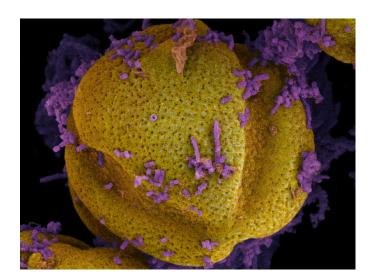


## **"Buzzing"** for Tomatoes & Beneficial Microbes for Bee Health

Stephen L. Buchmann

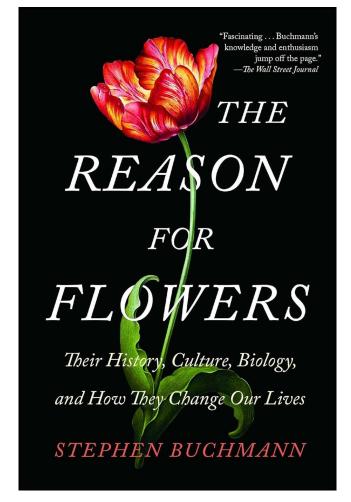
Southern Arizona Beekeepers Association

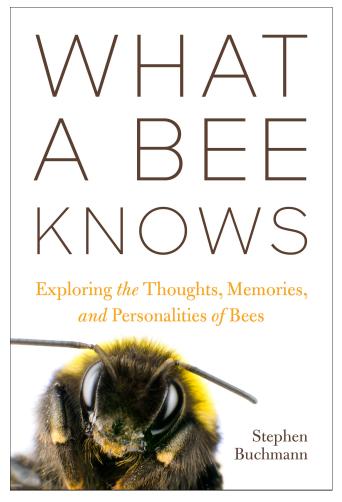
August 12, 2025



#### **Stephen Buchmann**

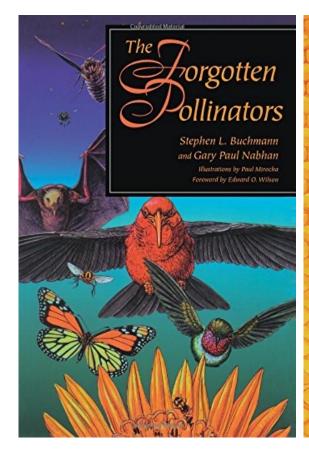
contact: buchmann.stephen@gmail.com Adjunct Professor, UA Entomology, Ecology & Evol. Biol. Depts. (Pollination ecology, bee nesting & mating biology)

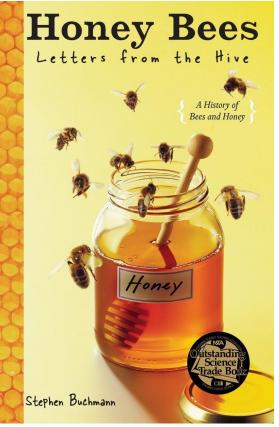


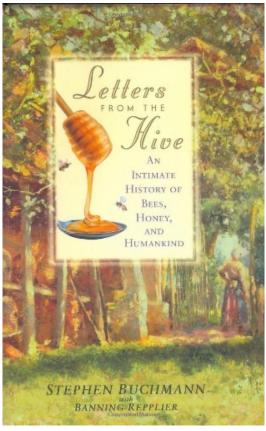


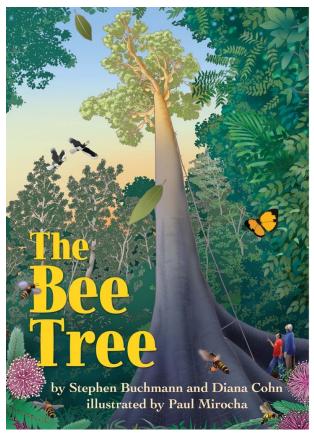


Also, a new Wikipedia page









(teen reader edition)

(a children's book)

### Some of my Earlier Bee Books

# I take pleasure in dedicating this talk to the late Charles Shipman, Geophysicist, Biological Technician

I was honored to be Charle's supervisor at the USDA-CHBRC in Tucson for 22 years, publishing many papers together.

Charles earned a degree in Geophysics from Stanford University before joining the Tucson bee lab working with Joe Moffett.

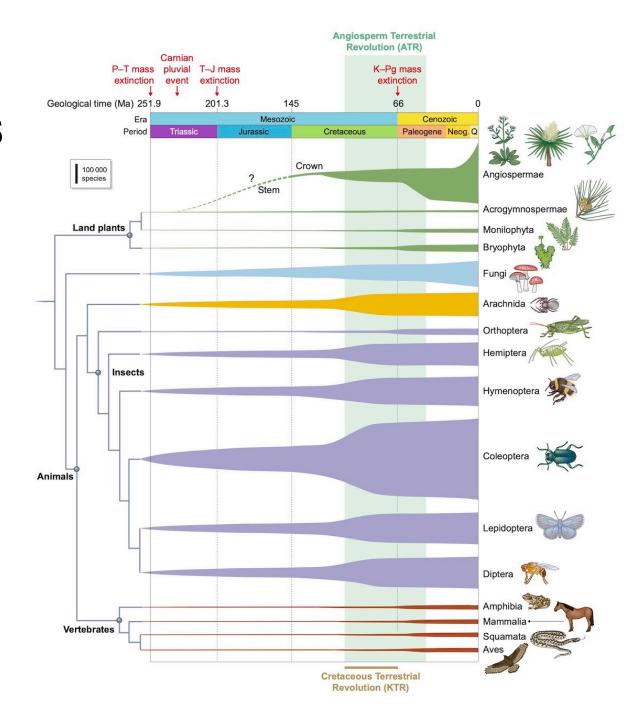


### Rise of Flowering Plants & Pollinators

Just look at the pale green vertical column.

Notice how all these pollinator groups exploded in biological diversity along with the rise of the flowering plants!

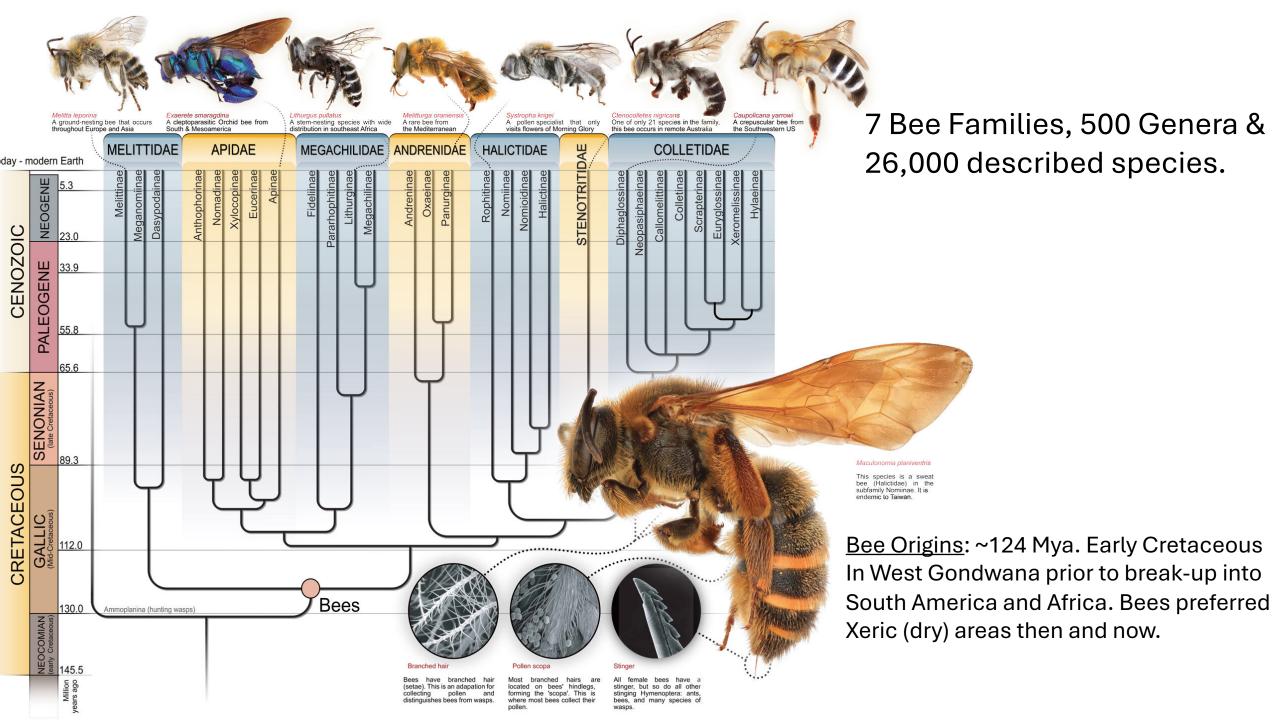
\*Coevolution & speciation

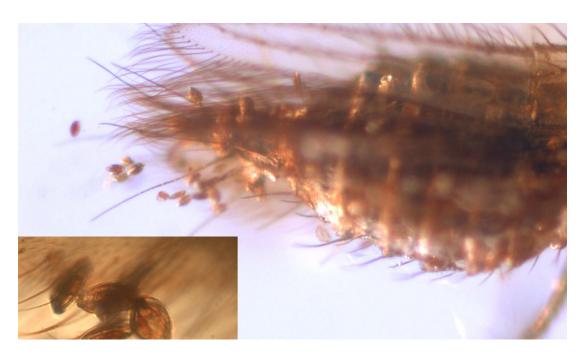


### Bee Diversity



Arizona has ~ 1,300 native bee species



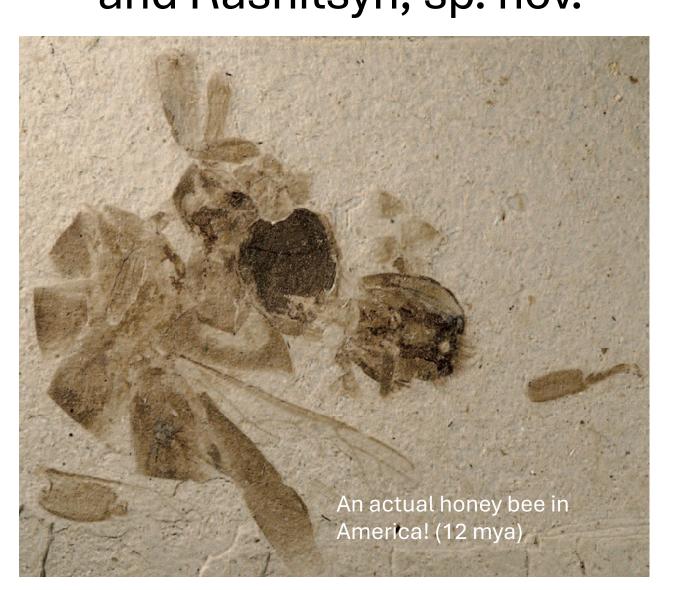




#### Bee Origins: Cretaceous Thripshunting Wasps

 Bees evolved from ancient predatory wasps that lived 130 million years ago. These wasps (Ammoplanina) hunted thrips covered in pollen. They are the closest living relatives of bees. Gradually, ancestors of these wasps gave up their "thrips meat treats" and foraged exclusively on pollen and nectar. Vegan Bees!

Apis (Cascapis) nearctica, Engel, Hinojosa-Diaz, and Rasnitsyn, sp. nov.



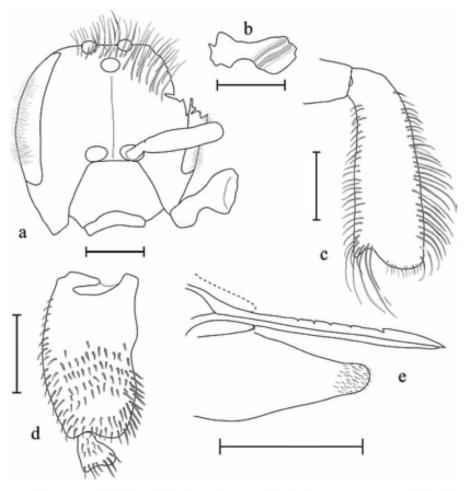
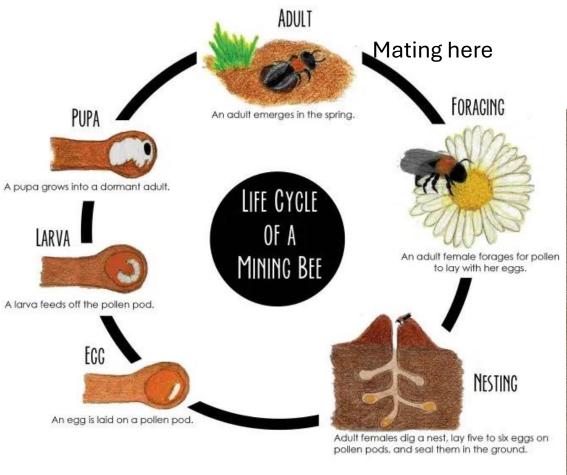
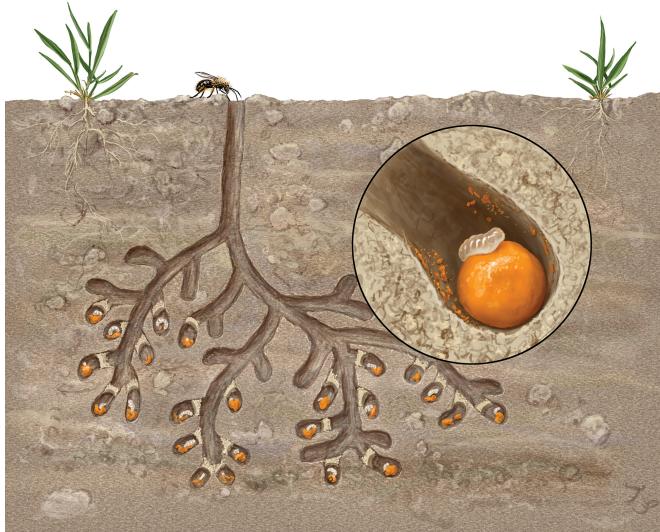


FIGURE 5. Line illustrations of holotype worker of *Apis* (*Cascapis*) nearctica sp. nov. (CAS #236) – a) head and left mandible; b) right mandible; c) metabasitarsus; d) corbicula; e) sting. Scale bars = 1 mm.

### A Typical Ground-Nesting Bee Life Cycle





#### **Most Bees Are Solitary Ground Nesters**

- Probably 90% of all bees nest in bare flat ground, or in banks or cliffs.
- About 10% nest in larger hollow cavities (trees, rocks), or in hollow or pithy twigs. Honey bees, bumblebees, leafcutter bees.
- Some make pebble and resin nests (Anthidiellum etc.).
- Other bees locate abandoned beetle burrows and move in as "renters." They can't dig their own nests in the wood like carpenter bees. These include leafcutter bees (*Megachile*, *Osmia*, *Hoplitis*, *Chelostoma*), and resin bees (e.g. *Heriades*).
- About 10% of bees make no nests (they are cleptoparasitic "cuckoo" bees that invade the nests of other bees. Think of cowbirds.



### Back to Buzzing & Vibrations:

#### Why Buzz?

- To stay warm (shivering flight muscles).
- To <u>incubate</u> brood (*Bombus* queens).
- To perform work (digging nests).
- A by-product of airborne <u>flight</u> sounds.
- <u>Defensive</u> (alarm buzzes) when caught by a bird, or pesky researcher.
- <u>Communication</u>, *Apis* waggle dance, *Bombus*, *Melipona* (intracolonial foraging signals).



#### Not All Bees Use Floral Sonication

- Small (*Lasioglossum*) to large bees (*Bombus*, *Xylocopa*) sonicate blooms to harvest pollen. \*82 bee genera in all 7 bee families.
- Not all bees sonicate flowers (e.g. <u>Apis</u> does not).
- Very common in Apidae (e.g. Anthophora, Centris etc.), with its ~ 5,900 spp.
- Rare in families like Megachilidae (only 1 Osmia, possibly 2 Megachile), and Andrenidae (only Protandrena and ~ 4 Andrena). Megs may be cute but they don't give good vibrations. :-(



### Non Bees that Sonicate (extremely rare!)

- Syrphid (flower flies): Copestylum mexicanum, Ornidia obessa, Aneriophora aureorufa.
- Masarid (pollen) wasps: Pseudomasaris, but needs confirmation.

### Floral Sonication by Female Bees



• Females bite into anthers and hang on. Leaves brown marks "bee kisses" on anthers.

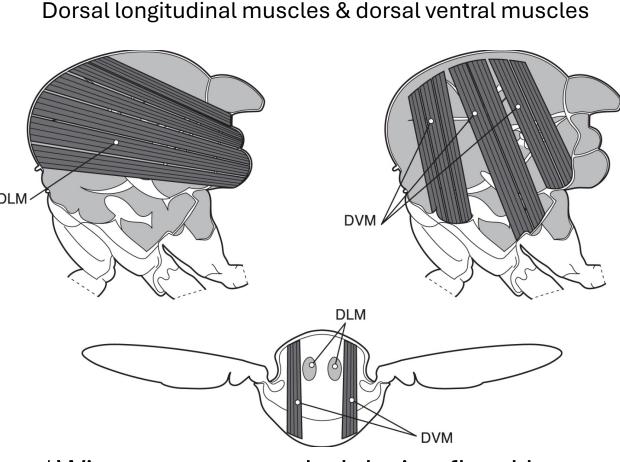
 Bees curl over pores. Pollen strikes legs and metasoma. Groom pollen into scopa while hovering or hanging by a leg.

Bees vibrate during each bite. Buzz, move to new anther, buzz again. Deliver up to 30 G acceleration to pollen.

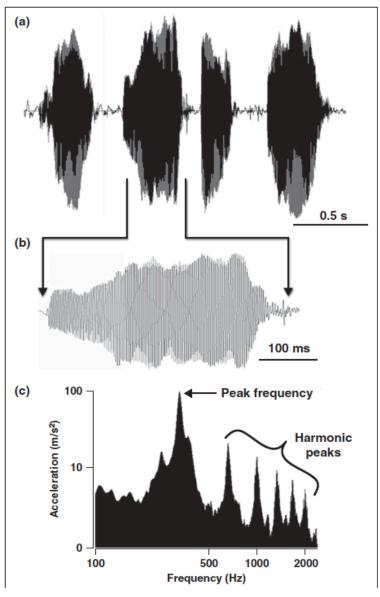
• Buzzes are 220 - 280 ms long. In the lab, 400+ buzzes in 200 seconds (*B. impatiens* on *Solanum*). Pollen ejected during first 3 - 5

### Vibrational Power Plant! The Indirect Flights Muscles: Bees are Living Tuning Forks)





\*Wings are uncoupled during floral buzzes and do not flap.



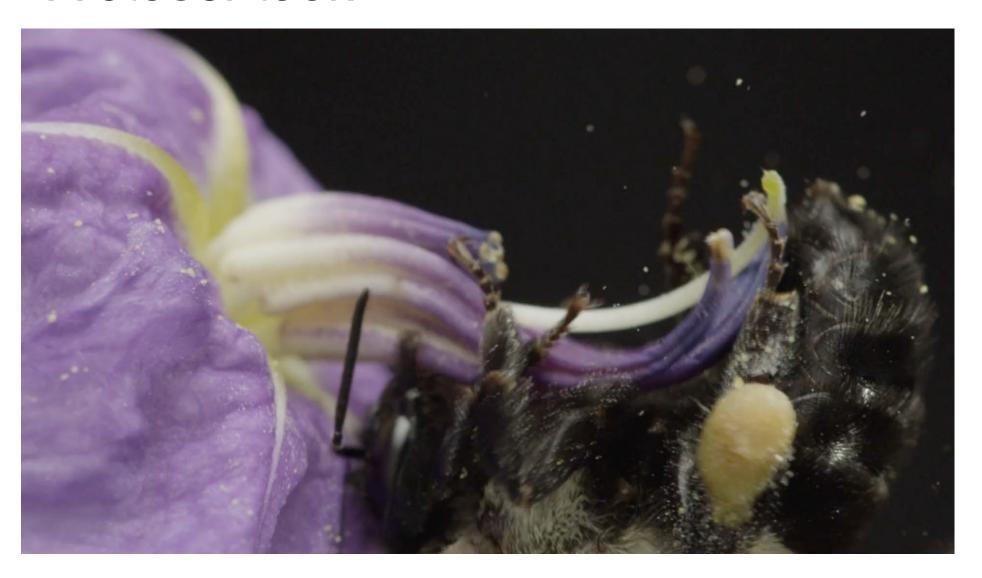
A typical set of floral sonicatory buzzes (B. terrestris on Solanum rostratum).

- (a) waveform, 4 buzzes
- (b) expanded view, 2nd buzz
- (c) Power (Fast Fourier Transform) spectrum of 2nd buzz.

\*The fundamental (or peak freq.) is 300 Hz. Note, five harmonic frequencies up to 2,000 Hz

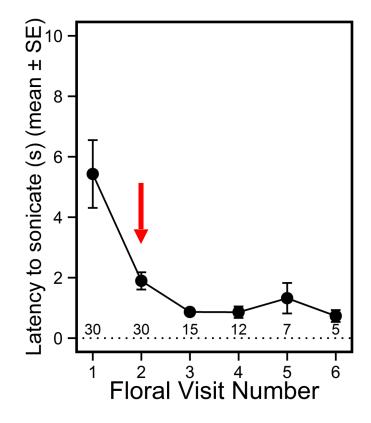


### A closer look



### I always believed Floral Sonication was Learned (Wrong!, its innate....)

Avery Russell experiments with naive lab-reared *Bombus impatiens* 





By the **2<sup>nd</sup> floral visit**, the latency to sonicate is at its minimum

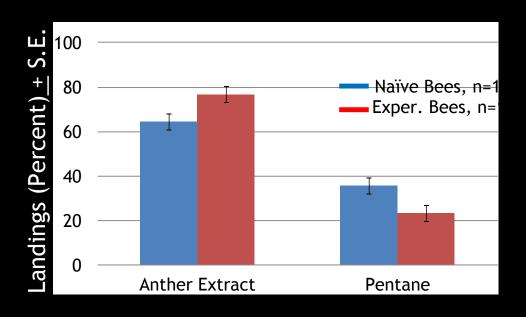
### What Floral Cues Elicit Buzzing: Visual, Tactile, Odors?

### Bees Land and Buzz Extract-Treated Foam Anthers

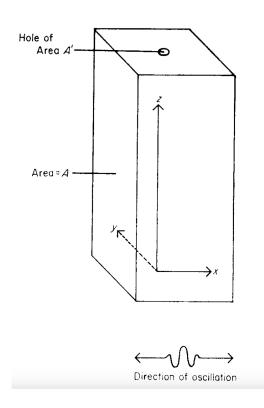
#### **Anther Solvent Extracts**

- 300 anthers into pentane solvent for 5 min
- Applied at 6x single flower equivalent to surrogate yellow foam anthers





### A First Biophysical Model of Pollen Ejection During Buzz Pollination (Buchmann & Hurley, 1978)



Buchmann, S.L., J.P. Hurley. 1978. A Biophysical Model for Buzz Pollination in Angiosperms. J. theor. Biol. 72:639 - 657.

ponent of velocity lies between  $v_x$  and  $v_x + dv_x$  then the number of such particles which strike the wall of area A during the time  $\tau/2$  is given by:

$$A(V-v_x)\frac{\tau}{2}n(v_x)\,\mathrm{d}v_x.$$

Thus, the net increase in energy due to the advancing anther wall is

$$\Delta E_{+} = \int_{-\infty}^{V} \left[ \frac{1}{2} m (2V - v_{x})^{2} - \frac{1}{2} m v_{x}^{2} \right] A(V - v_{x}) \frac{\tau}{2} n(v_{x}) \, \mathrm{d}v_{x}. \tag{3}$$

Particles whose x-component of velocity is greater than V will not impact with the wall and hence the upper limit on the integral is V.

Similarly, the energy loss due to particles striking the receding wall during the second half of the cycle is given by

$$\Delta E_{-} = \int_{-\infty}^{-V} \left[ \frac{1}{2} m (-2V - v_{x})^{2} - \frac{1}{2} m v_{x}^{2} \right] A (-v_{x} - V) \frac{\tau}{2} n(v_{x}) dv_{x}.$$

Since  $n(v_x)$  is an even function of  $v_x$ , we may write

$$\Delta E_{-} = \int_{V}^{\infty} \left[ \frac{1}{2} m (2V - v_{x})^{2} - \frac{1}{2} m v_{x}^{2} \right] A(V - v_{x}) \frac{\tau}{2} n(v_{x}) dv. \tag{4}$$

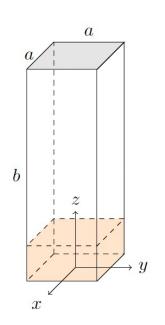
By adding equations (3) and (4) we obtain the net energy gain over one complete cycle

$$\Delta E = \int_{-\infty}^{\infty} \left[ \frac{1}{2} m (2V - v_x)^2 - \frac{1}{2} m v_x^2 \right] A(V - v_x) \frac{\tau}{2} n(v_x) \, dv_x.$$

Never mind the math!

### New (2024) Pollen Expulsion Biophysics Modelling from Jankauski lab (MSU, Bozeman)

By: Caelen Boucher-Bergstedt, Mark Jankauski, Erick Johnson



.5

pollen grains inside one anther. Virtually all grains expelled in 0.5 second.

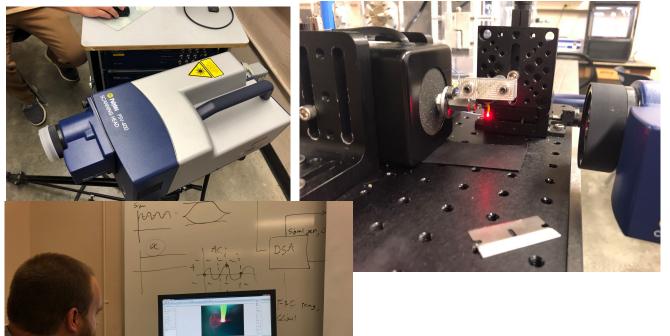
Model system: 10,000

**FIG. 4.** Examples of both poricidal and pseudoporicidal geometries pollen expulsion at  $\eta=0.4~mm$  and  $\omega_1=150~Hz$ . The thin lines designate the initial expulsion rate of the particles,  $\dot{P}$ , seen in FIG. 5.

(a) Base Geometry

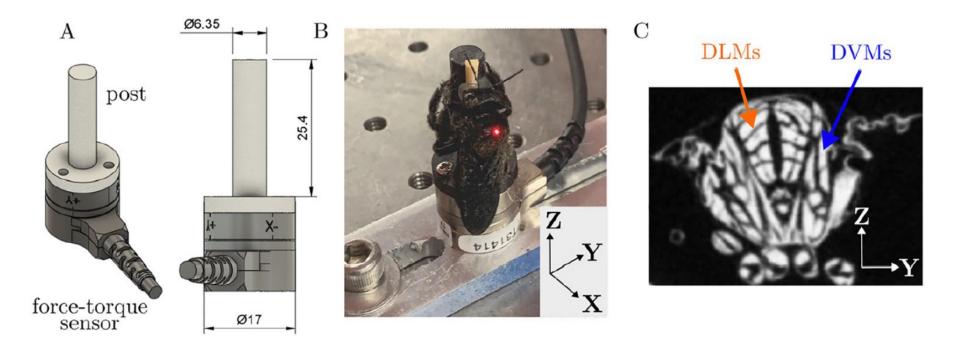
With a new NSF engineering grant in the Jankauski lab, we are investigating vibrations made by bees and delivered to anthers.

Jankauski, Cox, Johnson (Mont. State Univ) & Buchmann \*A new \$750K NSF 3 yr. grant to examine Solanum anther biomechanics.





New Research with Mark Jankauski (MSU) and Kathryn Busby (UA) Defensive Buzzing in *Xylocopa* & Biophysics of Anther Vibration and Pollen Expulsion



**Figure 2.** Experimental set-up. (**A**) CAD model. Dimensions are in millimeters. (**B**) Carpenter bee mounted to a carbon fiber post. Note the vibrometer laser spot on the dorsal surface of the bee thorax. The cartesian basis shown in the lower right corner defines force directions. The x and z axes most closely align with the insect's DVM and DLM muscle groups, respectively. (**C**) MicroCT scan shows a transverse cross section of the insect thorax with flight musculature. The DVM and DLM muscle groups are indicated by arrows. Note that the Jankauski, Casey, Heveran, Busby, Buchmann. 2022. Carpenter bee thorax vibration and force generation

Inform pollen release mechanisms during floral buzzing. Scientific Reports, 12(1):12654.

#### From: "What a Bee Knows"

- Bees are self-aware, they're sentient and likely have a primitive form of consciousness.
- They solve problems and can think. Memory can last days.
- May have a simple form of subjective experiences.
- They can discriminate between complex geometric shapes, even recognizing human faces.
- Bees can count, use tools, have spatial memory and can learn to navigate mazes. Bees have selective attention.
- Bees learn to associate colors and odors with rewards.
- Can seemingly plan ahead (resin mines or biting leaf holes).



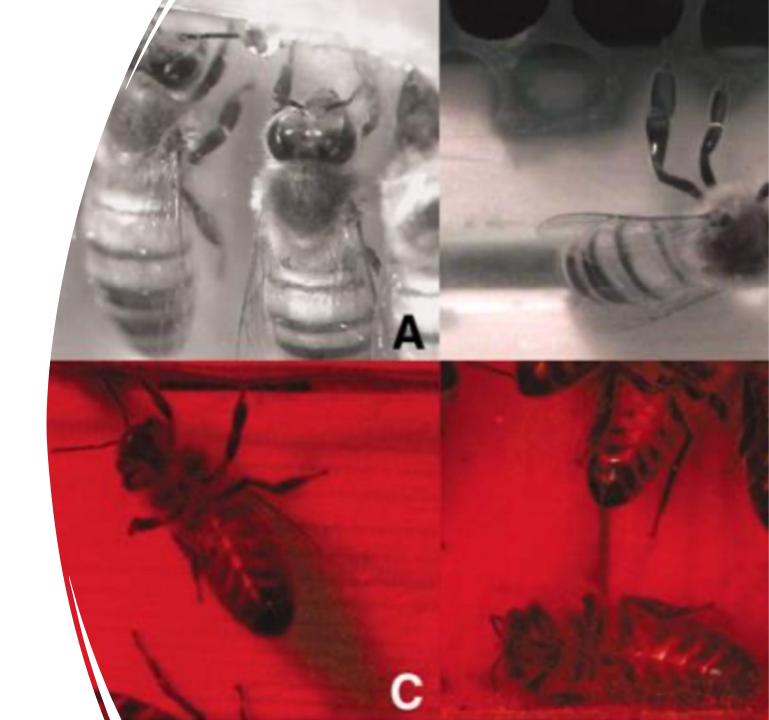
### Another Bee Vision Super Power: "Flicker Fusion Frequency"

- Bees see their world while flying fast (15 mph). For us, still images projected on a movie screen only need to be shown 20 – 24 fps, and they appear as continuous motion.
- For a bee, you would need to speed up the projector to about 200 to 250 fps. A movie-goer bee would be bored.
- Next time you try to swat a fly, remember that you are moving in slow motion and its easy for the fly to escape.



### **Bees Sleep**

- Bees sleep a lot.
- They have characteristic sleep postures, body parts droop with respect to gravity.
- Memories are likely consolidated during their sleep periods.
- We are far from understanding what happens when humans sleep!

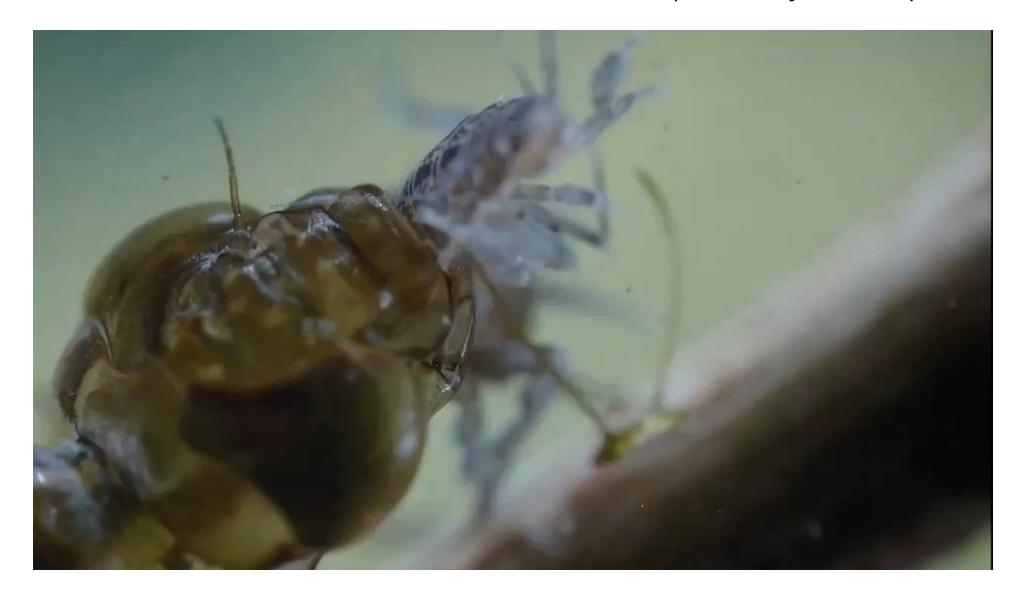


### Do Bees Dream?

- We don't know. No current way to do a mini MRI on their brains during sleep.
- I'd like to think they might dream. Perhaps about a rich field of flowers and its tasty delights...



#### From the TV Series PLANET INSECT (Curiosity Stream)



### Bee Husbandry: What Can You Do?

- Plant locally-adapted <u>native plants</u> for bees.
   Year-round blooms. Avoid big box store nursery plants or hybrids (may contain systemic insecticides) and they may not have adequate pollen and nectar.
- Avoid insecticides and herbicides.
- Provide bare ground in some areas.
- Consider creating a small mud source.
- Leave dead branches or even dead trees (beetle holes for bees, good wildlife habitat) in place.
- Consider making or buying bee hotels and placing them.



#### **Habitats for Beemanity!**

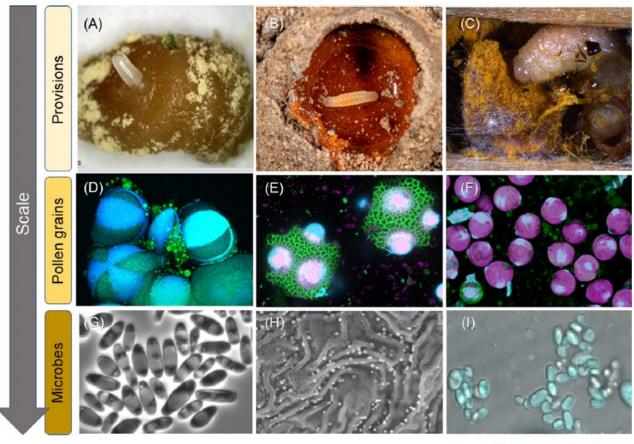
(Tucson Botanical Gardens)

 Several years ago I was honored to work with Tucson landscape architect and bee lover Greg Corman to create a solitary bees habitat at TBG.



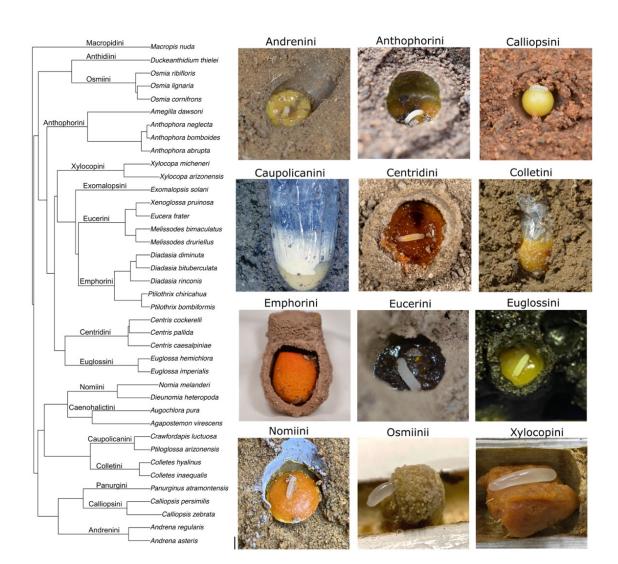


Solitary Bees Need Gut Microbiomes (bacteria, fungi) Just Like Us... to Stay Healthy

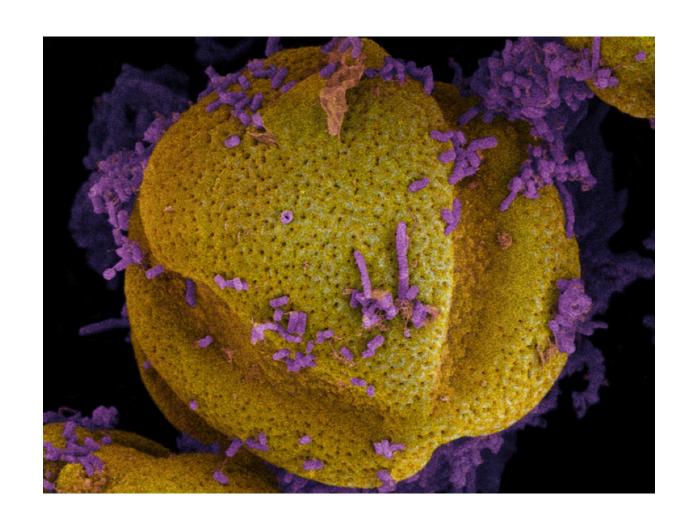


Trends in Ecology & Evolution

### Bee Brood Cells Contain Pollen + Nectar, sometimes floral oils & lots of microbes!



### Bacterial cells on outside of pollen grain



### Functions in bee biology and health

- Pathogen protection (bacterial, fungal, potentially RNA viruses)
- Protection against bacterial pathogens (H. alvei, E. coli)
- Protection against *Crithidia bombi*, *Bombella api*s. Specific antifungal metabolites.
- Unclear: protection against *Vairimorpha* (formerly *Nosema*).
- Role in development and behavior

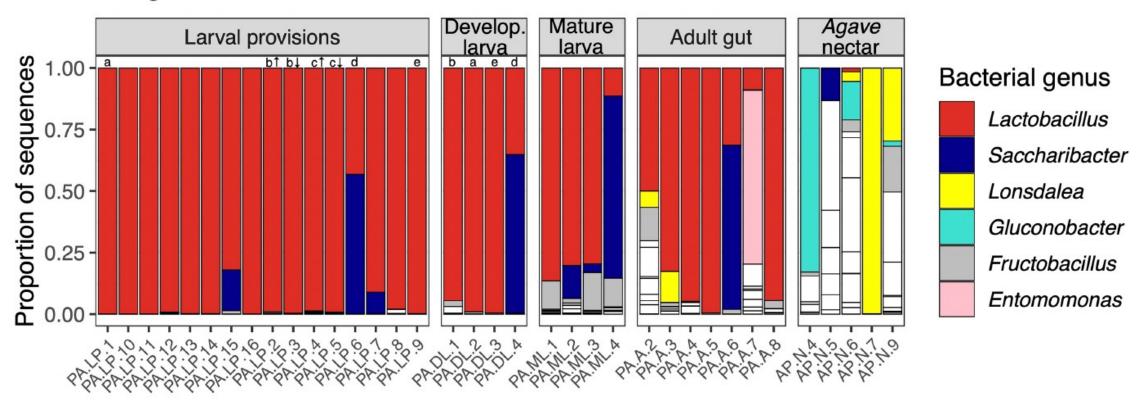
Bee bread from honey bees (*Apis mellifera*) contains a diverse community of bacteria, with the most consistently reported and abundant groups being:

- Lactic acid bacteria: These include several species of the genus Lactobacillus (such as Lactobacillus kunkeei, L. apis, and L. mellis) and Bifidobacterium spp. These are considered core symbionts of honey bees and are involved in fermentation and preservation of bee bread
   1 8 . Lactobacillus kunkeei and Acetobacteraceae (often referred to as "Alpha 2.2") are highly osmotolerant and acid-resistant bacteria especially common in stored pollen and bee bread
   5 .
- Bacillus species: *Bacillus* spp. are regularly found in bee bread and related hive products, contributing to fermentation and possibly protecting pollen stores from spoilage 1 3 6 7.
- Other Firmicutes: Families such as Clostridiaceae and Enterococcaceae (e.g., *Enterococcus* spp.) are also dominant in bee bread 3.
- **Proteobacteria**: This phylum is also well represented, including genera such as *Acinetobacter*, *Buttiauxella*, *Pantoea*, and *Oenococcus* 3 10.
- **Actinobacteria**: Representatives such as *Streptomyces* appear in bee bread, where they may help inhibit fungal growth and contribute to preservation 5.
- Other bacteria: Additional genera detected include *Frischella*, *Commensalibacter*, *Bombella*, and *Bartonella*, though these are more commonly part of the bee gut community and their prevalence in bee bread varies with environment and season 2 9.

## Lactic Acid Bacteria in *Ptilolossa* Bees

 \*Some of the same bacteria found in your breakfast cup of yogurt.

#### Ptiloglossa dataset



I'm part of a Brood Cell Microbiome Research Group at Cornell, UA, UW, UCSD, UCR, UCD



#### **OPEN ACCESS**

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SPECIALTY SECTION

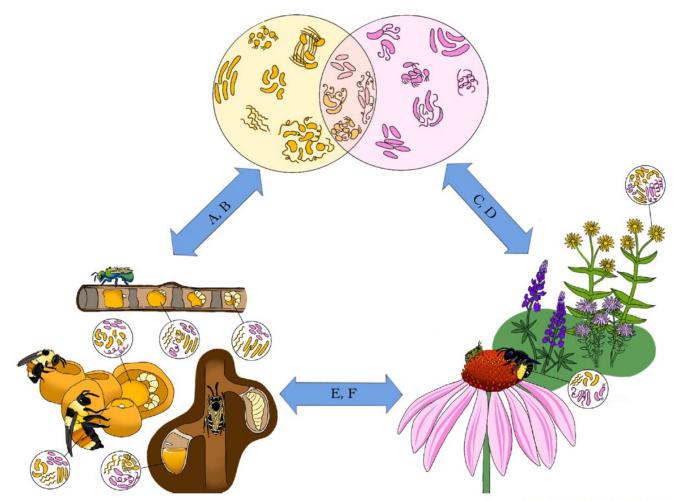
This article was submitted to Microbial Symbioses,

# Bee breweries: The unusually fermentative, lactobacilli-dominated brood cell microbiomes of cellophane bees

Tobin J. Hammer<sup>1\*</sup>, Jordan Kueneman<sup>2,3</sup>, Magda Argueta-Guzmán<sup>4</sup>, Quinn S. McFrederick<sup>4</sup>, Lady Grant<sup>5</sup>, William Wcislo<sup>3</sup>, Stephen Buchmann<sup>6,7</sup> and Bryan N. Danforth<sup>2</sup>

<sup>1</sup>Department of Ecology and Evolutionary Biology, University of California, Irvine, Irvine, CA, United States, <sup>2</sup>Department of Entomology, Cornell University, Ithaca, NY, United States, <sup>3</sup>Smithsonian Tropical Research Institute, Panama City, Panama, <sup>4</sup>Department of Entomology, University of California, Riverside, Riverside, CA, United States, <sup>5</sup>Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO, United States, <sup>6</sup>Department of Entomology, The University of Arizona, Tucson, AZ, United States, <sup>7</sup>Department of Ecology and Evolutionary Biology, The University of Arizona, Tucson, AZ, United States

## Microbes: the "silent third partners" of beeangiosperm mutualisms



#### ARTICLE IN PRESS

## Trends in **Ecology & Evolution**



#### Review

## Microbes, the 'silent third partners' of bee-angiosperm mutualisms

Shawn A. Steffan , <sup>1,2,\*</sup> Prarthana S. Dharampal, <sup>2</sup> Jordan G. Kueneman, <sup>3</sup> Alexander Keller, <sup>4</sup> Magda P. Argueta-Guzmán, <sup>5</sup> Quinn S. McFrederick, <sup>5</sup> Stephen L. Buchmann, <sup>6,9</sup> Rachel L. Vannette, <sup>7</sup> Anna F. Edlund, <sup>8</sup> Celeste C. Mezera, <sup>2</sup> Nolan Amon, <sup>2</sup> and Bryan N. Danforth <sup>3</sup>

While bee–angiosperm mutualisms are widely recognized as foundational partnerships that have shaped the diversity and structure of terrestrial ecosystems, these ancient mutualisms have been underpinned by 'silent third partners': microbes. Here, we propose reframing the canonical bee–angiosperm partnership as a three-way mutualism between bees, microbes, and angiosperms. This new conceptualization casts microbes as active symbionts, processing and protecting pollen–nectar provisions, consolidating nutrients for bee larvae, enhancing floral attractancy, facilitating plant fertilization, and defending bees and plants from pathogens. In exchange, bees and angiosperms provide their microbial associates with food, shelter, and transportation. Such microbial communities represent co-equal partners in tripartite mutualisms with bees and angiosperms, facilitating one of the most important ecological partnerships on land.

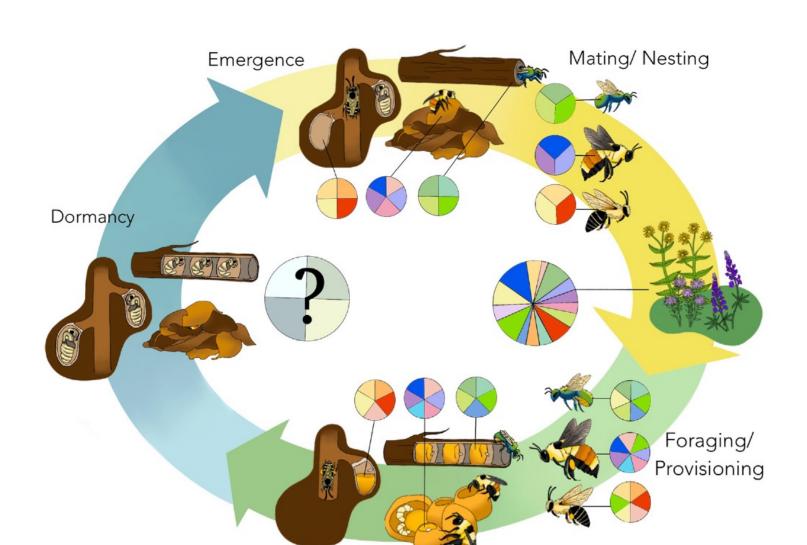
#### Highlights

Bee and angiosperm communities engage in myriad symbioses with microbial communities, conferring benefits, enduring costs, and exchanging fitness trade-offs.

Microbiota facilitate bee development by processing, protecting, and preserving the pollen provisions of young bees, while suppressing pathogen establishment. For angiosperms, microbes influence floral attractancy, increase fertilization, and antagonize pathogens.

Bee and angiosperm communities

## Annual cycling & movement of bee, microbes, flowering plants



### Martha Gilliam, honey bee microbiologist

- 1980's, Martha Gilliam, Brenda Lorenz, Buchmann, Roubik
- Gilliamella apicolla named in her honor, 2012
- 1980's, Klungenness, Peng (UCD)
- Later, Kirk Anderson at CHBRC, Apis results
- Controversy about whether microbes "pre-digest" pollen in bee bread, or if its just a preservational environment due to nectar etc.
- Role of probiotic supplements not clear (at least not yet)
- Recent summary paper by Erick Motta and Nancy Moran

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Review Article | Published: 04 December 2023

#### The honeybee microbiota and its impact on health and disease

Erick V. S. Motta & Nancy A. Moran ☑

Nature Reviews Microbiology 22, 122–137 (2024) Cite this article

10k Accesses | 86 Citations | 90 Altmetric | Metrics

#### **Abstract**

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Honeybees (*Apis mellifera*) are key pollinators that support global agriculture and are long-established models for developmental and behavioural research.

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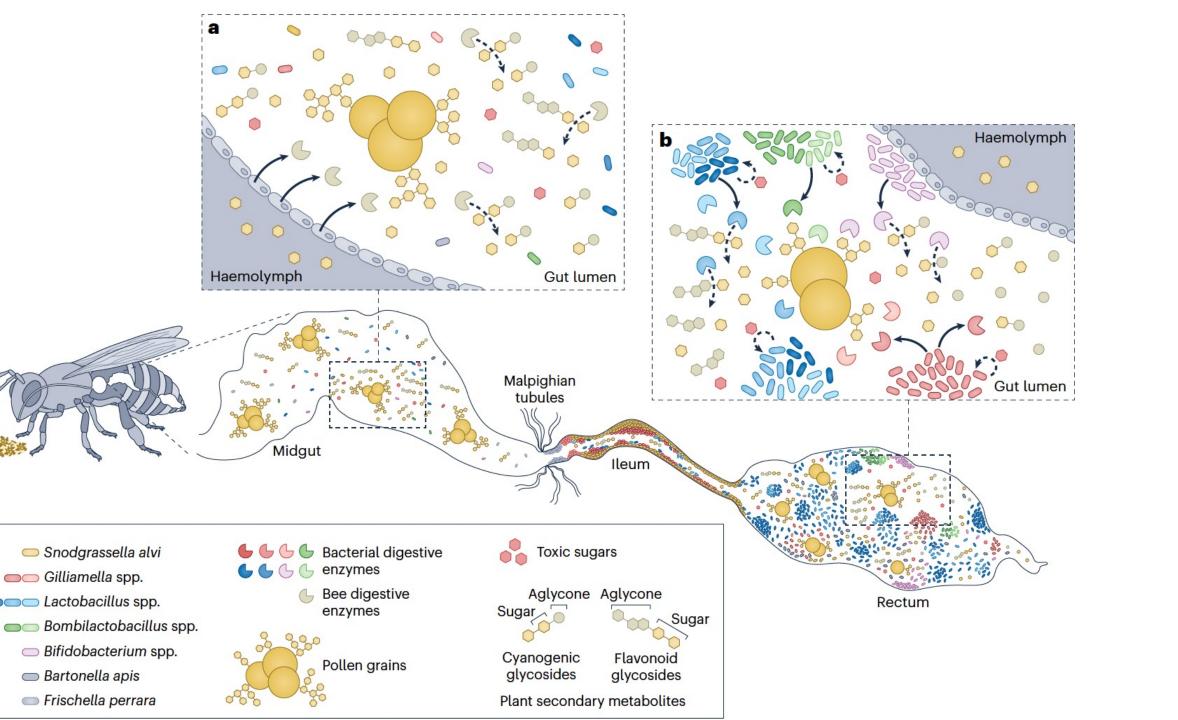
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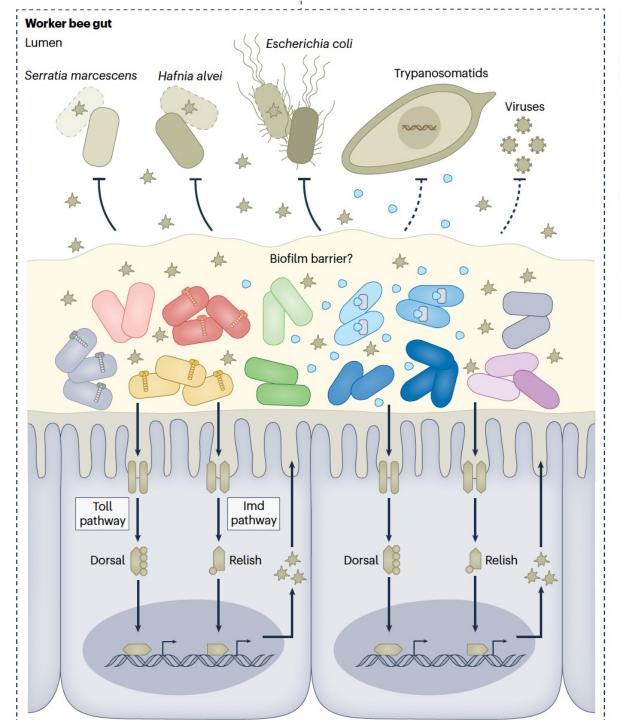
#### **Associated content**

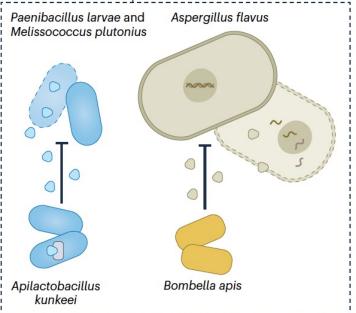
**Series** 

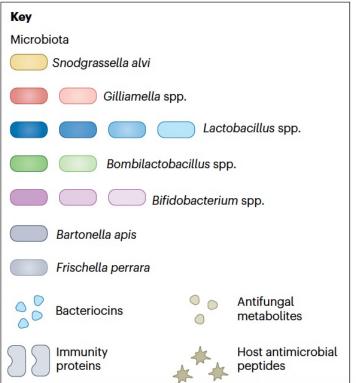
#### **Microbiome**

Sections **Figures** References

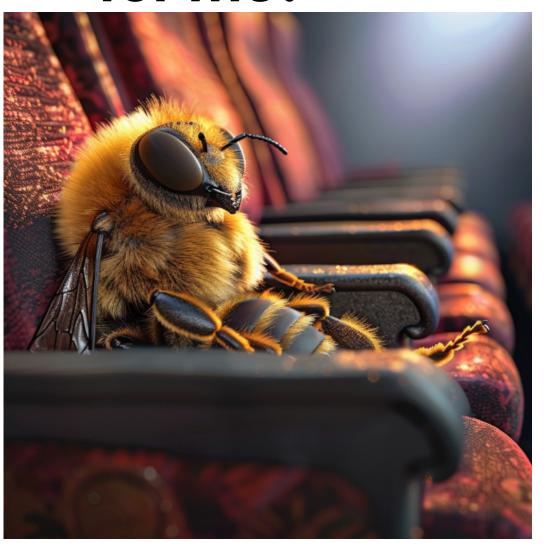








## Bee Questions for me?





Oil-collecting bee: Centris caesalpiniae

#### Potential for probiotics in bees

The use of probiotics that aim to treat or prevent microbial infections in hives is common in beekeeping. Recent reviews have summarized the studies in the bee probiotics field<sup>51,159</sup>. Most commercially available bee probiotics consist of non-native microorganisms, including bacteria and fungi from the food industry, which are marketed as promoting bee health, although they do not stably colonize bees 51,160. An alternative approach involves probiotics that consist of native microorganisms that colonize and persist in the bee gut<sup>51</sup>. Orally delivered gut homogenates are one way to transfer bacteria from healthy worker bees to bees that lack microbiota or those with perturbed microbiota. Gut homogenate treatments lead to stable colonization in young bees under laboratory conditions, but potentially introduce pathogens from donor bees. Defined communities of isolates of native core bacteria are another approach 48,70,82,125. Such defined communities can counteract perturbations caused by agrochemicals and other environmental stressors and prevent the proliferation of opportunistic pathogens that often follows perturbation 48,82,125. However, these studies have been primarily conducted in laboratory settings, and further hive-level studies us massassaure to avaluate the affice are of muchicities for healessains

#### **Dr. Anderson's Conclusions**

Our combined results do not support the hypothesis that hive-stored pollen of honey bees involves nutrient conversion or predigestion by microbes prior to consumption.

- The bacterial communities found in hive-stored pollen did not differ from those of newly collected pollen, but both sample types varied significantly by season. This result indicates the lack of an emergent 'core' bacterial community co-evolved to predigest pollen.
- Relative to other plant material involving microbial digestion or extensive fermentation, hive-stored pollen contains very few microbes.
- The absolute number of bacteria in hive-stored pollen decreases with storage time, indicating that it is not a suitable medium for microbial growth.
- The preferential consumption of freshly collected pollen indicates that bees have not evolved to rely on microbes or other time-related factors for pollen predigestion.
- The microbe to pollen grain ratio is many orders of magnitude removed from that required to alter hive-stored pollen.
- Regardless of sampled season or the taxonomic character of microbial communities, microscopic examination revealed no intermediate stage of pollen digestion in hive-stored pollen.

Based on these collective findings, we suggest that stored pollen is a preservative environment governed largely by nonmicrobial additions of nectar, honey and bee glandular secretions.