Diet-induced mating preference in *Drosophila*

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Diet-induced mating preference was initially observed by Dodd (1). Subsequently, we reported that diet-induced mating preference occurred in *Drosophila melanogaster*. Treatment of the flies with antibiotics abolished the mating preference, suggesting that fly-associated commensal bacteria were responsible for the phenomenon (2). The hypothesis was confirmed when it was shown that colonizing antibiotic-treated flies with *Lactobacillus plantarum* reestablished mating preference in multiple-choice mating assays with CMY (0.65% agar, 7.6% cornmeal, 7.6% molasses, 5% inactivated brewer's yeast, 0.1% methyl-4-hydroxybenzoate, 0.76% ethanol, and 0.4% propionic acid) flies. Furthermore, analytical studies indicated that the flies grown on different media had distinct levels of cuticular hydrocarbon sex pheromones. The difference in their levels was reduced when the flies were treated with antibiotics.

Najarro et al. (3) replicated our study with inbred *D. melanogaster* strains Oregon-R and Canton-S, reporting significant diet-induced homogamic mating preferences. The microbiome has also been reported to play a role in postzygotic reproductive success in *Nasonia* (4). Others have demonstrated that both intracellular (*Wolbachia*) and commensal symbionts contribute to female attractiveness in *D. melanogaster* (5).

Recently, Leftwich et al. (6) published a well-powered, well-controlled replication experiment on diet-induced mating preference in *D. melanogaster*. They reported no homogamic reproductive isolation. One possible explanation for their failure to reproduce mating preference is that microbiomes at the start of their experiments may have been affected by the media that was employed. Fly media utilize preservatives to prevent spoilage due to unwanted growth of fungi and bacteria. In our experience, the level of methylparaben, a common antifungal agent with bactericidal properties, has a profound impact on mating preference, practically eliminating it when present in too high concentrations. The initial fly populations in the Leftwich et al. study were maintained on 0.3% methylparaben before the initiation of the experiment, transitioning to 0.1% methylparaben for the experiment. This could potentially explain the different results between Leftwich et al. (6) and the other three reports (1–3), in which methylparaben was not used or present in much lower concentrations before the initiation of the experiments.

Leftwich et al. (6) conclude, based on their results, that "... there is no general role for gut bacteria in driving the evolution of [reproductive isolation] in this species. . . ". We, on the other hand, suggest that the initial microbiome is important in the adaptation of the holobiont to environmental changes; microorganisms adapt faster than their hosts. The microbiome can serve as a mechanism for evolutionary "memory" (on short or longer time scales). As a result, bottlenecks can change the trajectory of the host-associated microbiome.

We appreciate Leftwich et al.'s (6) effort in addressing the complexity of diet-induced, microbiome-mediated reproductive isolation and publishing their negative results. In light of the mixed results in the literature, our tentative conclusion is: The role microorganisms play in driving reproductive isolation is an unsolved riddle, one that has profound implications for our understanding of evolutionary processes, and thus warrants further examination.

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The authors declare no conflict of interest.

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Published online February 20, 2018.