Reproducibility of experimental studies is a cornerstone of scientific progress. When studying intact animals, variability comes from many sources, including the microbial organisms that associate with the animals being studied. The list of animal physiological traits known to be affected by host-associated microbial communities, collectively called microbiota, keeps growing. Not only immunity and metabolism, but also traits as diverse as bone density, blood-brain barrier integrity, and behavior are influenced by the non-pathogenic microbes (bacteria and eukaryotes) with which animals come into contact.

Some effects are seen when examining the differences between microbiologically sterile (axenic) and conventional animals, whereas others are observed between animals with natural variations in the microbiome. Some microbiota are transmitted from parent to offspring, making microbiota a potential source of nongenetic heritability. Other microbes can be acquired from the environment or from other animals, making them an invisible environmental factor. Environmentally acquired microbes can also be transferred to offspring or to animals that are housed together. The effect of microbes on a certain trait can be independent of the host’s genotype, or the microbiota composition may be shaped by traits of the host and in turn confer a phenotypic trait. Furthermore, some microbiota may have a different phenotypic effect in host animals with different genetic backgrounds, and vice versa. In a genome-wide association study of nutritional traits of flies, for example, some genetic polymorphisms resulted in phenotypic differences only in the presence of microbiota, whereas others did only in the absence of microbiota (1). The diversity of these effects means that the microbiota may contribute to unexplained variation either within or between experiments in a number of different, often difficult-to-predict, ways.

Clearly, the best way to take microbiota into account when designing experiments depends on the research question being asked and the parameter being studied. In some cases, it may be necessary to standardize the microbiota of the experimental animals—for example, by artificially creating a standard microbial mixture (similar to but even more rigorously standardized than the Schaedler flora sometimes used in mice) or by autoclaving or irradiating food and water to prevent the introduction of new microbes. Changes in microbiota over the course of an experiment may need to be monitored, which involves culturing and sequencing microbial communities. Parallel experiments using axenic animals may also be necessary. In other types of research, such measures may obscure important differences between genotypes, produce a biologically irrelevant phenotype, or simply be ineffective. For many experiments, it may be sufficient to randomize the effects of microbiota across treatment groups, such that variation in microbiota may add a certain amount of noise but not create a spurious signal. Natural variation in microbiota between experiments or facilities may be important for replication efforts to ascertain that a given effect is robust to microbial background (for example, when testing a drug that may be administered to patients with different microbiota).

The confounding of environment, genotype, and microbiota effects that occurs when parents transmit microbiota to their offspring may be the most problematic aspect of microbiota-mediated effects for researchers. Fortunately, many of the measures that control for the effects of microbiota are similar to measures already implemented in experiments in which it is important to reduce the influence of maternal effects (that is, phenotypic variation caused by variation in the environment experienced by the parental generation). These measures include rearing animals in identical conditions for several generations prior to the start of an experiment, co-housing or cross-fostering animals before assigning them to treatments, or conducting experiments in which replicates are matched pairs from the same litter (littermate controls or “split-brood” designs). As we increasingly understand the importance of epigenetic and nongenetic inheritance and the ways in which the experiences of one generation persistently affect one or more subsequent generations, these measures are likely to be the best practices for many types of animal experiments, even ones in which microbiota specifically do not have an effect.

Including details that affect microbiota in the methods sections of animal studies—for example, by describing chow source, bedding material, room temperature, housing information, animal acclimation to facilities, or randomization methods—should improve reproducibility (2, 3). The specific methodological details relevant to each organism will vary. Until such reporting becomes standard, researchers should remember that microbes are a cryptic dimension of both genotypes and environments, and consider their experimental design from a microbial point of view.

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Citation: A. A. Mushegian, Sources of variation: Animal microbiota. Sci. Signal. 10, eaam9011 (2017).
Sources of variation: Animal microbiota
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Sci. Signal. 10 (467), eaam9011.
DOI: 10.1126/scisignal.aam9011