Symbiosis: Gut Bacteria Manipulate Host Behaviour

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Bacteria resident in the gut of *Drosophila* modify the fly's innate chemosensory responses to nutritional stimuli. In effect, the gut microbiome compels the host to forage on food patches that favour particular assemblages of bacteria.

Microbial threads are intricately woven into the fabric of multicellular life [1]. In insects, these associations are widespread and contribute to the impressive success of this group, which dominates terrestrial ecosystems. Mutualistic symbiotic microorganisms are involved in several critical processes that increase the fitness of their insect hosts. Primary, obligate symbionts provide hosts with otherwise unavailable nutrients. Secondary, facultative symbionts, which may also provide essential nutrients to their hosts, contribute to a wide array of beneficial traits. These can include resistance to natural enemies, pathogens, predators and insecticides, adaptation to thermal stress, as well as an increase in host range and dispersal [2,3]. A new study by Wong and colleagues [4], published in this issue of *Current Biology* adds a novel facet to this emergent kaleidoscope. They show how the gut microbiota of the vinegar fly, *Drosophila melanogaster*, significantly affects food preferences and foraging behaviour of its host.

The microbiome of *Drosophila* (and that of many other flies) is dynamic, and the abundance and diversity of the community change as flies age, fluctuating according to the nutritional status and history of each host. In light of these observations, Wong and co-authors investigated the hypothesis that members of the microbial community directly bias the nutritional preferences and foraging behaviour of hosts towards food sources rich with similar bacteria. The dominant bacteria in *Drosophila* are species of *Acetobacter* and *Lactobacillus*. By manipulating the microbial community in the *Drosophila* gut and offering experimental flies diets seeded with combinations of different bacteria, changes in dietary preference could be monitored and quantified. Thus, whereas untreated flies preferred a cocktail of *Acetobacter* and *Lactobacillus*, flies raised on only one of these bacteria significantly preferred the diet containing the same bacteria. Although the magnitude of this behaviour varied according to the different bacterial species provided in the diet, the conclusion that microbial preferences in *Drosophila* are influenced by resident microbes is robust.

Previous work has shown that dietary preferences may be innate, exposure-dependent, or result from associative learning. Accordingly, Wong *et al.* [4] needed to nail down support for the hypothesis that resident bacteria are directly responsible for the observed changes in foraging behaviour. They inoculated eggs with combinations of *Acetobacter* and *Lactobacillus*, while rendering others axenic, and then monitored the food preferences of emerging larvae. Larvae from untreated eggs (containing maternally derived *Acetobacter* and *Lactobacillus*) preferred media inoculated with both these bacteria. Conversely, axenic larvae demonstrated no preferences after hatching. Dramatically, when eggs were inoculated with *Acetobacter*, the emerging larvae oriented towards the *Acetobacter*-laced media. Similarly, larvae hatching from *Lactobacillus*-inoculated eggs converged on the food spiked with this bacterium. A series of supporting experiments with adult flies show that the mechanism of attraction towards bacteria is based on olfaction, and that in the absence of microbes the olfactory-guided response is altered.

The paradigm of nutritional geometry has been useful in illustrating how animals self-select optimal diets, balancing the competing needs of protein and carbohydrate [5]. Using this approach, Wong and colleagues [4] further show that the presence of bacteria on food changes fly foraging behaviour. Specifically, untreated, symbiotic flies (that is, those harbouring the complex, native microbiota dominated by *Acetobacter* and *Lactobacillus*) prefer diets containing a 1:2 ratio of protein to carbohydrates. However, when flies were monocolonized by single strains of either *Acetobacter* or *Lactobacillus*, their behaviour changed significantly. Both the direction and magnitude of change differed according to the bacterial species present in the fly. Thus, *Acetobacter*-colonized flies were highly averse to diets rich in protein, whereas *Lactobacillus*-colonized flies gravitated towards diets rich in carbohydrates. These bacteria-induced changes in host behaviour may reflect both the metabolic needs of the resident bacteria, and their ability to complement host nutrition by buffering amino acid deficiencies in the diet [6,7].

The question of who benefits from this manipulation cannot fail to tickle our curiosity, and Wong *et al.*, [4] provide a partial answer. They seeded a 2:1 protein:carbohydrate diet (which is *not* the optimal diet for flies) with a mixture of *Acetobacter* and *Lactobacillus*. Symbiotic flies exhibited a preference for this diet, rather than the fly-optimal diet containing protein and sugar in a 1:2 ratio. This suggests that bacteria manipulate the fly host to forage in a manner that favours the dominant bacteria in the gut, even when it does not necessarily coincide with the nutritional needs of the host.

These observations provide several strings for future studies to pull that may help unravel both the proximate mechanisms and ultimate consequences of microbe–host interactions. Within the symbiotic hyperspace, gut bacteria are — compared to lethal parasites or obligate intracellular symbionts — tentative intermediates. They benefit to some extent from associating with the host and provide some benefits in return, yet represent a point in evolutionary time where both partners can still back out of the symbiotic interaction. Despite this tentative link, these symbionts can, as shown here, manipulate their host's behaviour. Is this manipulation similar to that exerted by highly co-evolved sophisticated parasites [8,9] or mutualists [10,11]? If so, then *Drosophila* and its gut microbiota provide a model to explore the proximate mechanisms governing the microbiome–gut–behaviour nexus. A number of recent studies have suggested that the molecular pathway involved in mediating host-behavioural manipulation by microorganisms (be they bacteria, protozoa or fungi) starts with the host's immune system and progresses to olfactory receptors [12–15]. Indeed, there seems to be a primeval molecular pathway leading from gut microorganisms to behavioural changes of their host. For example, mosquitoes infected with the malaria parasite (a protozoan), exhibit blood feeding behaviour that increases the probability of parasite transmission to a novel human host. Intriguingly, the same behaviour can be elicited by challenging the immune system of the mosquito with heat inactivated *Escherichia coli*, a bacterium ecologically and evolutionarily unrelated to mosquitoes [16]. *Drosophila*, with its supporting genomic, transcriptomic and proteomic scaffolds, combined with the relatively simple and malleable associated microbiota, is an excellent model to decipher precisely how microbes manipulate the behaviour of their hosts.

On the ultimate level, insect symbionts are known to be agents of evolutionary change [17]. Specifically, the insect microbiome is known to affect life-history evolution [18], mate choice [19], and ultimately speciation [20]. The results suggest that gut bacteria, by eliciting subtle changes in food preferences and foraging behaviour, impose a trade-off that may not be immediately adaptive for the host. This can initiate a process that may lead to evolutionary change, by embedding flies associated with specific microorganisms in a discrete ecological niche. Accordingly, the experimental paradigm used in this study will be a useful tool to study the forces that maintain the partnership between host and microbiota, the apparent conflict of interest between the organisms involved, and its dynamic resolution.

REFERENCES

1. Douglas, A.E. (2014). Symbiosis as a general principle in eukaryotic evolution. Cold Spring Harb. Perspect. Biol. *6*, a016113.
2. Bourtzis, K., and Miller, T.A., eds. (2009). Insect Symbiosis, Volume 3 (Boca Raton: CRC Press)).
3. Douglas, A.E. (2015). Multiorganismal insects: Diversity and function of resident microorganisms. Annu. Rev. Entomol. *60*, 17–34.
4. Wong, A.C.M., Wang, Q.-P., Morimoto, J., Senior, A.M., Lihoreau, M., Neely, G.G., Simpson, S.J., and Ponton, F. (2017). Gut microbiota modifies olfactory-guided microbial preferences and foraging decisions in Drosophila. Curr. Biol. *27*, 2397–2404.
5. Simpson, S.J., Clissold, F.J., Lihoreau, M., Ponton, F., Wilder, S.M., and Raubenheimer, D. (2015). Recent advances in the integrative nutrition of arthropods. Annu. Rev. Entomol. *60*, 293–311.
6. Ben-Yosef, M., Pasternak, Z., Jurkevitch, E., and Yuval, B. (2014). Symbiotic bacteria enable olive flies (*Bactrocera oleae*) to exploit intractable sources of nitrogen. J. Evol. Biol. *27*, 2695–2705.
7. Leitao-Goncalves, R., Carvalho-Santos, Z., Francisco, A.P., Fioreze, G.T., Anjos, M., Baltazar, C., Elias, A.P., Itskov, P.M., Piper, M.D.W., and Ribeiro, C. (2017). Commensal bacteria and essential amino acids control food choice behavior and reproduction. PLoS Biol. *15*, e2000862.
8. Adamo, S.A. (2013). Parasites: evolution's neurobiologists. J. Exp. Biol. *216*, 3–10.
9. Libersat, F., Delago, A., and Gal, R. (2009). Manipulation of host behavior by parasitic insects and insect parasites. Annu. Rev. Entomol. *54*, 189–207.
10. Kenyon, S.G., and Hunter, M.S. (2007). Manipulation of oviposition choice of the parasitoid wasp, *Encarsia pergandiella*, by the endosymbiotic bacterium *Cardinium*. J. Evol. Biol. *20*, 707–716.
11. Dion, E., Polin, S.E., Simon, J.-C., and Outreman, Y. (2011). Symbiont infection affects aphid defensive behaviours. Biol. Lett. *7*, 743–746.
12. Benoit, J.B., Vigneron, A., Broderick, N.A., Wu, Y.N., Sun, J.S., Carlson, J.R., Aksoy, S., and Weiss, B.L. (2017). Symbiont-induced odorant binding proteins mediate insect host hematopoiesis. eLife *6*, e19535.
13. Fischer, C., Trautman, E.P., Crawford, J.M., Stabb, E.V., Handelsman, J., and Broderick, N.A. (2017). Metabolite exchange between microbiome members produces compounds that influence *Drosophila* behavior. eLife *6*, e18855.
14. Broderick, N.A., Buchon, N., and Lemaitre, B. (2014). Microbiota-induced changes in *Drosophila melanogaster* host gene expression and gut morphology. mBio *5*, e01117–14.
15. de Bekker, C., Ohm, R.A., Loreto, R.G., Sebastian, A., Albert, I., Merrow, M., Brachmann, A., and Hughes, D.P. (2015). Gene expression during zombie ant biting behavior reflects the complexity underlying fungal parasitic behavioral manipulation. BMC Genomics *16*, 620.
16. Cator, L.J., George, J., Blanford, S., Murdock, C.C., Baker, T.C., Read, A.F., and Thomas, M.B. (2013). 'Manipulation' without the parasite: altered feeding behaviour of mosquitoes is not dependent on infection with malaria parasites. Proc. Biol. Sci. *280*, 1–7.
17. Goodacre, S.L., and Martin, O.Y. (2012). Modification of insect and arachnid behaviours by vertically transmitted endosymbionts: infections as drivers of behavioural change and evolutionary novelty. Insects *3*, 246–261.
18. Macke, E., Tasiemski, A., Massol, F., Callens, M., and Decaestecker, E. (2017). Life history and eco-evolutionary dynamics in light of the gut microbiota. Oikos *126*, 508–531.
19. Sharon, G., Segal, D., Ringo, J.M., Hefetz, A., Zilber-Rosenberg, I., and Rosenberg, E. (2013). Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA *107*, 20051–20056.
20. Shropshire, J.D., and Bordenstein, S.R. (2016). Speciation by symbiosis: the microbiome and behavior. mBio *7*, e01785.