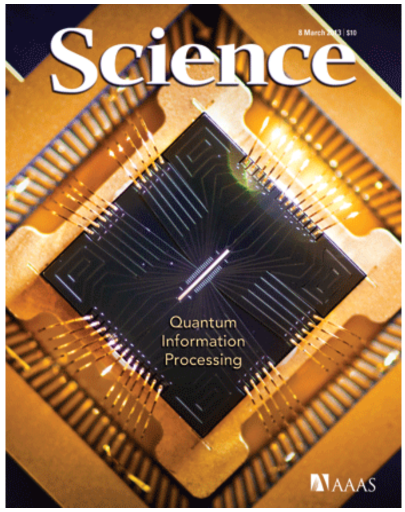
Teacher Resource for:

Caffeine in Floral Nectar Enhances a Pollinator's Memory of Reward



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GENERAL USE OF *Science* in the Classroom

Student Learning Goals:

"One fundamental goal for K-12 science education is a scientifically literate person who can understand the nature of scientific knowledge."[1]

The U.S. National Academy of Sciences defines science as: "Any new finding requires independent testing before it is accepted as scientific knowledge; a scientist is therefore required to honestly and openly report results so that they can readily be repeated, challenged, and built upon by other scientists. Proceeding in this way over centuries, the community effort that we call science has developed an increasingly accurate understanding of how the world works. To do so, it has had to reject all dogmatic claims based on authority, insisting instead that there be reproducible evidence for any scientific claim."

An important student learning goal, central to any understanding of "the nature of scientific knowledge," is to give each student an appreciation of how science is done.

This includes knowing why:

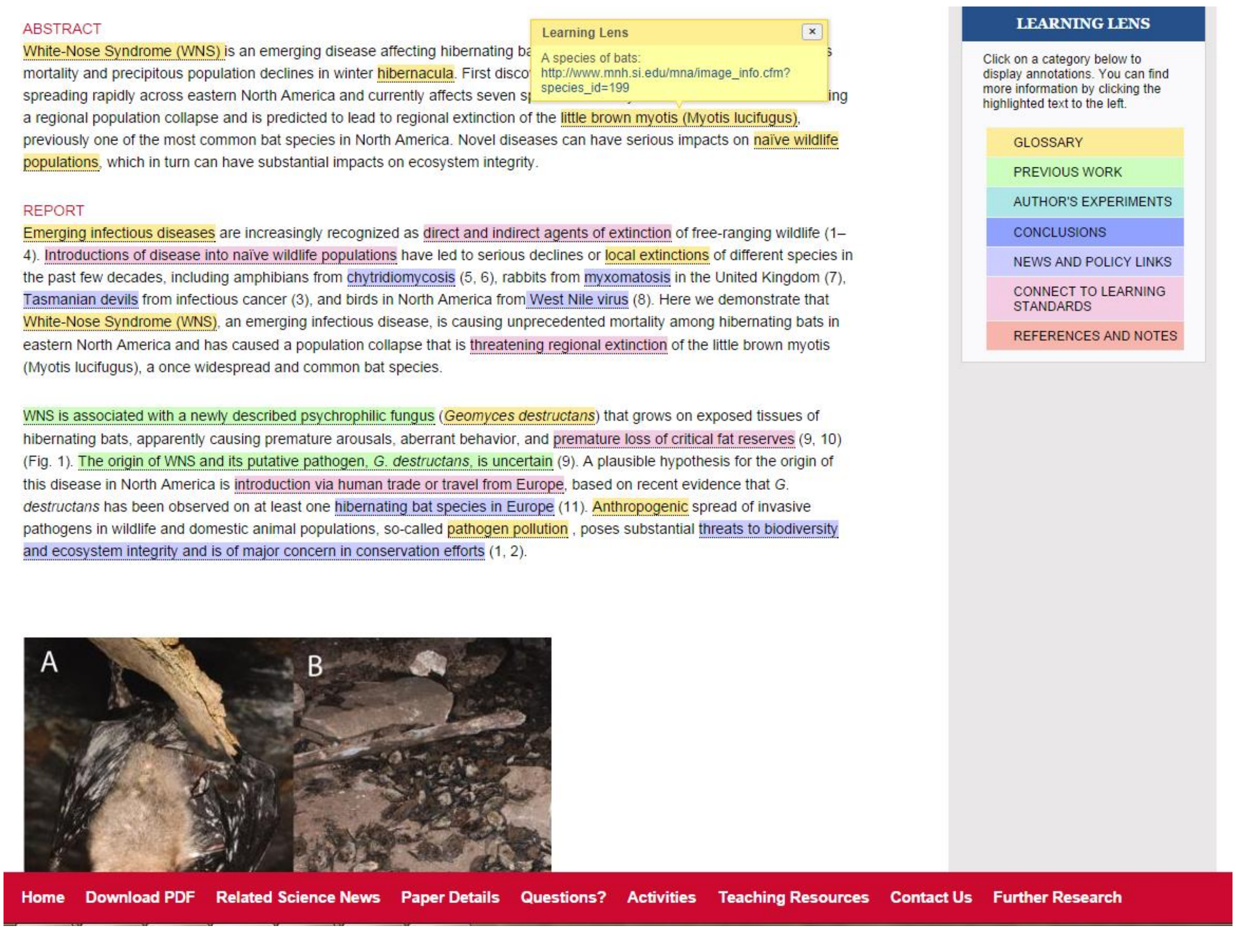
- Scientists must be independent thinkers, who are free to dissent from what the majority believes.
- Science can deal only with issues for which testable evidence can be obtained.
- All scientific understandings are built on previous work
- It is to be expected that one scientist's conclusions will sometimes contradict the conclusions of other scientists.
- Science is a never-ending venture, as the results from one study always lead to more questions to investigate.

[1] *A Framework for K-12 Science Education*, National Research Council, 2012

Using This Resource

<u>Learning Lens:</u>

The Learning Lens tool can be found on the right sidebar of each resource and is the source of annotations. Click on the headings to highlight portions of the text of the corresponding research article. A subsequent click on the highlighted text will produce a text box containing more information about that particular piece of text. Below is an example of the Glossary function of the Learning Lens.


ABSTRACT

White-Nose Syndrome (WNS) is an emerging disease affecting hibernating ba… mortality and precipitous population declines in winter hibernacula. First disco… spreading rapidly across eastern North America and currently affects seven s… ing a regional population collapse and is predicted to lead to regional extinction of the little brown myotis (Myotis lucifugus), previously one of the most common bat species in North America. Novel diseases can have serious impacts on naïve wildlife populations, which in turn can have substantial impacts on ecosystem integrity.

Learning Lens

A species of bats: http://www.mnh.si.edu/mna/image_info.cfm?species_id=199

REPORT

Emerging infectious diseases are increasingly recognized as direct and indirect agents of extinction of free-ranging wildlife (1–4). Introductions of disease into naïve wildlife populations have led to serious declines or local extinctions of different species in the past few decades, including amphibians from chytridiomycosis (5, 6), rabbits from myxomatosis in the United Kingdom (7), Tasmanian devils from infectious cancer (3), and birds in North America from West Nile virus (8). Here we demonstrate that White-Nose Syndrome (WNS), an emerging infectious disease, is causing unprecedented mortality among hibernating bats in eastern North America and has caused a population collapse that is threatening regional extinction of the little brown myotis (Myotis lucifugus), a once widespread and common bat species.

WNS is associated with a newly described psychrophilic fungus (*Geomyces destructans*) that grows on exposed tissues of hibernating bats, apparently causing premature arousals, aberrant behavior, and premature loss of critical fat reserves (9, 10) (Fig. 1). The origin of WNS and its putative pathogen, *G. destructans*, is uncertain (9). A plausible hypothesis for the origin of this disease in North America is introduction via human trade or travel from Europe, based on recent evidence that *G. destructans* has been observed on at least one hibernating bat species in Europe (11). Anthropogenic spread of invasive pathogens in wildlife and domestic animal populations, so-called pathogen pollution, poses substantial threats to biodiversity and ecosystem integrity and is of major concern in conservation efforts (1, 2).

A B

LEARNING LENS

Click on a category below to display annotations. You can find more information by clicking the highlighted text to the left.

GLOSSARY
PREVIOUS WORK
AUTHOR'S EXPERIMENTS
CONCLUSIONS
NEWS AND POLICY LINKS
CONNECT TO LEARNING STANDARDS
REFERENCES AND NOTES

Home | Download PDF | Related Science News | Paper Details | Questions? | Activities | Teaching Resources | Contact Us | Further Research

An example of the resource with the Glossary, Previous Work, Author's Experiments, News and Policy Links, and References and Notes tools turned on. The Glossary tool is in use.

Learning Notes:

Learning Notes accompany each figure and are designed to help students deconstruct the methods and data analysis contained within each figure.

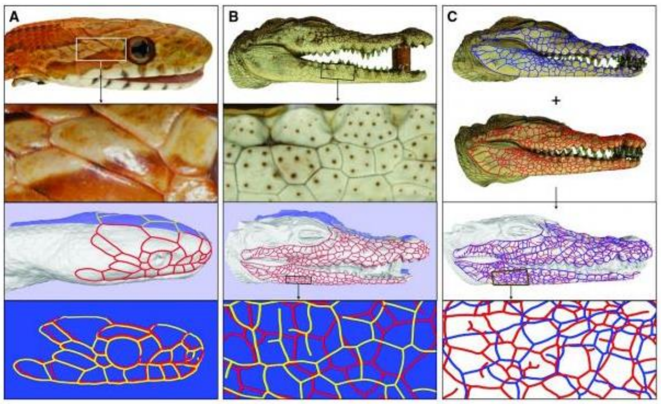


Fig. 1. **Spatial distribution of head scales. (A) Head scales in most snakes (here, a corn snake) are polygons (two upper panels) with stereotyped spatial distribution (two lower panels): left (yellow) and right (red) scale edges overlap when reflected across the sagittal plane (blue). (B) Polygonal head scales in crocodiles have a largely random spatial distribution without symmetrical correspondence between left and right. (C) Head scales from different individuals have different distributions of scales' sizes and localizations (blue and red edges from top and bottom crocodiles, respectively).**

Method: 3D geometry and color-texture reconstruction | Panel A | Panel B | Panel C

The authors took 120 color pictures of each animal to create detailed, three-dimensional models of reptile heads. Watch this video in which the authors further explain their modeling methods:

http://www.sciencemag.org/content/suppl/2012/11/29/science.1226265.DC1/1...

LEARNING LENS

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- GLOSSARY
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- NEWS AND POLICY LINKS
- CONNECT TO LEARNING STANDARDS
- REFERENCES AND NOTES

References:

The Reference section of each resource is annotated with a short statement about how or why each reference relates to the current research study.

6. L. Lewejohann, B. Zipser, N. Sachser, “Personality” in laboratory mice used for biomedical research: A way of understanding variability? Dev. Psychobiol. 53, 624 (2011).
7. G. Kempermann, The neurogenic reserve hypothesis: What is adult hippocampal neurogenesis good for? Trends Neurosci. 31, 163 (2008).
8. Lewejohann et al., Behavioral phenotyping of a murine model of Alzheimer's disease in a seminaturalistic environment using RFID tracking. Behav. Res. Methods 41, 850 (2009).
9. B. Steineret al., Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. Glia 46, 41 (2004).
10. Garthe, J. Behr, G. Kempermann, Ad flexible use of spatially precise learning strategies. PLoS ONE 4, e54
11. G. Kempermann, New neurons for ‘su 27 (2012).
12. G. Kempermannet al., Why and how brain plasticity. Front Neurosci 4, 189 (2010).

Learning Lens

This paper showed that while both physical activity, like running, and living in an enriched environment can result in the generation of new hippocampal neurons in mice, a combination of the two activities leads to even greater rates of neurogenesis.

13. K. Fabelet al., Additive effects of physical exercise and environmental enrichment on adult hippocampal neurogenesis in mice. Front. Neurosci. 3, 50 (2009).
14. Amrein, H. P. Lipp, Adult hippocampal neurogenesis of mammals: Evolution and life history. Biol. Lett. 5, 141 (2009).
15. G. Kempermann, Why new neurons? Possible functions for adult hippocampal neurogenesis. J. Neurosci. 22, 635 (2002).
16. C. Crabbe, D. Wahlsten, B. C. Dudek, Genetics of mouse behavior: Interactions with laboratory environment. Science 284, 1670 (1999).
17. Lewejohann et al., Environmental bias? Effects of housing conditions, laboratory environment and experimenter on behavioral tests. Genes Brain Behav. 5, 64 (2006).
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19. R. Lathe, The individuality of mice. Genes Brain Behav. 3, 317 (2004).
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21. R. P. Talenset al., Epigenetic variation during the adult lifespan: Cross-sectional and longitudinal data on monozygotic twin pairs. Aging Cell 11, 694 (2012).
22. P. B. Baltes, J. R. Nesselroade, S. W. Cornelius, Multivariate antecedents of structural change in development: A simulation of cumulative environmental patterns. Multivariate Behav. Res. 13, 127 (1978).
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26. D. P. Wolferet al., Laboratory animal welfare: Cage enrichment and mouse behaviour. Nature 432, 821 (2004).
27. K. Lewin, Dynamic Theory of Personality (McGraw-Hill, New York, 1935).
28. K. L. Jang, R. R. McCrae, A. Angleitner, R. Riemann, W. J. Livesley, Heritability of facet-level traits in a cross-cultural twin

LEARNING LENS

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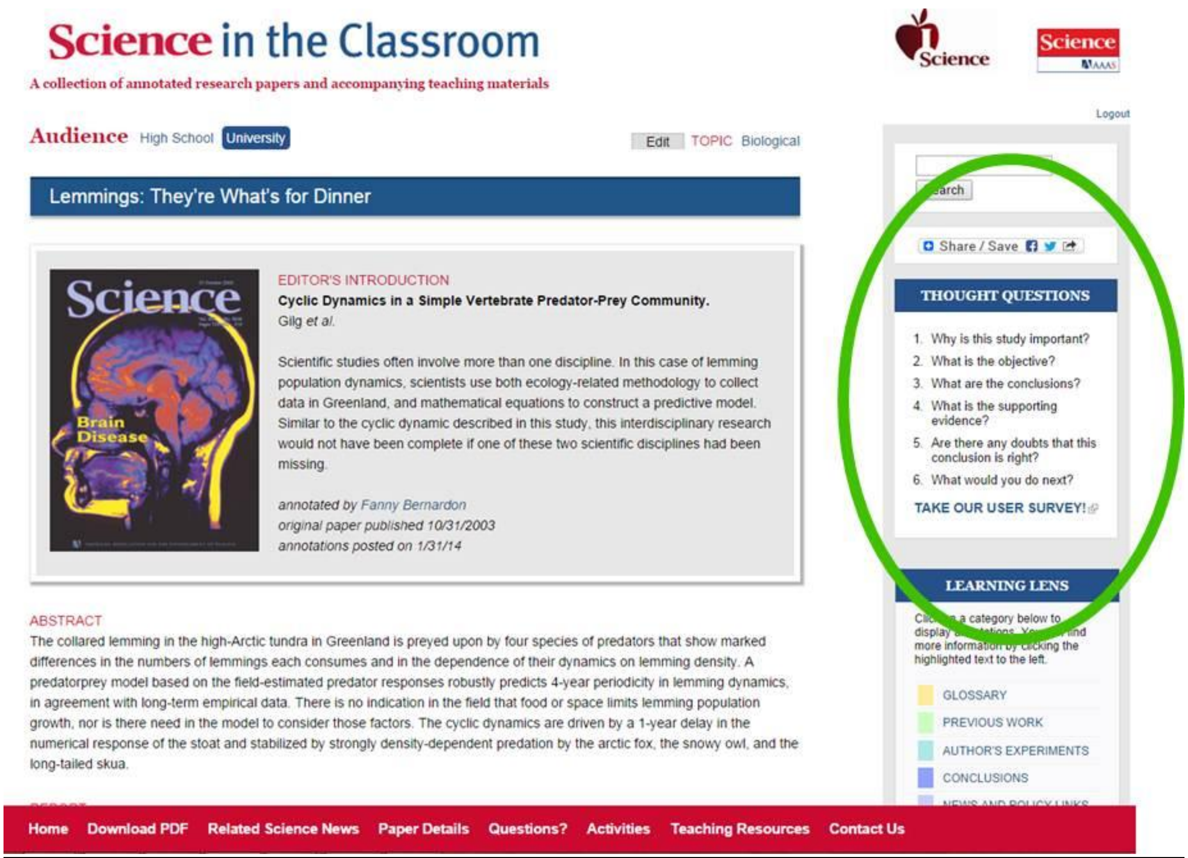
Thought Questions

Thought Questions are located above the Learning Lens in the right sidebar of each resource. These questions were written to be universal and applicable to any primary research paper. Thought questions do not have a single answer, or a correct answer for that matter, and can be used to stimulate discussion among students.



Suggestions for Classroom Use:

In addition to the thought questions discussed above, other resources are provided for use in the classroom. These can be found toward the end of the teacher guides associated with each specific article and include:

1. Discussion questions specific to the article, related to the standards, and/or associated with the figures.

2. Activities tied to the articles.

Some ways to use the *Science* in the Classroom articles:

1. Assign to student groups to read and discuss during class.

2. Assign small sections of the article to student groups to read and discuss during class, with the expectation that they will present or use jigsaw to teach the entire class what is in their part of the article.

3. Assign to individual students to complete during class or as homework.

4. Assign reading as an extra credit project.

Some ideas for interactive student engagement after reading the article:

1. Students write answers to discussion questions (for example, those linked to the standards or those linked to the diagrams).

2. Go over the abstract, as well as information about the purpose and structure of an abstract, and have students write their own abstracts for the articles in language that could be understood by their peers.

3. Have students edit the article, or parts of the article, to a simpler reading level.

4. Have students, alone or in small groups, use the annotated list of references to explain how the scientists who wrote this article built on the published work of at least one independent group of scientists in making their discoveries. In the process, did they produce data that supports the findings of the earlier publication that they have cited in the text? In what way does this article support the statement that scientific knowledge is built up as a “community effort”?

5. Use the article and discussion questions linked to the standards and the diagrams for a teacher-led classroom discussion. The discussion can focus on the nature of science and scientific research, as well as on the science in the article itself.

6. Have students give a classroom presentation about the article, parts of the article, or their answers to discussion questions.

ARTICLE-SPECIFIC MATERIALS

Connections to the nature of science from the article

- This paper links pollinator behavior with defense compounds produced by flowering plants.
- The results of this study have important implications for plant-pollinator interactions and how these interactions can drive selection in plant populations.

The importance of this scientific research

- This study shows that compounds produced by plants can manipulate pollinator behavior and memory in order to improve reproductive success. Additionally, it implies that pollinator behavior can drive selection within plant populations.

The actual science involved

- Neurobiology
- Animal learning behavior
- Liquid chromatography-mass spectrometry

Connect to Learning Standards:

The Next Generation Science Standards

- Practice 1: Asking questions (for science) and defining problems (for engineering)
- Practice 2: Developing and using models
- Practice 3: Planning and carrying out investigations
- Practice 4: Analyzing and interpreting data
- Practice 6: Constructing explanations (for science) and designing solutions (for engineering)
- Practice 7: Engaging in argument from evidence
- Practice 8: Obtaining, evaluating, and communicating information

The AP Biology Standards

- Practice 1: The student can use representations and models to communicate scientific phenomena and solve scientific problems.
- Practice 3: The student can engage in scientific questioning to extend thinking or to guide investigations within the context of the AP course.
- Practice 4: The student can plan and implement data collection strategies appropriate to a particular scientific question.
- Practice 5: The student can perform data analysis and evaluation of evidence.
- Practice 6: The student can work with scientific explanations and theories.
- Practice 7: The student is able to connect and relate knowledge across various scales, concepts, and representations in and across domains.

Common Core English Language Arts

- 11-12.1: Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account.
- 11-12.2: Determine the central ideas or conclusions of a text; summarize complex concepts, processes, or information presented in a text by paraphrasing them in simpler but still accurate terms.
- 11-12.3: Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks; analyze the specific results based on explanations in the text.
- 11-12.4: Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 11–12 texts and topics.

- 11-12.6: Analyze the author's purpose in providing an explanation, describing a procedure, or discussing an experiment in a text, identifying important issues that remain unresolved.
- 11-12.8: Evaluate the hypotheses, data, analyses, and conclusions in a science or technical text, verifying the data when possible and corroborating or challenging conclusions with other sources of information.

Summary of the Article for the Teacher:

It is recommended that this not be used by students in place of reading the article.

General Overview:

Many drugs regularly used by humans, including caffeine and nicotine, are produced by plants as toxic defenses against herbivores. These compounds may deter mammals from eating those plants, but how do they affect the pollinating insects that help these plants reproduce? Because these pollinators play such an important role in flowering plant reproduction, do they affect the production of these toxic defense compounds in plants?

Caffeine-producing plants like coffee and citrus plants produce more flowers and fruits when pollinated by bees, improving their reproductive success. Caffeine in the nectar of these plants exists at lower concentrations than in their vegetative material — enough to affect bee neuronal function without being bitter-tasting or toxic. Caffeine in plant nectar stimulates bee neurons, increasing their ability to remember the reward of sugary nectar derived from visiting the caffeinated plant. As a result, the bees visit the same plants more often, improving the reproductive success of that plant. Together, these results show that plant compounds can affect pollinator behavior. In turn, pollinator behavior can also drive selection within plant populations for the production of defense compounds at levels that will have an effect on, but not deter, pollinators.

Topics Covered:

- Animal behavior
- Memory formation
- Neuron action potential/neuroelectrophysiology
- Liquid chromatography-mass spectrometry

Why this research is important:

Understanding how associative learning behaviors affect interactions among natural partners like pollinators and flowering plants gives us important information on how such partnerships may drive selection of a species. Organisms within an ecosystem adapt and evolve to each other's behavior, producing compounds to ward off predators or to encourage mutualistic behavior by symbionts. It is known that caffeine present in plant vegetative material serves to discourage herbivory; however, it is unknown whether caffeine present in plant nectar plays an ecological role.

This study showed that caffeine present in plant nectar affects pollinator learning behavior, thereby improving pollinator fidelity and increasing reproductive success. Further, this affect on pollinator associative learning in turn drives selection of the concentration of the compound specifically in plant reproductive tissues to levels that are pharmacologically active but not repulsive to pollinators. The implications of this research are that interactions between species can be very complex and can direct the evolution of a species. A thorough understanding such complexities is crucial to understand related issues, such as optimizing effective agricultural practices and anticipating and mitigating the effects of human actions on ecosystems.

Methods used in the Research:

- Liquid chromatography-mass spectrometry
- Whole-cell patch clamp electrophysiology
- Classical conditioning

Conclusions:

- Alkaloids like caffeine are not just defense compounds; they also increase reproductive success by pharmacologically manipulating pollinator behavior.
- Bees simultaneously exposed to caffeine, floral scent, and a reward (nectar) are more likely to associate that scent with the reward and return to that plant.

Areas of Further Study:

- Does caffeine have a similar affect on associative learning of other important pollinator species?
- Do non–caffeine-producing plants have compounds that similarly increase pollinator fidelity, or do they rely on different mechanisms to draw pollinators and ensure reproductive success?

<u>**Discussion Questions:**</u>

1. What are plant defense compounds and how have they been used by humans? What are some examples of plant defense compounds other than caffeine that you might encounter in your daily life?

2. What is a *selective advantage*?

3. Why did the authors of the paper expect to find caffeine in the nectar of the flowering plants tested?

4. Explain “classical conditioning” as used by the study’s authors. How would this method help reveal the effect caffeine has on memory formation in honey bees?

5. What is an action potential firing threshold?

Discussion questions associated with the figures

Fig. 1:

1. Describe the technique the authors used to measure caffeine concentrations in Figure 1. What could be some other potential uses for this technique?

Fig. 2:

1. How did adding caffeine affect honey bees’ ability to learn? (Be descriptive! For example, did it help with short- or long-term memory? Did it help them learn faster, cause them to learn more slowly, or neither?)

Fig. 3:

1. What technique did the authors use to obtain the trace recordings in Figure 3? What does this technique measure?

2. What is a representative sample? Why would a representative sample be shown instead of the sum of all the data available?

3. Why were the study’s authors interested to find that adding caffeine to honey bee neurons pushed the membrane potential toward the action potential firing threshold?

4. Why did adding DPCPX eliminate the changes in holding current and membrane potential induced by caffeine? What did the authors learn as a result of this observation?

Fig. 4:

1. What is the significance of a caffeine concentration of 1 mM? How does that concentration correlate to the concentrations observed in plant nectar in Figure 1?
2. Explain the conclusions drawn by authors on the effect of honey bees on flowering plant selection based on the data presented in Figure 4. Do you agree with their reasoning? Why or why not? What evidence presented in this paper confirms their conclusion or calls it into question?