

REPLY TO ROSENBERG ET AL.:

Diet, gut bacteria, and assortative mating in *Drosophila melanogaster*Philip T. Leftwich^{a,b}, Naomi V. E. Clarke^a, Matthew I. Hutchings^a, and Tracey Chapman^{a,1}

Rosenberg et al. (1) suggest that the lack of evidence for assortative mating we reported (2) in comparison with a previous study (3) could be due to gut microbiomes in *Drosophila melanogaster* being affected by differing dietary history before culturing on the CMY (0.65% agar, 7.6% commeal, 7.6% molasses, 5% inactivated brewer's yeast, 0.1% methyl-4-hydroxybenzoate, 0.76% ethanol, and 0.4% propionic acid) and starch (3% starch, 5% inactivated brewer's yeast, 1% agar, 0.5% propionic acid) diets used by both studies (2, 3). Variation in methyl paraben (mp; also known as methyl 4-hydroxybenzoate or Nipagin) concentration is highlighted. Before initiating our experiments (2), flies were maintained on a diet containing 0.3% mp and then, as in Sharon et al. (3), on CMY or starch diets containing 0.1% or 0% mp, respectively. Propionic acid was the same (0.4%) in both studies [not, as we originally reported in error in our report (2), as 4%] and hence cannot be the source of any differences.

The effects of mp on *D. melanogaster* mating have not been systematically tested, but existing studies do not support consistent associations. There was no diet-associated mating in flies maintained for many generations on diets containing ~0.2% mp (4, 5) and diet-associated mating was found in inbred but not outbred *D. melanogaster* strains (6) maintained under the same CMY and starch diets as in Sharon et al. (3). Gut microbiomes were not characterized in these studies.

Mp is a commonly used antifungal and is not expected to directly affect gut bacterial composition. In agreement with this we observed, in line with Sharon et al. (3), consistent differences between the gut microbiomes of flies maintained on CMY versus starch diets, despite the initial minor variation in mp concentration. This is consistent with studies showing

D. melanogaster gut microbiomes are flexible and vary with ingested diets (e.g., refs. 7 and 8).

The central question is whether there is a general association between diet-induced divergent microbiomes and assortative mating by diet. We found a robust lack of evidence for this, strengthened by "add-back" experiments in which the putative causal bacteria, *Lactobacillus plantarum*, exerted no influence on mating behavior (2). We suggest that the emerging picture is of variability in diet-associated mating effects and that there is no consistent association between gut microbiomes and mating preferences. Hence, in cases where significant assortative mating by diet occurs in *D. melanogaster*, it would appear to have alternative causes.

There are undoubtedly instances in which obligate symbionts play important roles in host mating preferences and behavior (9). However, in species in which guts appear to be flexibly colonized by whatever is in or on the diet (7, 8), conditions do not exist for the establishment of stable, recurrent evolutionary associations with commensal gut bacteria (10, 11). This strongly suggests that the selective conditions permitting the formation of a recognizable holobiont, whose adaptation could be affected by divergent microbiomes, do not exist for *D. melanogaster* and its gut microbes.

We will reach a deeper understanding of the fascinating interplay between hosts and their microbes with the recognition that they have a wide spectrum of coincidences and conflicts of interest, underpinned by interdependence and by inheritance mechanisms (10, 11). These need to be understood on a case-by-case basis to assess both proximate and ultimate significance.

¹ Rosenberg E, Zilber-Rosenberg I, Sharon G, Segal D (2018) Diet-induced mating preference in *Drosophila*. *Proc Natl Acad Sci USA* 115:E2153.

² Leftwich PT, Clarke NVE, Hutchings MI, Chapman T (2017) Gut microbiomes and reproductive isolation in *Drosophila*. *Proc Natl Acad Sci USA* 114:12767–12772.

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The authors declare no conflict of interest.

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