What shapes the composition of microbes in a warbler's gut?

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Prothonotary warbler (Protonotaria citrea). Credit: Wikipedia

Differences among the collection of bacteria and other microorganisms that live within birds' digestive tracts—their gut microbiomes—are not primarily driven by diet diversity, contrary to a recently proposed hypothesis. Instead, a team of researchers from Penn State has found that evolution may play a larger role in explaining these differences, which could potentially have implications for how these species adapt to new habitats.

Depending on the species, an animal's microbiome can impact their host's digestion, immune function and response to disease, development, metabolism and behavior. In land mammals, there is a clear signal that evolutionary history plays an important role in determining their microbiomes, with species that are most related to each other tending to have the most similar microbiomes and those that are more distantly related tending to have more different microbiomes.

"By contrast, the differences between species' gut microbiomes in birds and bats is much less pronounced, and the drivers of these different patterns has remained unclear," said Marcella Baiz, postdoctoral researcher at Penn State, who led the research.

"It was recently suggested that the less pronounced differences among bird and bat gut microbiomes may be a result of changes to the digestive system as adaptations for powered flight. In this study, we investigated this hypothesis by examining the relative impact of a bird's evolutionary history and their environment, including diet, on the gut microbiomes of 15 species of wood warblers."

Birds and bats have a shorter and more simplified digestive system

compared to land mammals, meaning food passes through more quickly, which ultimately reduces weight when flying. A recent study had suggested that because of this higher turnover in food, there might also be higher turnover in the microbial community within the gut, and thus diet would have a stronger influence on the gut microbiome. However, recent studies that investigated the gut microbiome in birds either considered captive animals, who have artificial diets, or characterized diets using broad categories of diet type, which provides an incomplete picture.

In the current study, published in a paper appearing online Nov. 21 in the journal *Molecular Ecology*, the research team collected fecal samples from more than 400 birds from 15 species of wood warblers from central Pennsylvania and from the Adirondack region of New York.

"One of our study's strengths is that we were able to collect samples from many individuals of each species at two different localities," said Baiz. "While some previous studies may have included a greater number of species, they might have sampled only a few individuals from each species, so it is unclear if the sampling is representative of the species as a whole."

The researchers extracted and sequenced DNA from the fecal samples to identify the species of bacteria present in a bird's gut as well as the species of insects that each bird had eaten. The most common species of bacteria they identified are also present in other types of birds.

When the researchers examined the diversity of bacteria within the birds' microbiomes, the bird species' identity was the biggest explaining factor. The diversity of insects in their diet was not strongly related to the diversity of bacteria in their gut. Additionally, when the researchers grouped warblers by their gut microbiome similarity, these relationships were a closer match to the warbler tree of life—a tree grouping the

warblers by evolutionary relatedness among species—than to a tree that grouped the warblers by their diet similarity.

"Our results suggest that diet diversity may not be the most important factor shaping the gut microbiome of these birds, so the hypothesis that fast gut turnover and the evolution of a shortened digestive tract favor environmental influences in driving the composition of the gut microbiome may be too simple," said Baiz.

"We plan to continue pursuing how host evolution might shape the gut microbiomes of warblers, which are a very diverse group of more than 100 species of songbirds that rapidly diverged in the last 7 million years. If there is a signature of evolution, we might expect the microbiome to play a role in how birds adapt to new habitats as they are colonized, or—if two different species were to mate and produce a hybrid bird, which is common among warblers—it could impact the compatibility of the microbiome and ultimate success of the hybrid offspring."

The researchers acknowledge that much of the variation among the birds' microbiomes remains unexplained, and that future studies should continue to explore evolution and other ecological factors. Next, the team plans to investigate whether specific food items are related to specific bacteria in the gut.

"We would like to know whether bird microbiomes are influenced by bacteria associated with the organisms they are eating, or if they are picking up bacteria that are associated with the environment where they are foraging," said Baiz.

"We would also like to look across the bird's whole migratory cycle, since migration comes with a whole suite of physiological changes which could influence the chemistry of the gut. What the birds are eating when they migrate south would also be completely different than what they are

eating where we sampled in eastern North America."

In addition to Baiz, the research team at Penn State includes Andrea Benavides Castaño, an undergraduate at the time of the research; Andrew Wood, research technologist; and David Toews, assistant professor of biology. The team also includes Eliot Miller, collections development manager at the Cornell Lab of Ornithology.

More information: Marcella D. Baiz et al, Gut microbiome composition better reflects host phylogeny than diet diversity in breeding wood-warblers, *Molecular Ecology* (2022). DOI: 10.1111/mec.16762

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