Reply to Obadia et al.: Effect of methyl paraben on host-microbiota interactions in *Drosophila melanogaster.*

Leftwich PT[1,2], Clarke NVE[1], Hutchings, MH[1] & Chapman, T[1]*

[1]School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK.
[2]The Pirbright Institute, Woking, Surrey, GU24 0NF, UK.

*Author for correspondence.

Obadia et al. (1) suggest that variation in the concentration of the fly media fungicide methyl paraben (mp) (aka Tegosept or Nipagin) can restrict gut microbial growth and diversity (2, 3), and that this could provide insight into conflicting data on the role of the gut microbiome in generating positive assortative mating by diet in *D. melanogaster* (4-6).

mp is reported to reduce the culturable bacterial diversity of wild *Drosophila* to a level comparable with established laboratory populations (2), though the effect of mp on the microbial diversity of long established laboratory populations isn't yet clear. This is consistent with the greater gut microbiome diversity of wild in comparison to laboratory-reared *Drosophila* (7, 8). mp >0.1% is also reported to severely inhibit the growth of *Acetobacter* (3). However, this contrasts with data from our (5) and Sharon et al.'s (4) assortative mating experiments. In these tests gut microbiomes from 'CMY' diet (0.1% mp) flies were dominated by *Acetobacter*. These species also represented a greater proportion of the gut microbiome in CMY as compared to Starch diet (0% mp) flies. Hence, variation in *Acetobacter* was influenced more strongly by other factors (e.g. diet and sugar availability) than by differences in mp between diets (9). Obadia et al. (1) also show that mp of up to 0.3% has little effect on the growth of *L. plantarum,* the suggested putative causal agent of positive assortative mating in the host (4). Hence, variation in mp between studies cannot itself be the cause of differential effects on *L. plantarum* with the potential to generate host assortative mating.

As fly gut microbiota are generally transient in the laboratory (e.g. 3), variation in mp in diets prior to the start of mate choice tests should not affect their outcome. This suggestion is supported by the finding that our (5) and Sharon et al.'s (4) analyses of microbial community composition of CMY and Starch diet gut microbiomes showed convergence, despite different starting diets and mp concentrations prior to the initiation of the studies.

We agree that gut microbes could represent food, or contribute nutrients to their fly hosts (1). Hence, quantification of absolute microbial loads would be useful. Microbiome 'food' could influence a variety of host life history traits, including mate choice (e.g. if it influenced host body size). Given their transient nature, it is possible that nutrients from gut microbiomes could contribute to immediate proximate effects on hosts. However, they are unlikely to shape long-term evolutionary responses in host mating behaviour.

Additional studies using wild isolates of the fly microbiota would also be useful to resolve emergent patterns. For example, a recent report of greater colonization ability of wild over laboratory fly bacteria (3) could suggest that host-microbe associations are stronger in the wild. However, these results currently contrast with studies of wild caught *Drosophila,* of a flexible diet-determined microbiome (8, 10, 11).

Overall, these growing insights are useful in increasing the resolution of discussions into the significance of diet-associated mate choice versus diet-induced divergent microbiota.

Citations

1. Obadia B, Keebaugh ES, Yamada R, Ludington WB & Ja WW (2018) Diet influences host-microbiota associations in *Drosophila*. Submitted to *Proc Natl Acad Sci USA*.
2. Téfit MA, Gillet B, Joncour P, Hughes S & Leulier F (2017) Stable association of a *Drosophila*-derived microbiota with its animal partner and the nutritional environment throughout a fly population's life cycle. *J Insect Physiol* 10.1016/j.jinsphys.2017.09.003.
3. Obadia B, *et al.* (2017) Probabilistic invasion underlies natural gut microbiome stability. *Curr Biol* 27:1999–2006.
4. Sharon G, *et al*. (2010) Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 107:20051–20056.
5. Leftwich PT, Clarke NVE, Hutchings MI & Chapman T (2017) Gut microbiomes and reproductive isolation in *Drosophila*. *Proc Natl Acad Sci USA* 114:12767–12772.
6. Leftwich PT, Clarke NVE, Hutchings MI & Chapman T (2018) Reply to Rosenberg et al.: Diet, gut bacteria, and assortative mating in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 10.1073/pnas.1721804115.
7. Broderick NA & Lemaitre B (2012) Gut-associated microbes of *Drosophila melanogaster*. *Gut Microbes* 3:307–321.
8. Chandler JA, Lang JM, Bhatnagar S, Eisen JA & Kopp A (2011) Bacterial communities of diverse *Drosophila* species: ecological context of a host-microbe model system. *PLoS Genet* 7:e1002272.
9. Matos RC & Leulier F (2014) Lactobacilli-host mutualism: "learning on the fly". *Microb Cell Fact* 13 Suppl 1:S6.
10. Wong AC, Dobson AJ & Douglas AE (2014) Gut microbiota dictates the metabolic response of *Drosophila* to diet. *J Exp Biol* 217:1894–1901.
11. Staubach F, Baines JF, Künzel S, Bik EM & Petrov DA (2013) Host species and environmental effects on bacterial communities associated with *Drosophila* in the laboratory and in the natural environment. *PLoS One* 8:e70749.