

MICROBIOME

Littermate wanted: standardizing mouse gut microbiota requires more than cohousing

Robertson, S.J. et al. *Cell Rep* 27, 1910-1919 (2019)

A few years ago, a group of Dana Philpott’s mice at the University of Toronto came down with a parasitic infection. Their people just didn’t realize it right away. The lab had been working with two separate litters, one of wild-type control mice and the other, a transgenic line. Rather suddenly, a disease phenotype in the colitis model that had been taking a couple of months to appear was developing in just a couple of weeks in one of the litters. “We knew that something had changed,” says Philpott. They looked more closely, co-housing different animals and discovering that healthy mice soon became sick too. A colleague diagnosed the parasite not long after.

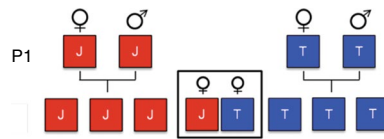
It raised an important question: what could have been? “If we hadn’t done a more intense investigation, we would have published the paper saying ‘this particular genotype leads to this extremely severe disease,’” Philpott says. “Where it really had nothing to do with the genotype; it was really just the presence of this parasite.”

“You can imagine, this can happen in any mouse colony,” she says. It’s a testament to the power of the microbiome, the community of bacteria, fungi, and viruses that live on and in an organism. The effects of gut microbiota can be drastic. “We’ve seen it completely switch phenotypes in mice. It seems to trump genotypic effects in certain cases,” says Philpott.

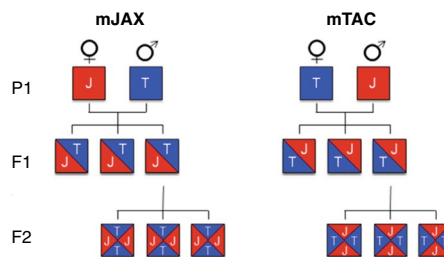
It’s a variable that merits attention from researchers working with mouse models. But how researchers chose to standardize the gut microbiota in their animals, a consideration for controlling for its effects in a study, can vary. “In the literature, you see people variously using littermates or cohousing methods to try to standardize the microbiota,” she says. Can both get the job done, or is one approach better than the other? To find out, Philpott and her colleagues decided to test how effectively littermate control and cohousing methods each manage to standardize microbiota between mice; their results are reported in a recent issue of the journal *Cell Reports*.

They started with two groups of C57BL/6 mice purchased from the Jackson Laboratory and Taconic Farms, the baseline gut microbiota of which differed substantially. To test the cohousing strategy—in which coprophagy is assumed

1. Co-housing



2. Littermates



Schematic of cohousing vs. littermate testing strategy. Credit: Robertson et al (2019). Elsevier

to transfer microbiota between non-related animals—they bred Jackson X Jackson and Taconic X Taconic mice and cohoused a subset of offspring from each pairing together for five weeks post-weaning; they then compared the microbiota of the cohoused mice against siblings that were not mixed together. For the littermate breeding strategy, they determined the microbiota present in offspring from reciprocal crosses from the two vendors over two generations, noting maternal origins. Microbiota were sampled from three locations along the gut: the terminal ileum, the proximal colon, and fecal pellets.

Cohousing normalized fecal microbiota among the mixed litter mice, but that was, unfortunately, about it. “You would see this same microbiota in the poop samples among a group of mice. But it doesn’t necessarily mean that those bacteria that you see in their poop actually colonized those animals,” explains Philpott. “The mucosal bacteria, so the bacteria associated with the intestines that are probably the ones that influence the phenotype the most, were not as normalized as when you do littermate mice.” The microbiota from the littermate mice were much more homogenous, across fecal pellets, the colon, and the ileum, though maternal signatures of a few bacteria could be detected.

Philpott would like to look more closely at that maternal transmission aspect, versus those microbes that can be passed mouse-to-mouse. Some, for example, are less aerobically tolerant than others. “What if those ones are really the, what we call the ‘pathobionts,’ that could be really important for causing a certain disease?” she asks. “Maybe those are the ones that are really oxygen sensitive and are only transmitted through the mom in these cases.”

The take-home messages remain the same: the microbiota matters, as does how it is standardized. “In my department, I think everyone has heard me enough times and now they are convinced that [littermates] is the right way to go.” Others may still need more convincing—particularly in areas where connections with the microbiome may seem less clear. But, “the microbiome is a huge variable in your experiments,” Philpott says. That’s something all mouse researchers should at least keep in mind, she says, with a note to editors too: authors need to include their breeding strategies. She recalls seeing papers with authors claiming to use littermates to standardize microbiomes, but the litters are only of a single genotype. “Their wildtype mice were all littermates; their knockout mice were all littermates, but that’s not what we need,” she says. “We want to have both being raised by the same mother.”

Relating to cohousing separate litters, standardization via littermates can be an expensive undertaking that can involve more mice—in reciprocal crossing of wildtype and knockout lines, for example, some pups in the litter will have a wildtype genome; others, the knockout genome, and a third group still will be heterozygous for the genetic change. Philpott suggests phenotyping the heterozygous group too to see if it definitively matches one of the others and can thus be pooled, to make use of what would otherwise be extra animals. But in any case, her lab has been using littermates ever since the parasite incident; the current proof-of-concept study is another piece of evidence in favor of the practice.

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