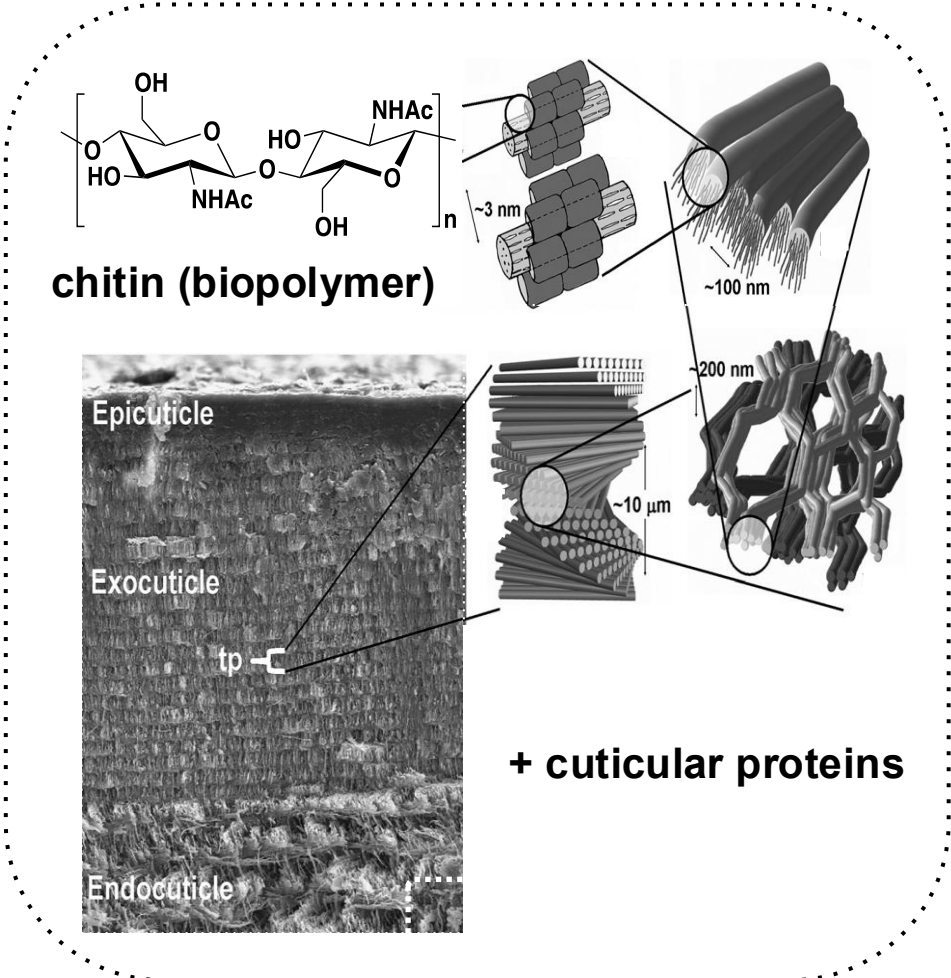
Paraponera clavata



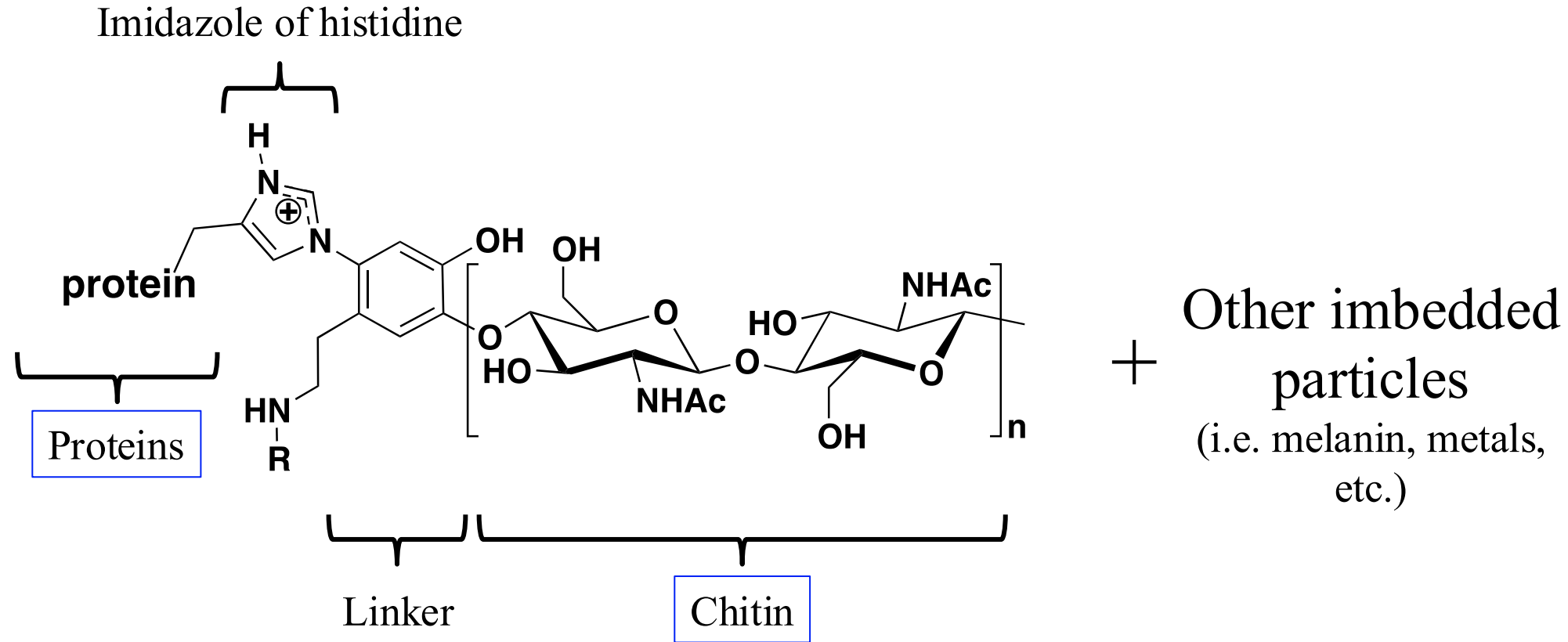
Apis mellifera



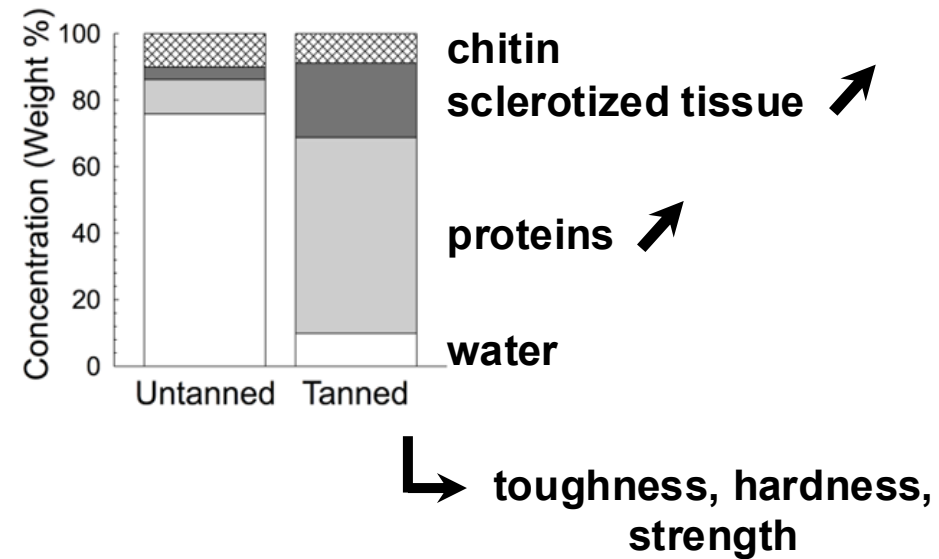
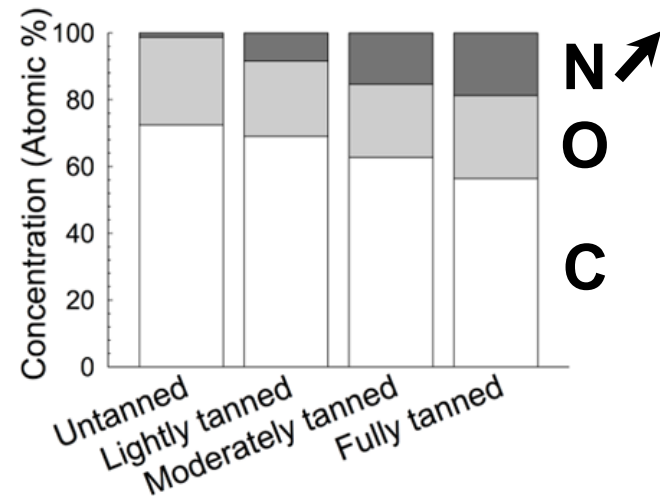
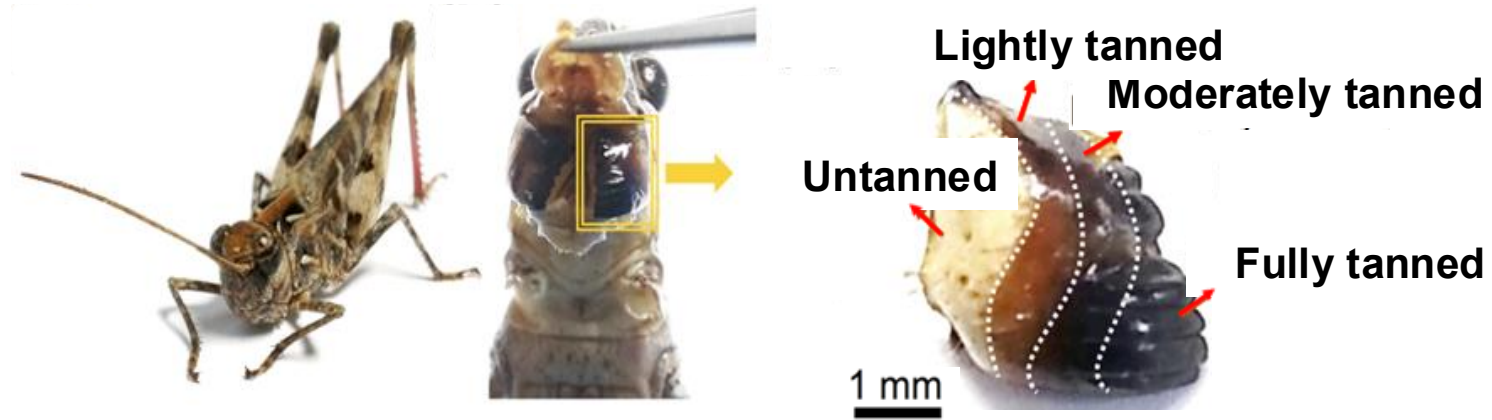
Composition and structure



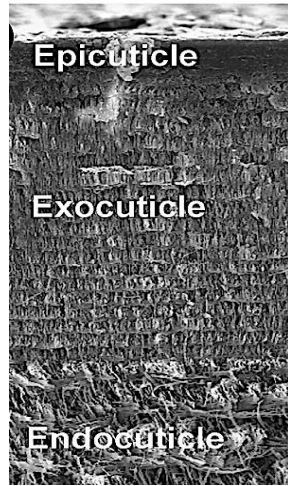
Insect cuticle



Example: Grasshopper mandibles

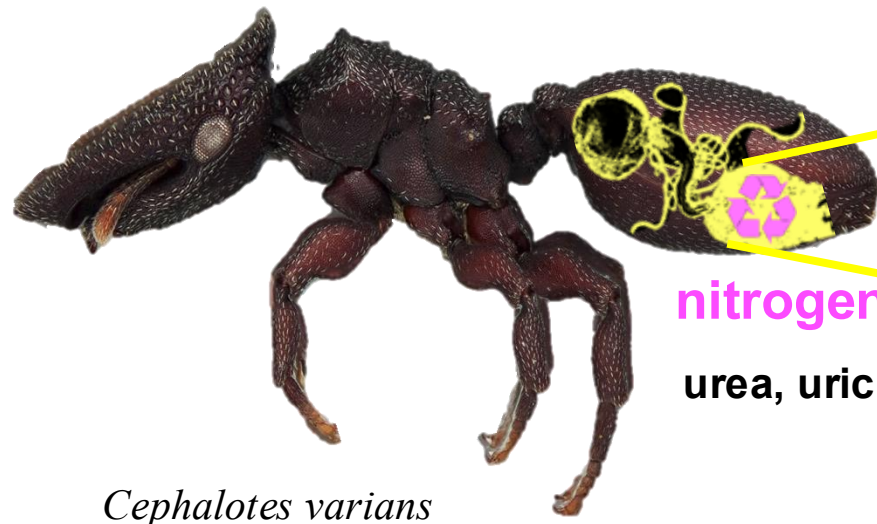


Hypothesis



Contribution?

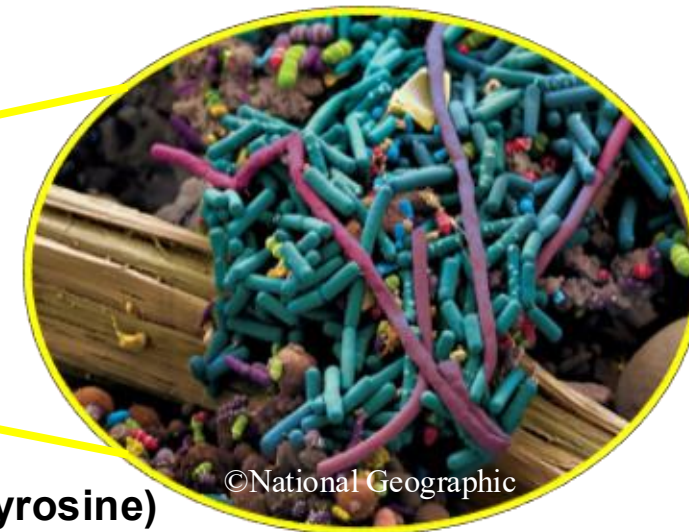
Burkholderiales, Pseudomonadales,
Xanthomonadales, Rhizobiales, and
Opitutales



Cephalotes varians

nitrogen

**urea, uric acid → amino acids
(phenylalanine, tyrosine)**



©National Geographic

Previous research

AS

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}



PMAS PLUS



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 Engl¹ | Nadia Eberl^{1*} | Carla Gorse^{1*} | Theresa Krüger^{1*} | Thorsten H. P. Schmidt² | Rudy Plarre³ | Cornel Adler⁴ | Martin Kaltenpoth¹

Current Biology 24, 2267–2273, October 6, 2014 ©2014 Elsevier Ltd All rights reserved

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,*}

¹Biologie Fonctionnelle Insectes et Interactions, UMR203 BF21, INRA, INSA-Lyon, Université de Lyon, 69621 Villeurbanne, France

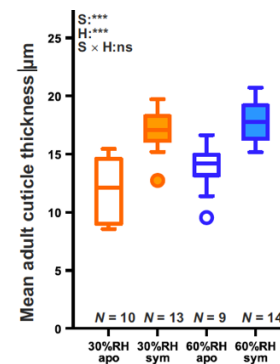
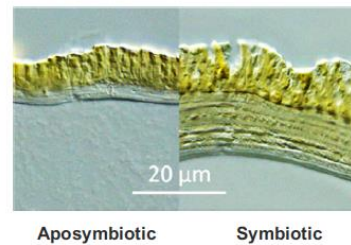
Previous research

Quantifying the impact of symbiont on cuticle formation

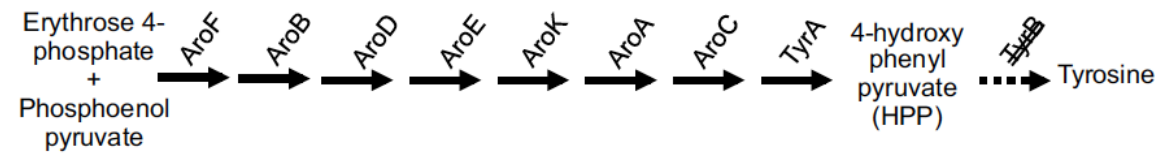
➤ Cuticle coloration



➤ Cuticle thickness



➤ One identified biosynthetic pathway from the symbiont



Small genome in beetles
 Hisashi Anbutsu^{a,b,1,2}, Minoru Masahiko Tanahashi^a, Xian-Manabu Fujie², Noriyuki Sato

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

SPECIAL ISSUE: THE HOST-PATTERN, PROCESS AND F

Ancient symbiosis of grain pest beetles

Tobias Engl¹ | Nadia Ebe
 Thorsten H. P. Schmidt² | F

ed, and
 biont
 of a Grain

Futahashi,^{*}
 Yuichi Koga,^{*}

Ltd All rights reserved

symbionts

r,¹

Previous research

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?**

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

SPECIAL ISSUE: THE HO...
PATTERN, PROCESS AND...

Ancient symbiosi...
grain pest beetle...


Tobias Engl¹ | Nadia Eberl^{1*} | Carla Gorse^{1*} | Theresa Krüger^{1*} |
Thorsten H. P. Schmidt² | Rudy Plarre³ | Cornel Adler⁴ | Martin Kaltenpoth¹

Carole Vincent-Monégat,¹ Emre Aksoy,¹
Etienne Aubailly-Giraud,¹ Anna Zaidman-Rémy,¹
and Abdelaziz Heddi^{1,*}

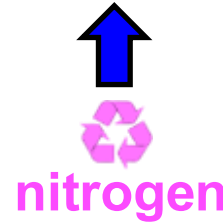
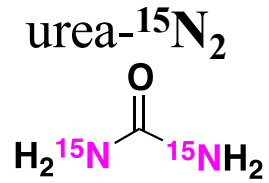
¹Biologie Fonctionnelle Insectes et Interactions,
UMR203 BF21, INRA, INSA-Lyon, Université de Lyon,
69621 Villeurbanne, France

Experimental design and methods



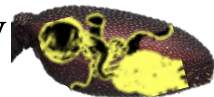
Colony n° 1:
No treatment 


Diet with labelled



Colony n° 2:
Antibiotics treatment 

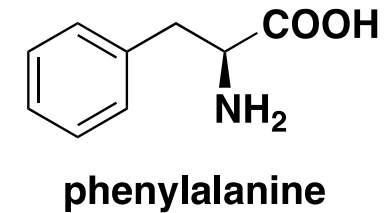
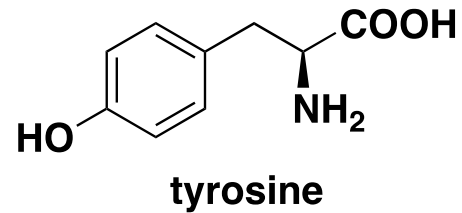
1) Two months experiment \rightarrow new adults emerge



2) Gut dissection \rightarrow solvent extraction \rightarrow liquid-state ^1H NMR spectroscopy 

3) Cuticle \rightarrow solid-state ^{13}C and ^{15}N NMR spectroscopy 

4) Cuticle \rightarrow scanning electron microscopy (SEM) 

Liquid-state ^1H NMR of gut chemical extracts



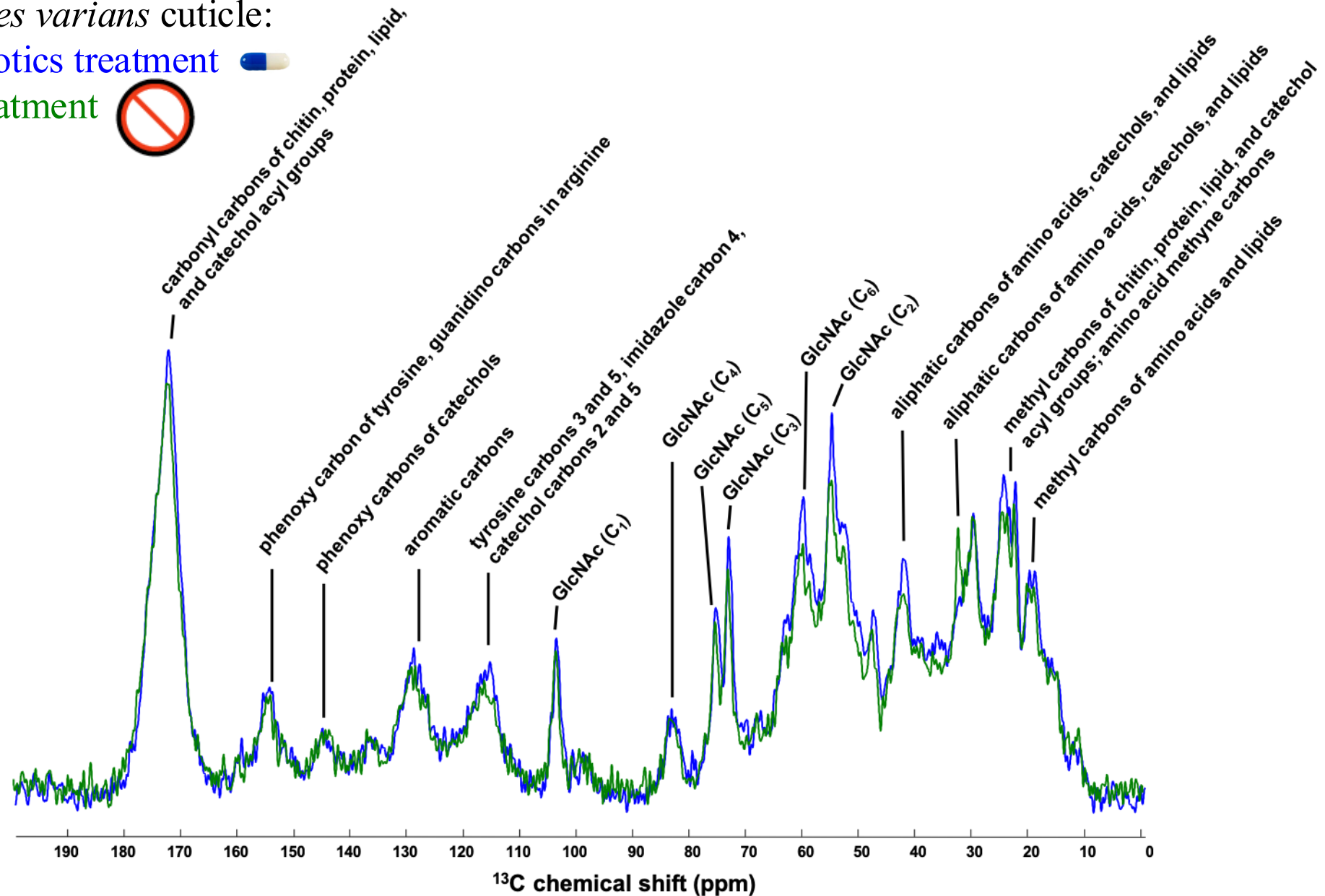
<i>C. varians</i> samples	Tyrosine concentration in μM (SD)	Phenylalanine concentration in μM (SD)
Untreated 	11.3 (0.1)	11.2 (0.3)
Antibiotic treated 	4.9 (0.3)	4.7 (0.3)

Solid-state ¹³C NMR of cuticle



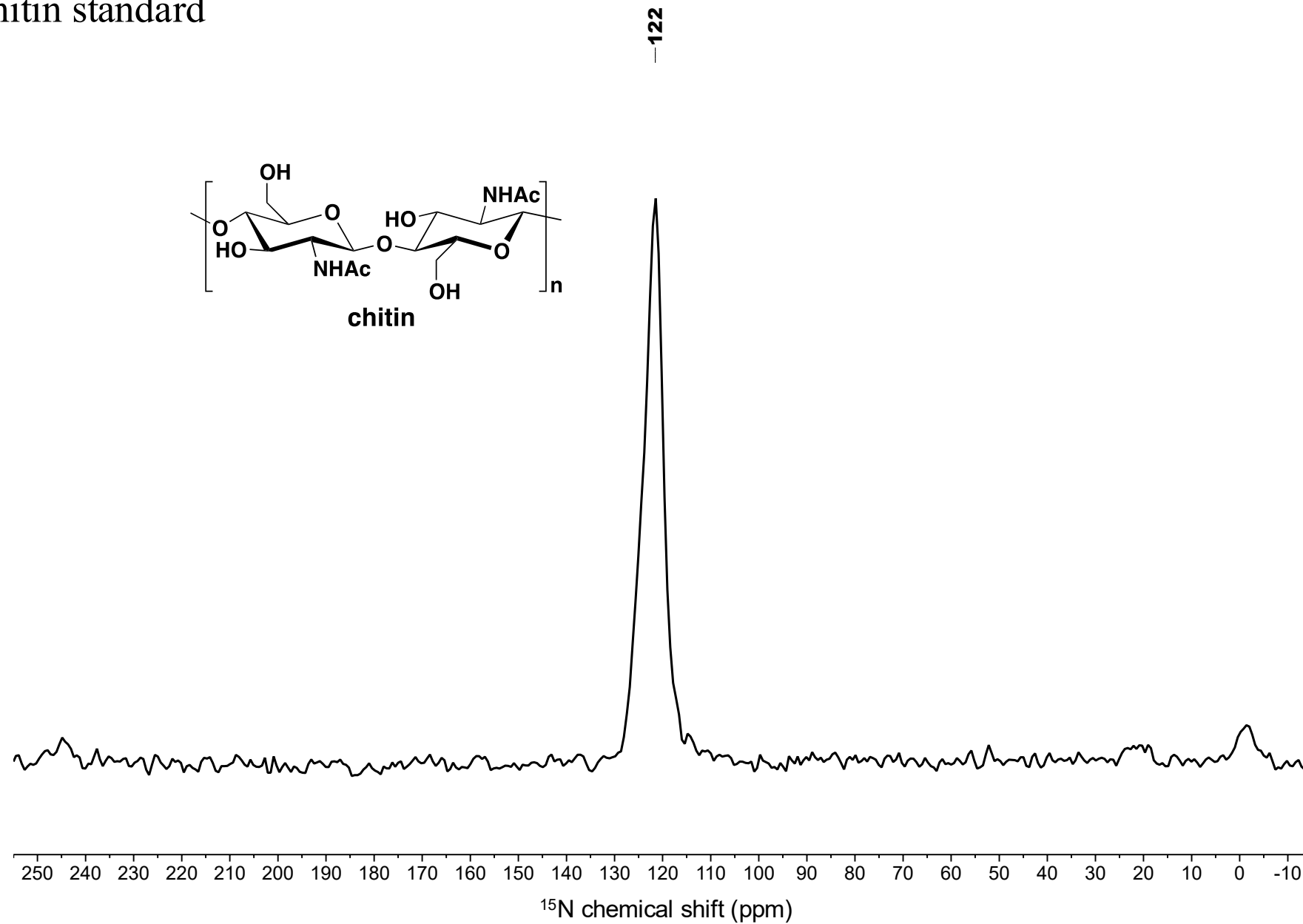
Cephalotes varians cuticle:

- antibiotics treatment 
- no treatment 



Solid-state ^{15}N NMR of cuticle

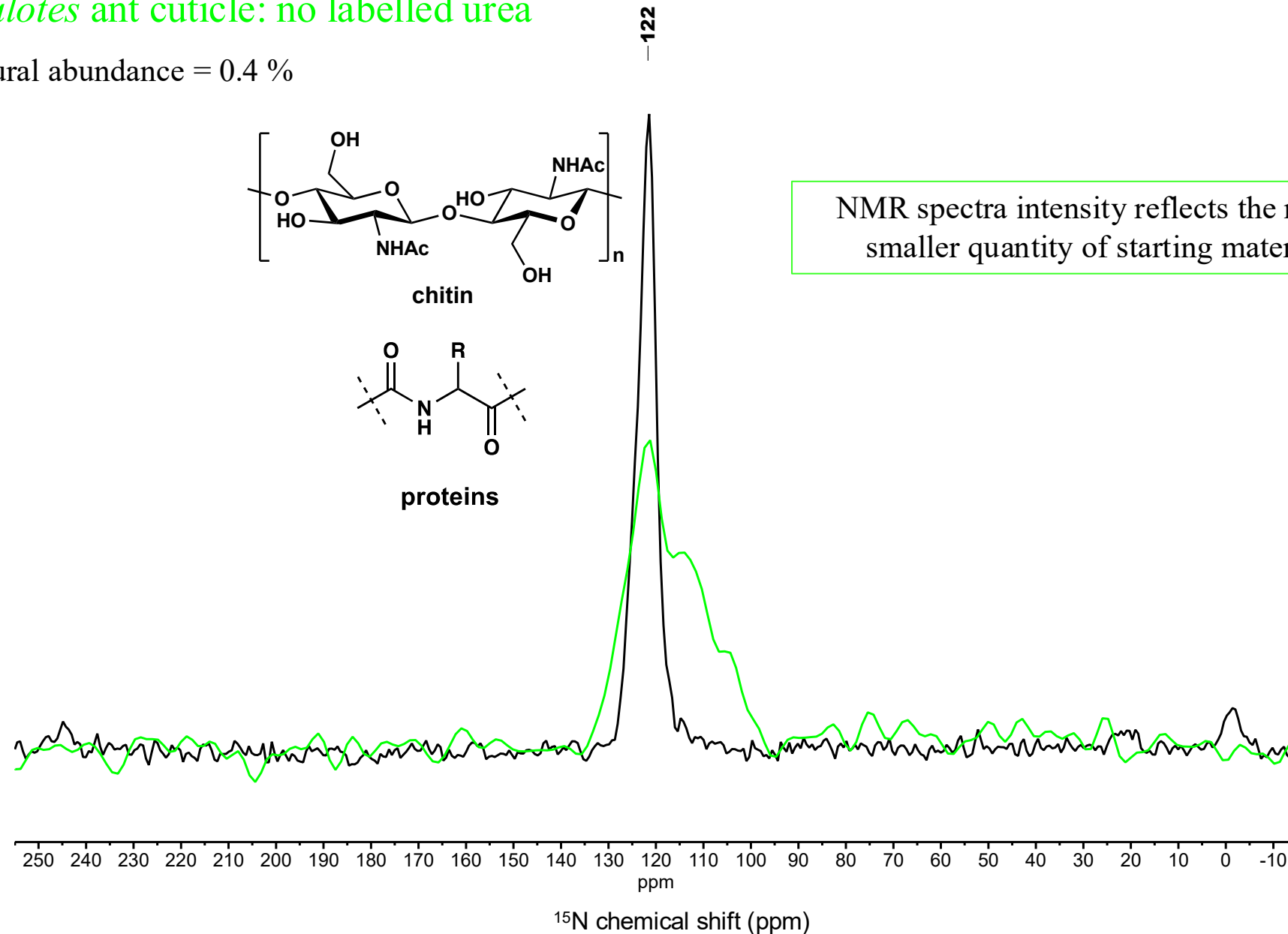
Pure chitin standard



Solid-state ^{15}N NMR of cuticle


Cephalotes ant cuticle: no labelled urea

^{15}N natural abundance = 0.4 %



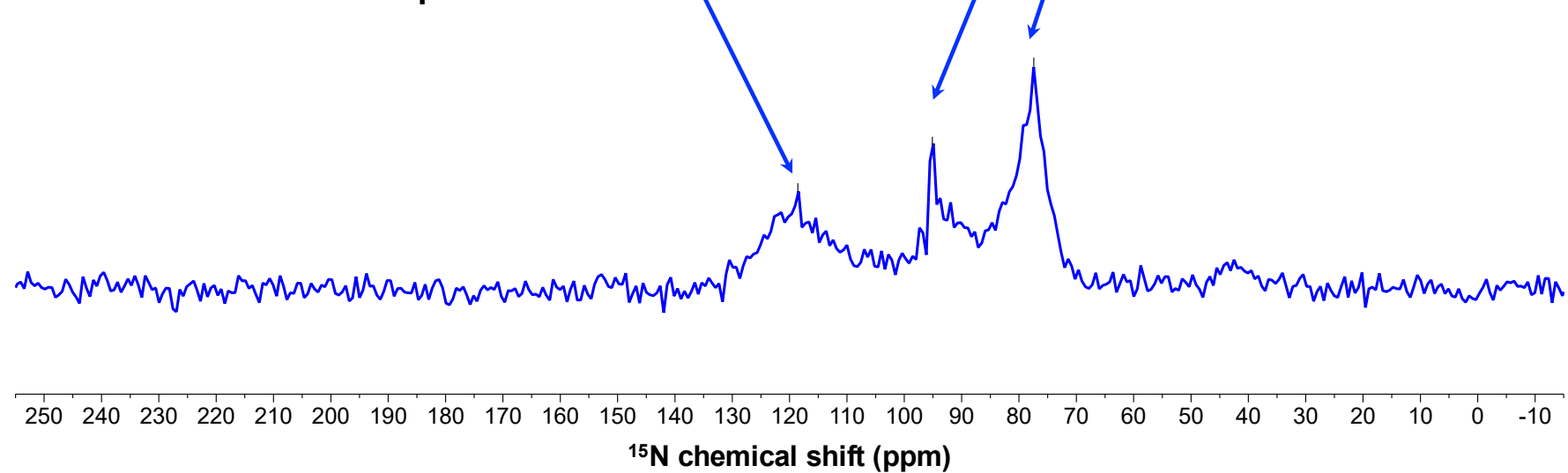
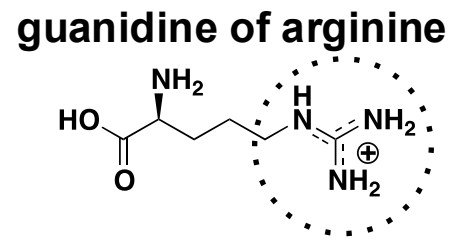
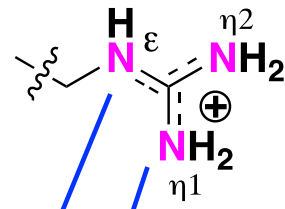
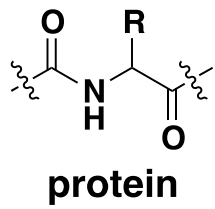
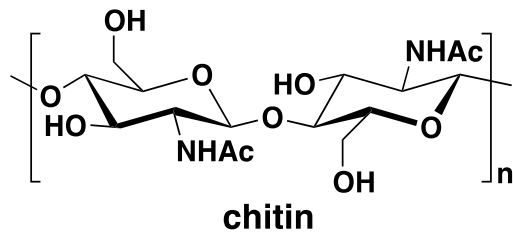
Solid-state ¹⁵N NMR of cuticle



C. varians cuticle:
labelled urea-¹⁵N₂
- antibiotics treatment 

119 95 77

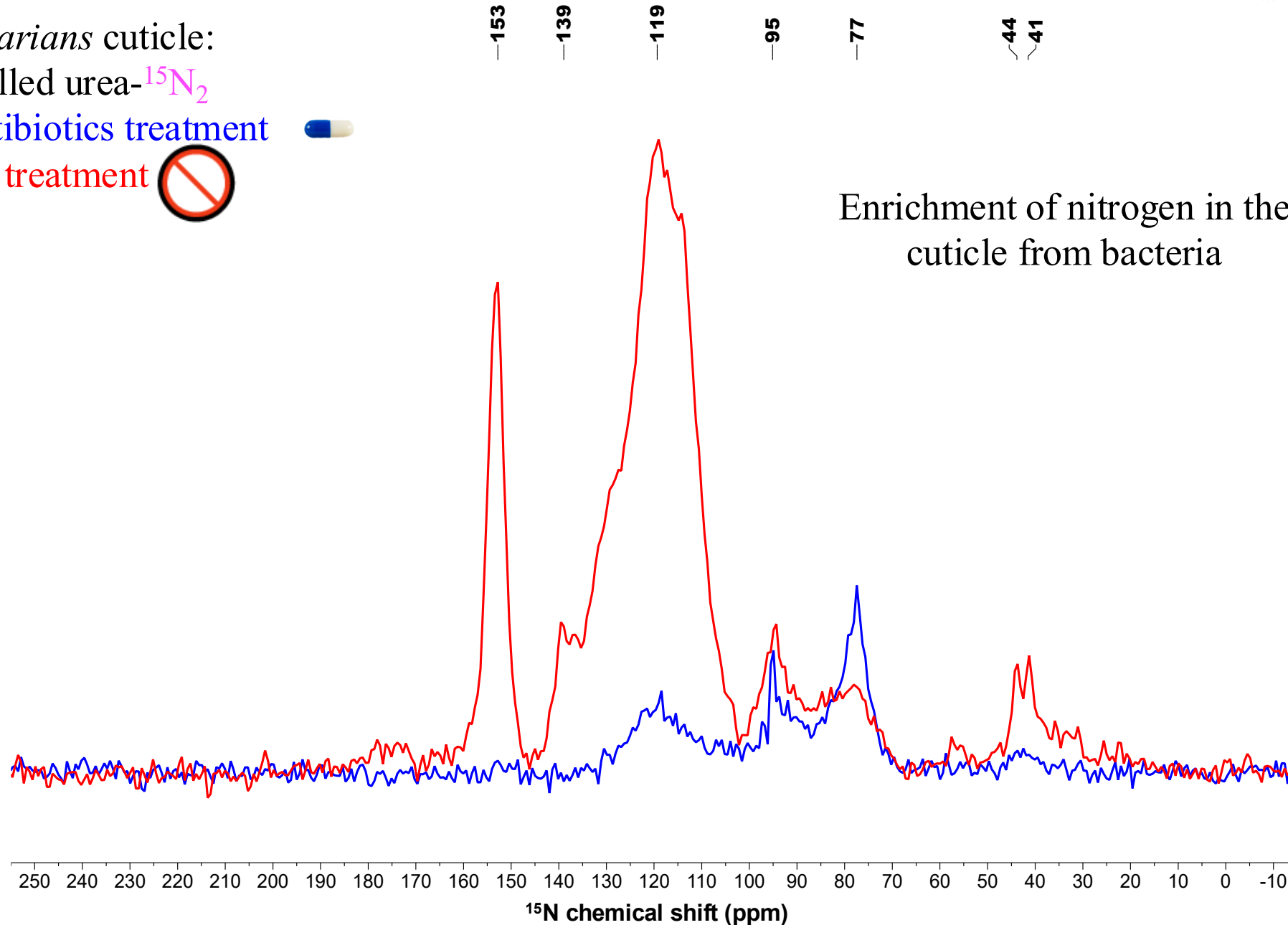
¹⁵N = enriched
¹⁴N = natural abundance



Solid-state ¹⁵N NMR of cuticle



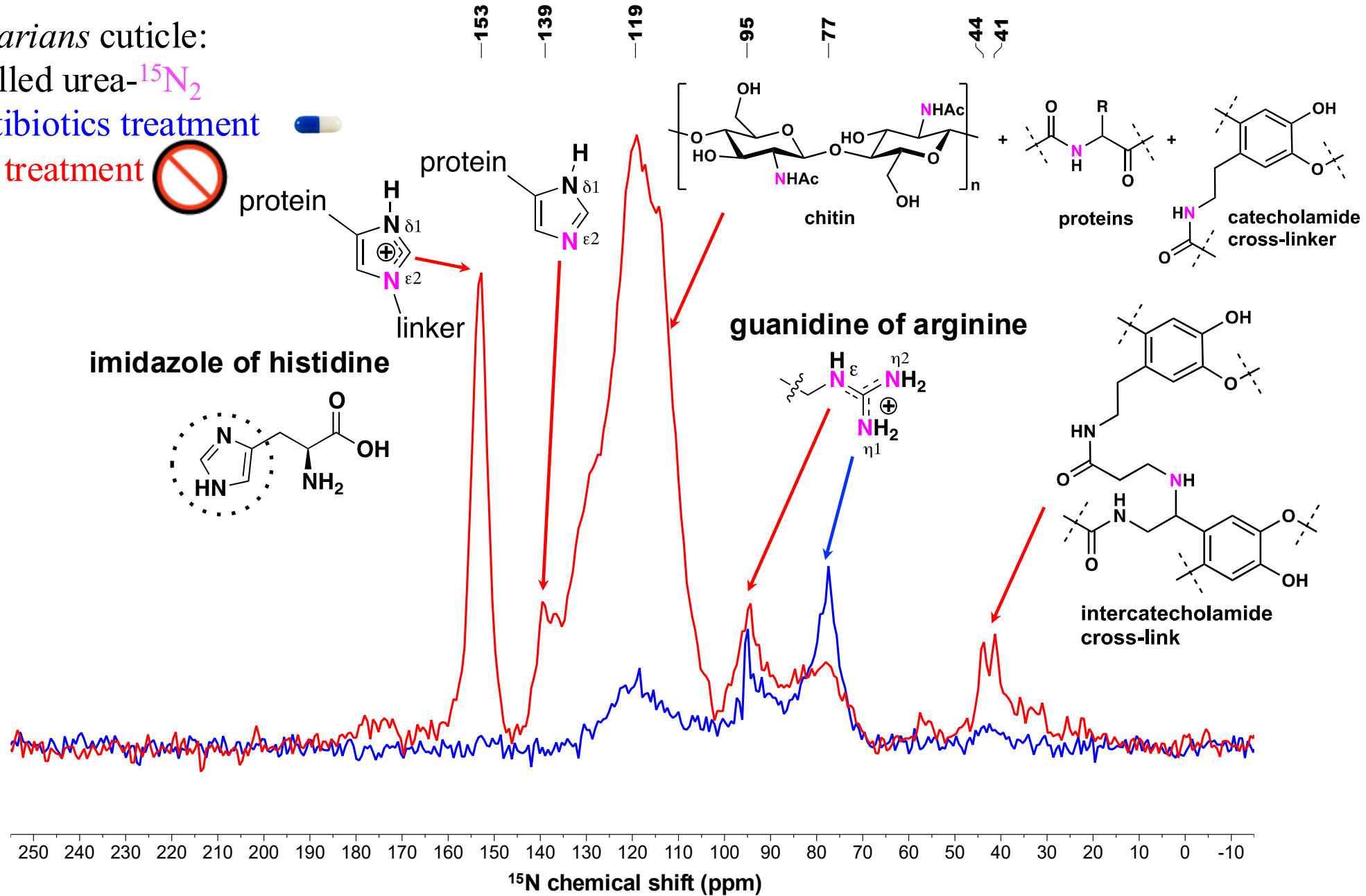
C. varians cuticle:
labelled urea-¹⁵N₂
- antibiotics treatment
- no treatment




Solid-state ^{15}N NMR of cuticle

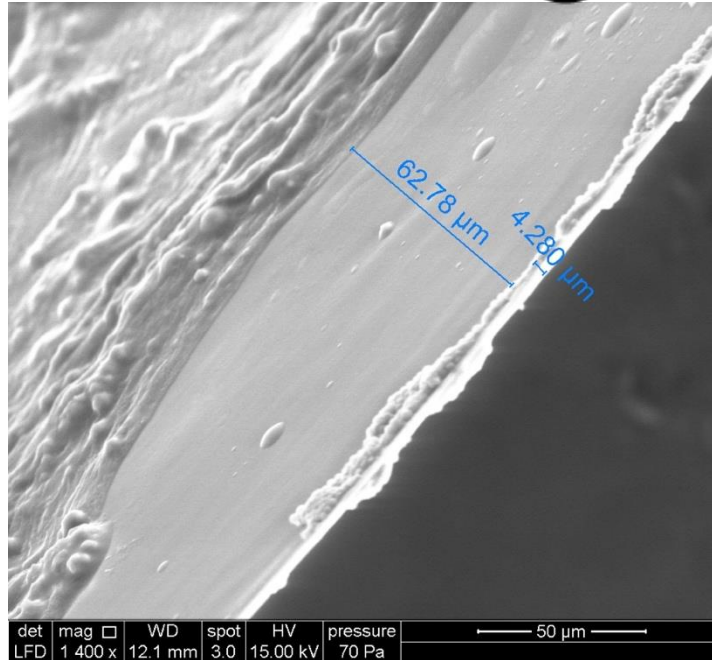



C. varians cuticle:
 labelled urea- $^{15}\text{N}_2$
 - antibiotics treatment
 - no treatment

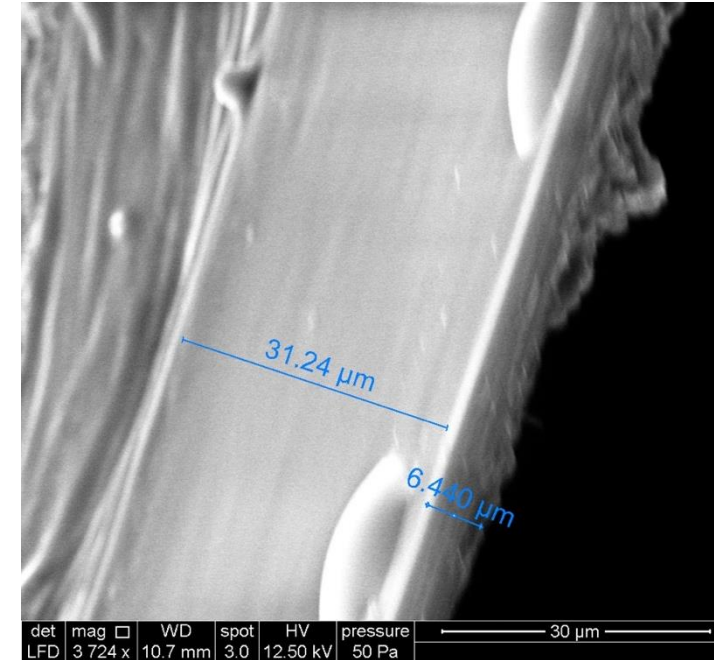


Electronic microscopy

Colony n° 1:
No treatment 



Colony n° 2:
Antibiotics treatment 

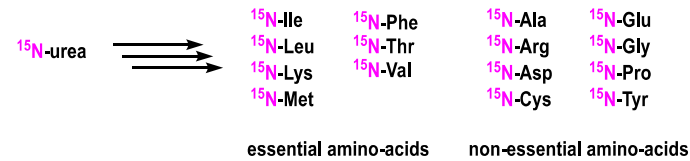


Cuticle thickness reduce by 50% in antibiotic treatment

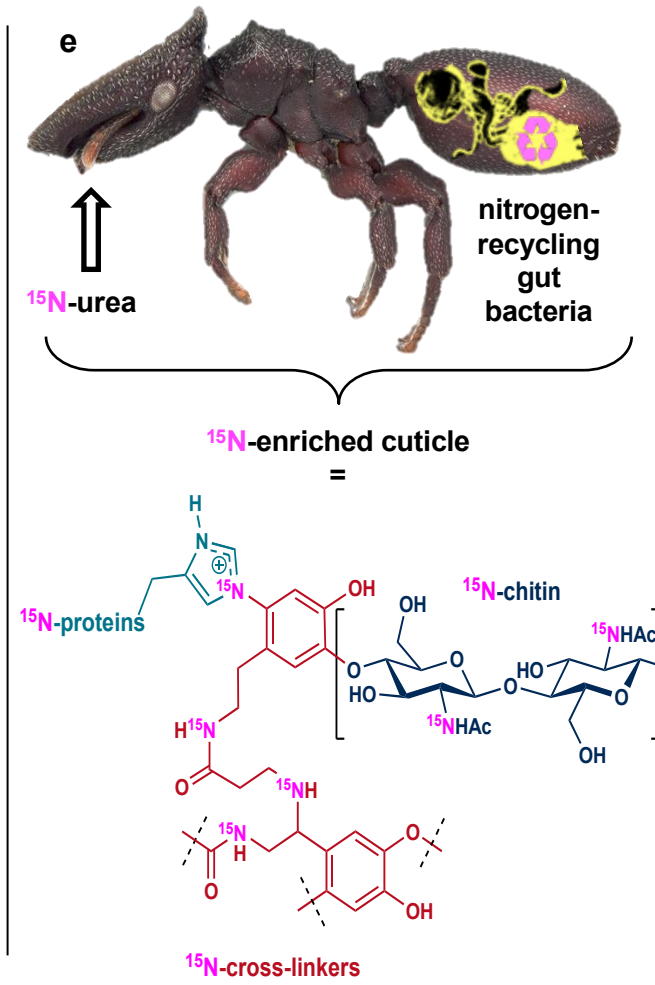
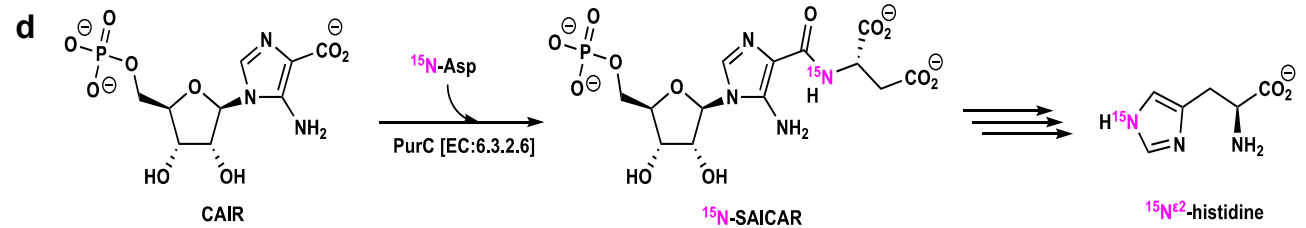
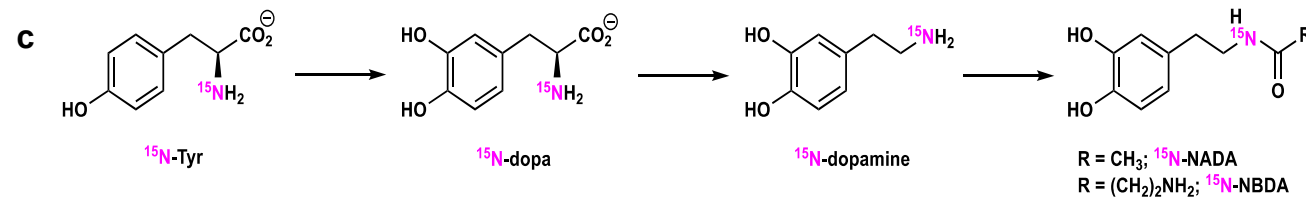
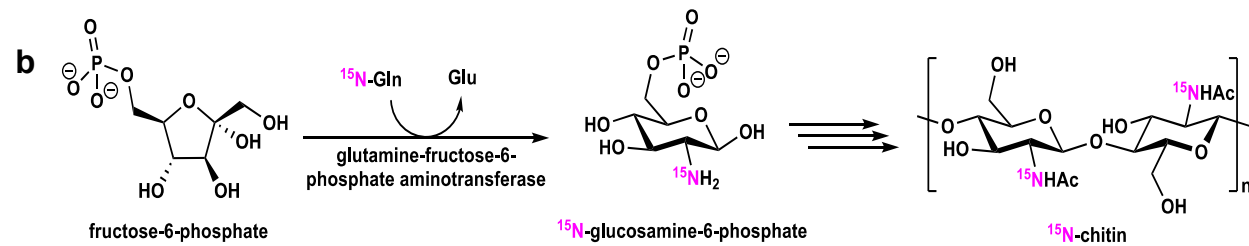
**Antibiotics treated individuals are fully black
with no sign of depigmentation**

Gut bacteria contribute to most cuticular components: protein, linker, & chitin ✓ melanin ✗

a Previous work



This work



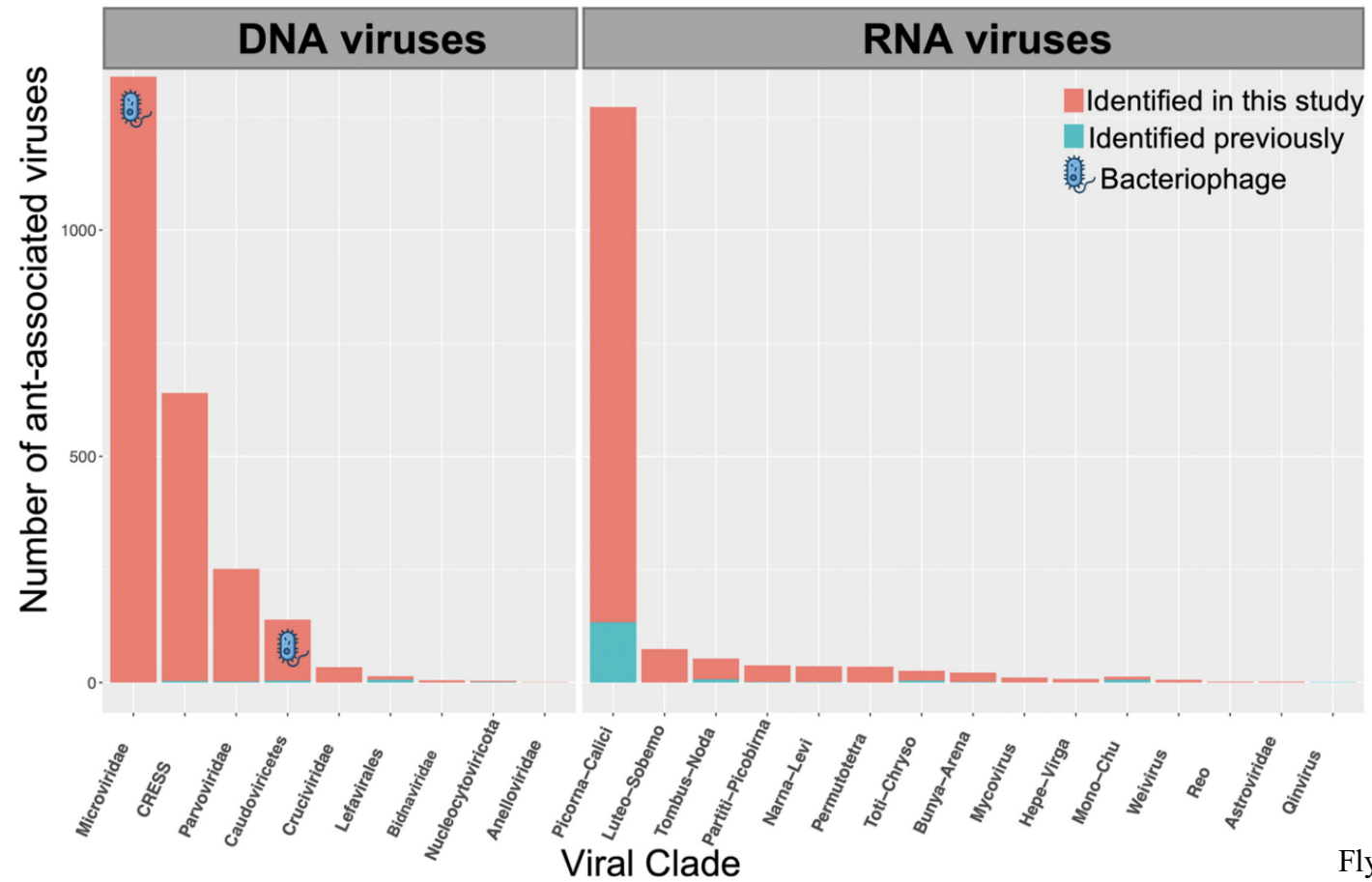
What about viruses?

ORIGINAL ARTICLE

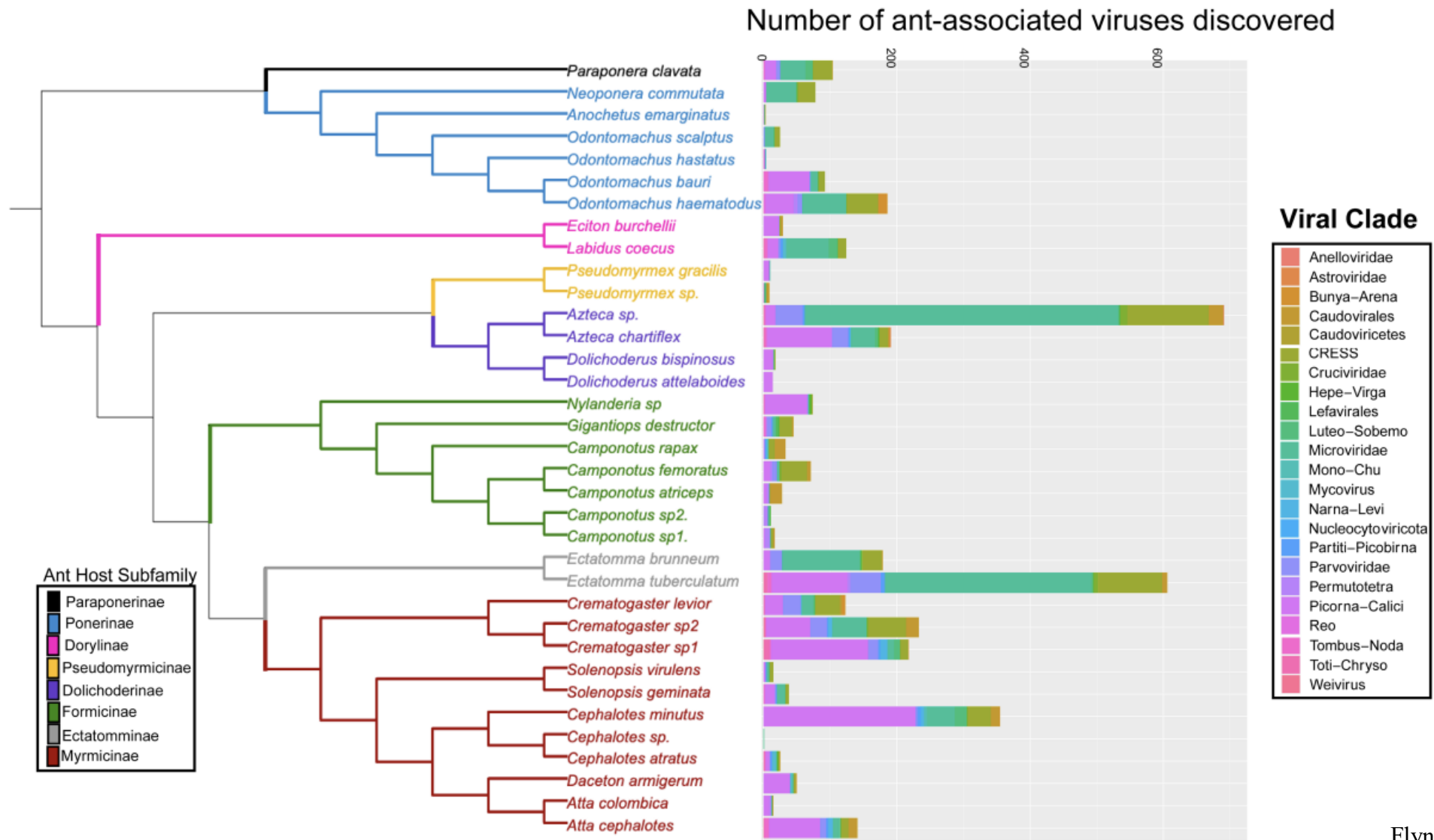
MOLECULAR ECOLOGY WILEY

Viral diversity and co-evolutionary dynamics across the ant phylogeny

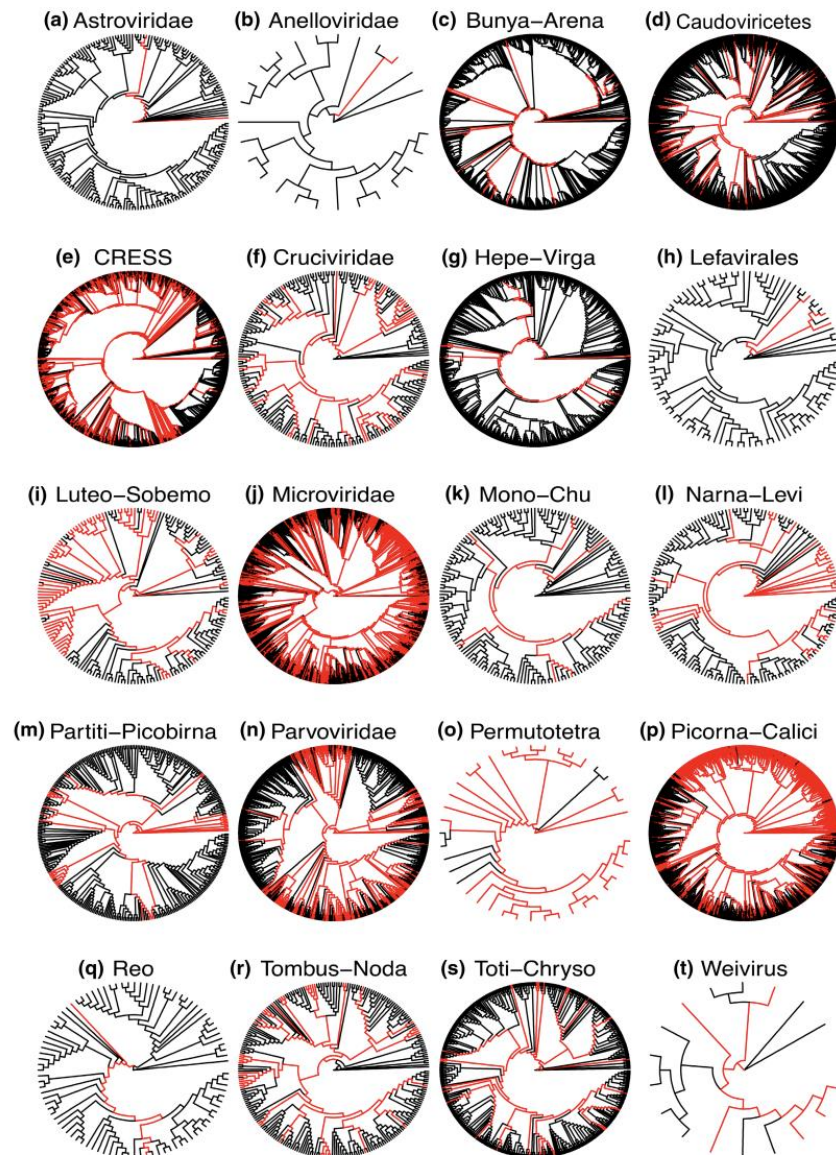
Peter J. Flynn^{1,2} | Corrie S. Moreau^{3,4}



Viruses are not evenly distributed across the ant phylogeny



Many diverse viral lineages are associated with the ants



- We discovered 3710 novel and unique ant-associated viral genomes
- Of these 659 were complete genomes
- Frequent host-switching and co-divergence

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
- Viruses in ants are far more diverse than previously appreciated

Acknowledgements

Collaborators on this work:

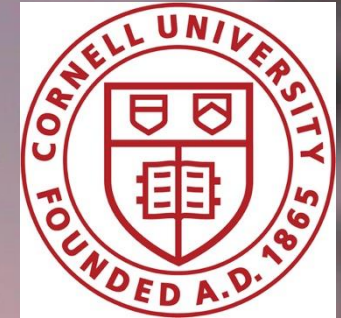
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