NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Got Blood?

The Evolution of Human-Biting Preference in Mosquitoes

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Part I – A Matter of Preference

"So, what do we do with all of these mosquitoes we collected?" James, a new undergraduate research student in the lab was anxious to get some hands-on experience, but was wondering what he had gotten himself into by joining this "mosquito preference" research lab.

"Well, first we have to sort out the females of course, because we'll only need them, not the males," Professor Leslie Vosshall, the Principal Investigator of the lab, said matter-of-factly.

"Then we let them decide whether to bite us and suck our blood!" laughed Carolyn, the senior post-doctoral fellow in the lab.

James felt even more puzzled, and a little bit anxious.

"Let me explain," began Dr. Vosshall. "These mosquitoes are offspring of mosquitoes that we originally collected from different sites around a village in the region of Rabai, Kenya. Some of the collection sites were in or around homes in the village, and other collection sites were a little way outside the village in forested areas."

"We brought the mosquitoes back to the lab and bred the males and females from each location separately," jumped in Carolyn, "so now we have these established colonies in the lab that are offspring from each of the original collection sites."

"Yes, so now we want to see if the mosquitoes from domestic colonies and forest colonies have different preferences for biting humans or biting other non-human animals, like you might find in the forest," added Dr. Vosshall.

"Why would they have different biting preferences?" asked James.

"Well, that's the six-million-dollar question," remarked a quiet graduate student who had been looking at some data on his laptop.

"Are the forest and domestic mosquitoes different species?" asked James. "I noticed that the ones marked 'forest' look a little darker."

"You have a good eye, James," replied Dr. Vosshall. "But that doesn't necessarily mean they are different species. Sometimes there is a lot of noticeable variation between populations. Just look at humans!"

"Plus, there is the question of just what makes a species one species," added Carolyn.

"What do you mean?" asked James.

"Never mind that right now," interrupted Dr. Vosshall. "We can talk about that concept later. Right now we need to get back to planning these experiments. We have to design a rigorous test to quantify biting preferences for mosquitoes from the different colonies."

A few weeks back, when James was first interviewing for an undergraduate research position, Carolyn had talked about how learning about the genetics and evolution of biting preference of mosquitoes could help with finding ways to stop the spread of Zika and other diseases spread by these mosquitoes. When he left the meeting for his class, James still wasn't sure if he completely understood the "big picture." He had felt a little lost when the research group had gone over details of the experiments they were planning. But he vowed to spend some time that evening learning about mosquitoes.

That night, James spent hours on the Internet. He found himself getting surprisingly interested in learning about mosquitoes. Here is some of what he learned:

- There are more than 3,500 known species of mosquitoes, which belong to the order Diptera and family Culicidae. There is speculation that there may be hundreds or thousands of additional species as yet undiscovered.[*] Although different species live in diverse habitats, a common feature is the requirement of standing or flowing water for larval development from eggs.
- Mosquitoes are thought to have diverged from other dipterans at least 100 million years ago (Cretaceous Period), possibly even further back, during the Jurassic Period. Recently, researchers actually found a 46-million-year-old mosquito fossil with traces of hemoglobin in its abdomen, indicating that the blood-sucking trait dates to long before human origins and that the non-human biting (forest) form is an ancestral trait.[†]
- Many different species of mosquitoes preferentially bite humans to obtain a blood "meal," and in the process often transmit a variety of diseases, including malaria, yellow fever, chikungunya, dengue, West Nile, and Zika. However, not all species are human selective; consider the fossil find mentioned above (i.e., dated earlier than human origins) and the spread, via mosquitoes, of the West Nile virus in birds.

As James got more immersed in the topic, he stumbled on references to some older studies from the 1960s and '70s on yellow fever mosquitoes *(Aedes aegypti)* that had been conducted near Rabai, Kenya, and realized this was the same study site that had been mentioned in the lab group meeting. One paper[‡] described distinct populations that could be distinguished by morphology (differences in coloration), as well as by their natural histories. This article described a forest-dwelling feral form (named *Aedes aegypti formosus)* with black coloration and a behavioral trait of laying its eggs in natural rainwater sources, such as tree holes and small ground depressions, and a "domestic" form *(Aedes aegypti aegypti)* with brown coloration and a behavioral pattern of entering human dwellings and laying eggs in drinking water storage containers. The two forms seemed to have distinct, genetically determined preferences for either forest or domestic (human dwellings) habitats. The paper also described a third, peridomestic population, living in zones between the domestic and forest populations and occasionally entering homes and biting humans.

James was particularly excited about the research when he realized that the same species of mosquito the lab was studying was the vector for Zika virus, which was becoming a big story in the news.

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*. Rueda, L.M. (2008). Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater. *Hydrobiologia* 595: 477–487.

† Greenwalt, D.E., Gorevab, Y.S., Siljeströmb, S.M., Roseb, T., and Harbache, R.E. (2013). Hemoglobin-derived porphyrins preserved in a Middle Eocene blood-engorged mosquito. *Proc. Nat. Acad. Sci.* 110: 18496–18500.

‡ Trpis, M., and Hausermann, W. (1978). Genetics of house-entering behavior in East African populations of *Aedes aegypti* (L.) (Diptera: Culicidae) and its relevance to speciation. *Bull. Entomol. Res.* 68: 521–532.

Part II – Why the Preference?

1. In groups, start with a brainstorming session on what you know and don't know about mosquitoes in general. The class will compare lists to see how much collective information we can gather. Include, specifically, these questions in your brainstorming:
   a. Why were the researchers planning to use only female mosquitoes?
   b. Why do female mosquitoes bite for a blood "meal" in the first place?
   c. What do male and female mosquitoes live on as their main nutrient source (if only females take blood meals)?
2. Continuing in your groups, work on the following:
   a. Based on the information above, write a plausible *hypothesis* that describes a possible *mechanism* to explain the differences between the forest and domestic forms in terms of their specific preferences for humans versus non-human animals. Your hypothesis should describe a genetic (heritable) difference between the populations.
   b. Based on your hypothesis, write one or more specific *predictions* that you could experimentally test.
   c. Draw a *cartoon* of a specific experiment you would want to design to test your predictions.
3. During the lab meeting described in Part I of this case study, Carolyn brought up the concept of "what makes a species one species." The most common way that scientists define species is called the "biological species concept" (BSC).
   a. Define the BSC. What are the criteria used in the BSC to define a species?
   b. Refer back to the information James found from earlier research describing a hybrid "peridomestic" population that seemed to have a mix of domestic and forest form traits. Based on this information, do you think the "forest" and "domestic" forms of *Aedes aegypti* should be considered as separate species? Explain.
   c. Make a list of the differences (morphological, behavioral, ecological) between the forest and domestic forms, as mentioned in the 1978 paper that James read. Try to hypothesize a mechanism that might eventually lead to separation of the two forms into distinct species.
4. The Vosshall research team was able to cross-breed forest and domestic mosquitoes in the laboratory to obtain *viable and fertile* first (F1) and second (F2) generation offspring. Why is this an important experiment? What kind of information might be gained from this approach?

Part III – Preference Indices

By the time James met with the lab group for the second time a couple of weeks later, he had gotten some "hands-on" experience for sure! He had volunteered to be one of the human subjects in the biting-preference studies. He also was beginning to have a better grasp of what the Vosshall research group was trying to understand.

As Carolyn had explained to him, the laboratory mosquitoes were continuously inbreeding colonies, each derived from one of the original collection sites, either domestic or forest. James was surprised to learn that the color differences that had been observed in the wild were maintained in these breeding colonies. James had observed some of the "biting assay" experiments in which female mosquitoes from an individual colony were put in a chamber and allowed to bite and obtain blood over a 10-minute period, selecting between a human arm and an anesthetized guinea pig. Carolyn also explained to him how they presented results of the biting assays as a *preference index* for each colony.

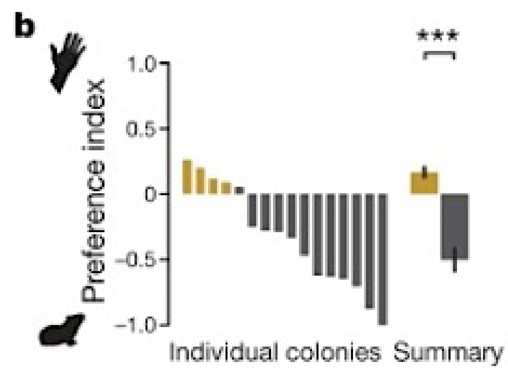

Figure 1. Biting preference index results (human versus guinea pig) for domestic and forest mosquitoes. This graph shows the average preference indices for 16 colonies, 4 derived from the domestic forms (yellow bars) and 12 from the forest form (black bars). The bars on the far right are overall means for all colonies from the two forms with error bars (standard error of means). The triple asterisk indicates a significant difference between these means with a P value of < 0.001. Data from McBride et al. (2014).

Questions

5.
   a. How do you think the "preference index" is calculated, and why would you use this value to show the preference data? (Consider that a preference index of +1.0 means that all trial mosquitoes from that colony that bit at all bit the human arm and not the guinea pig.)
   b. How would you end up with a preference index of −1?
   c. Why did the researchers show the preference index for each individual colony instead of showing only the overall averages (which are also presented)?
6. Consider how the above experiment was done and the results that were obtained.
   a. What are some potential pitfalls in interpreting this experiment?
   b. Based on these results, would you revise your group hypothesis?
   c. Try to think of what you would do for the next experiment, based on these results.

Reference

McBride, C.S., Baier, F., Omondi, A.B., Spitzer, S.A., Lutomiah, J., Sang, R., Ignell, R., and Vosshall, L.B. (2014). Evolution of mosquito preference for humans linked to an odorant receptor. *Nature* 515: 222–227

Part IV – Finding the Cue

It didn't take long for James to get totally immersed in the project. In talking with Dr. Vosshall and the others, he now understood the big picture much better. In fact, for a different class, he had written a paper about the research project, which included a clear statement of hypothesis.

> *The domestic form of* Aedes aegypti *prefers to bite humans over other animals because they have undergone a genetic change in their olfactory sensory receptors (sense of smell), making them highly sensitive to a distinctly human odorant (a volatile chemical molecule released from the skin).*[*]

However, he realized, thinking back, that the previous experiment (Figure 1) was difficult to interpret because there are *multiple* cues that might cause mosquitoes to prefer a human hand to an anesthetized guinea pig besides odor.

Question

7. How would you modify the biting-preference assay so that human smell was the only variable that differed between the human and non-human (guinea pig) choice?
8. What kind of control treatments would you want to include and why?
9. Do the data in Figure 2 support the authors' hypothesis (see statement of hypothesis above)? Explain.
10. What criteria would have to be met to argue that human biting preference found in the domestic form of *Aedes aegypti* resulted from an evolutionary process?
11. If you had a well-equipped lab, good funding, and collaborators with expertise in chemical methods as well as molecular and genetic methods, what would you want to do next?

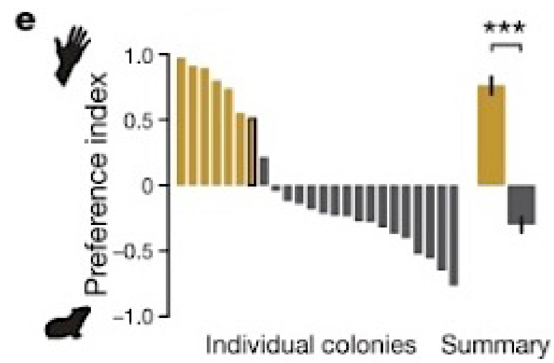

Figure 2. Olfactometer preference index results. This figure shows the results of the second experiment, in which odor was the *only* cue driving mosquito preference. In this experiment, there was no actual biting; instead the preference index was based on mosquitoes entering a trap connected to either a human or non-human (guinea pig) odorant source. Dual-port olfactometer results from McBride et al. 2014

* James' hypothesis essentially summarizes the focus of the *Nature* paper (McBride et al., 2014).

Part V – The Big Picture

Understanding the basic biology and ecology of an organism is critical for finding strategies to control or modify impacts on human health. Build a concept map to show the connections between these (and possibly other) concepts: *olfactory receptor genes, human biting preference, vector-born disease transmission, heritable traits, phenotypic divergence, forest versus domestic water availability (or quality), reproductive isolation, speciation, human odorants, Zika virus, evolution.*