The Science of Nature (2018) 105:59
https://doi.org/10.1007/s00114-018-1584-7

SHORT COMMUNICATION

Microbiota disruption leads to reduced cold tolerance in *Drosophila* flies

Youn Henry[1] · Hervé Colinet[1]

Received: 18 May 2018 / Revised: 3 July 2018 / Accepted: 5 September 2018

Abstract

It is now acknowledged that bacteria from gut microbiota deeply interact with their host by altering many physiological traits. Such interplay is likely to consequently affect stress tolerance. Here, we compared cold and heat tolerance of *Drosophila melanogaster* flies with undisrupted (control (*Co*)) versus disrupted gut microbiota (dechorionated eggs (*De*)). The disrupting treatment strongly reduced bacterial load in flies' guts, though 16S sequencing analysis did not evidence strong diversity changes in the remaining bacterial community. Both chill coma recovery and acute cold survival were repeatedly lower in De than in Co flies under our experimental conditions. However, heat tolerance was not consistently affected by gut disruption. Our results suggest that microbiota-related effects on the host can alter ecologically relevant traits such as thermal tolerance.

Keywords Gut microbiota · Fruit flies · Thermal stress · Chill coma

Introduction

Over the last two decades, studies have linked variation in microbiota composition to variation in numerous host characteristics, including immune response, metabolism, morphology, fitness, and development (Brummel et al. 2004; Ryu et al. 2008; Shin et al. 2011; Wong et al. 2014). Most of these microbiota-mediated changes are caused by the production of primary or secondary metabolites or by the presence of bacteria per se (Matos et al. 2017). All these molecular signals can up- or downregulate multiple cellular pathways of the host and alter its whole physiology. Despite their functional importance, commensal gut bacteria communities vary considerably with time and environmental parameters (Engel and Moran 2013; Wong et al. 2013). For instance, in *Drosophila melanogaster*, bacterial load and diversity are strongly altered by nutrition (Staubach et al. 2013) or aging (Clark et al. 2015). Impoverished microbiota may lead to dysbiosis and reduce benefits provided by microorganisms (e.g., amino acids, vitamins, and other metabolites) (Ridley et al. 2012; Yamada et al. 2015). Thus, microbiota appears as a highly labile component of the holobiont, which could be the source of ecologically relevant phenotypic variations that are not under the control of the host's genome.

Given the range of beneficial functions provided by microbiota, it may also shape the ability of hosts to tolerate stressful situations (Soen 2014). In particular, coping with temperature variations is critically important for ectotherms. Alteration of energy reserves, metabolism, or gene expression by microbiota may indirectly affect thermal tolerance, which strongly depends on these traits (Teets and Denlinger 2013). In humans, the presence of gut bacteria such as *Lactobacillus* sp. is correlated with the production of heat shock proteins, a family of chaperone molecules able to repair cellular damages generated by thermal stress (Liu et al. 2014). In insects, some studies investigated microbiota effects on thermotolerance. For instance, intracellular heritable symbionts such as *Hamiltonella* and *Serratia* can promote the fitness of their thermally stressed aphid hosts (Russell and Moran 2006). Recent data suggest developmental temperature strongly affects microbial composition of *D. melanogaster* (Moghadam et al. 2017). However, whether gut microbiota have a role to play in thermotolerance remains unclear.

Communicated by Volker Loeschcke

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00114-018-1584-7) contains supplementary material, which is available to authorized users.

✉ Hervé Colinet
herve.colinet@univ-rennes1.fr

[1] UMR CNRS 6553 Ecobio, Université de Rennes1, 263 Avenue du Général Leclerc, 35042, F-35000 Rennes, France

Published online: 17 September 2018

Here, we hypothesized that *D. melanogaster* harboring a disrupted gut microbiota would be more susceptible to stressful temperatures (cold and heat), compared to flies with intact microbiota. To test this assumption, we disrupted flies' microbiota, evaluated the outcome of this treatment with colony counting and 16S sequencing methods, and then assessed thermotolerance using replicated experiments.

Material and methods

Fly culture

We conducted the experiments on an outbred *D. melanogaster* population derived from wild individuals collected in 2015 in Brittany (France). This lineage did contain *Wolbachia* symbionts. We routinely maintained fly stocks at 25 °C (12L:12D), on standard yeast-sugar-agar fly medium (16% yeast, 5% sugar, 8 mL L^{-1} Nipagin). Before experiments, one generation was reared on decaying organic fruits to maximize bacterial diversity.

Generation of dysbiotic flies

We performed the experiments over two independent replicates. Adults were collected and allowed to lay eggs for 6–12 h on agar plates. Eggs were collected with a paint brush and transferred to one of the two following treatments for development:

Dechorionation (*De*): eggs were successively immersed in 2.7% hypochlorite for 2 min, 70% ethanol for 2 min, and rinsed twice in autoclaved water (Koyle et al. 2016). Dechorionated eggs were then aseptically transferred into autoclaved food vials under a sterile laminar flow ($N = 30$, approx. 50 eggs per vial). At emergence and every other day thereafter, adults were transferred to new autoclaved food vials.

Control (*Co*): eggs were transferred after water wash in vials ($N = 30$, approx. 50 eggs per vial) containing autoclaved food recontaminated with 50 μL of homogenized flies. Recontamination was assumed to guarantee bacterial colonization of autoclaved sterile food. Emerging adults were then maintained as above.

In all experiments, we used 4–6-day-old females that were visually sexed without CO_2 to avoid stress (Colinet and Renault 2012). Additional details on the experimental protocol are presented in ESM and Fig. S1.

Thermal tolerance

For chill coma recovery (CCR), we exposed 50 females to 0 °C for 12 h in an incubator (MIR-154, Sanyo, Japan), and time to recover at 25 °C was individually monitored. For acute cold stress, flies placed in glass vials were immersed into a water-glycol bath set at − 3.5 ± 0.1 °C for cold stress and 38.5 ± 0.1 °C for heat stress. Exposure lasted for maximum 120 min, and every 15 min starting from $t = 0$ min, 20–30 individuals were removed from the bath, resulting in nine exposure durations. We scored survival after 24 h at 25 °C.

Microbiota quantification and sequencing

We used plating to evaluate bacterial load in flies, following a standard protocol (Koyle et al. 2016).

We performed Illumina MiSeq sequencing of microbiota in three randomly picked biological replicates of *De* and *Co* flies, from whole body DNA extracts. Individuals were surface-sterilized with hypochlorite and ethanol to avoid the extraction of external bacteria. Sequencing was only performed in the second replicated experiment.

Complete experimental procedures are described in ESM.

Statistical analyses

CCR were analyzed with log-rank method using Prism software (Graphpad, La Jolla, USA). Other analyses were performed using R (R Core Team 2017). Binomial generalized linear models (GLMs) with a logit link function followed by Tukey tests were used for acute stress survival. Differences in bacterial load were tested with *t* tests. Clustering OTU at genus level and computation of *alpha* and *beta* diversity was performed using "phyloseq" package (McMurdie and Holmes 2013).

Data availability The datasets generated during the current study are available in the Figshare repository https://figshare.com/projects/Data_accessibility_Microbiota_disruption_leads_to_reduced_cold_tolerance_in_Drosophila_flies_/28995.

Results

Plating of flies' gut bacteria confirmed an effective bacterial load reduction by dechorionation treatment ($t = 4.15$, $df = 17$, $p < 0.001$; Fig. 1a). MiSeq sequencing generated 39,000 to 50,000 reads per sample. After removal of *Wolbachia* reads, *De* flies still presented reads corresponding to common OTU in fruit flies. The number of reads in these *De* flies was however drastically reduced (Fig. 1b), suggesting again a quantitative reduction of gut bacteria by the treatment. Bacterial community information obtained by 16S sequencing revealed a large dominance of *Acetobacteraceae* order in *Co* flies, which was less clear in *De* flies (Fig. 1b). We found a low representation of *Commensalibacter* in *De* flies, while this genus was largely represented in *Co*. Yet, we could not detect

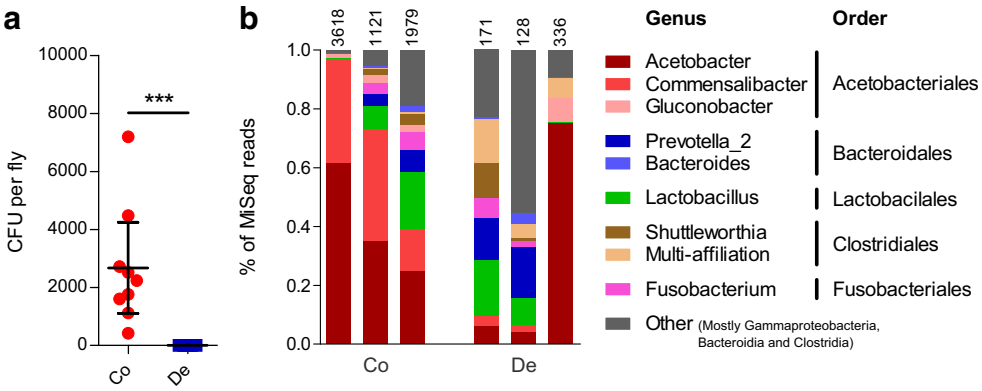

Fig. 1 Microbiota characteristics of flies from the second replicate. **a** Colony forming unit (CFU) per fly in *Co* and *De* individuals. **b** Stacked relative abundance of bacteria from flies' gut represented at the genus level. The number of reads effectively used for each replicate is displayed on top of bars

any significant microbiota structure or diversity alteration caused by the treatment (*Co* vs. *De*: Shannon alpha diversity comparison, $F = 0.25$, df = 1, $p = 0.643$; Bray-Curtis beta diversity comparison, $F = 3.08$, df = 1, $p = 0.1$).

Cold tolerance was significantly lower in *De* compared to *Co* flies. CCR was longer in *De* than in *Co* flies, in both replicated experiments ($X^2 = 12.78$, df = 1, $p < 0.001$; $X^2 = 14.55$, df = 1, $p < 0.001$; in Fig. 2(A, D) respectively). Survival rate after cold exposure was also negatively impacted by dechorionation ($X^2 = 28.68$, df = 1, $p < 0.001$; $X^2 = 35.71$, df = 1, $p < 0.001$; in Fig. 2(B, E) respectively). However, we did not observe a consistent effect of microbiota disruption on acute heat stress survival. The treatment significantly reduced heat tolerance in the first, but not in the second replicate ($X^2 = 8.18$, df = 1, $p = 0.004$; $X^2 = 1.23$, df = 1, $p = 0.27$; in Fig. 2(C, F) respectively). Overall, the amplitude of heat tolerance variation between *Co* and *De* was small compared to that observed for cold tolerance assay.

Discussion

Our results support the hypothesis that microbiota disruption can alter the ability to cope with thermal stress. The drastic reduction of bacterial load in dechorionated flies was associated with delayed recovery after mild cold stress and reduced survival to acute cold stress. Heat tolerance, on the other hand, remained almost unaffected. This result is unsurprising given cold tolerance is generally more plastic than heat tolerance (Schou et al. 2017). Although our experimental design did not allow separating effects of microbiota from effects of microbiota elimination treatment, dechorionation is assumed to have limited non-specific effects (Ridley et al. 2013). Consequently, our observations are likely due to actual microbiota disruption.

Mechanisms underlying these stress tolerance divergences can be very diverse. Loss of ion homeostasis across gut epithelia is directly responsible for chill injuries (Overgaard and

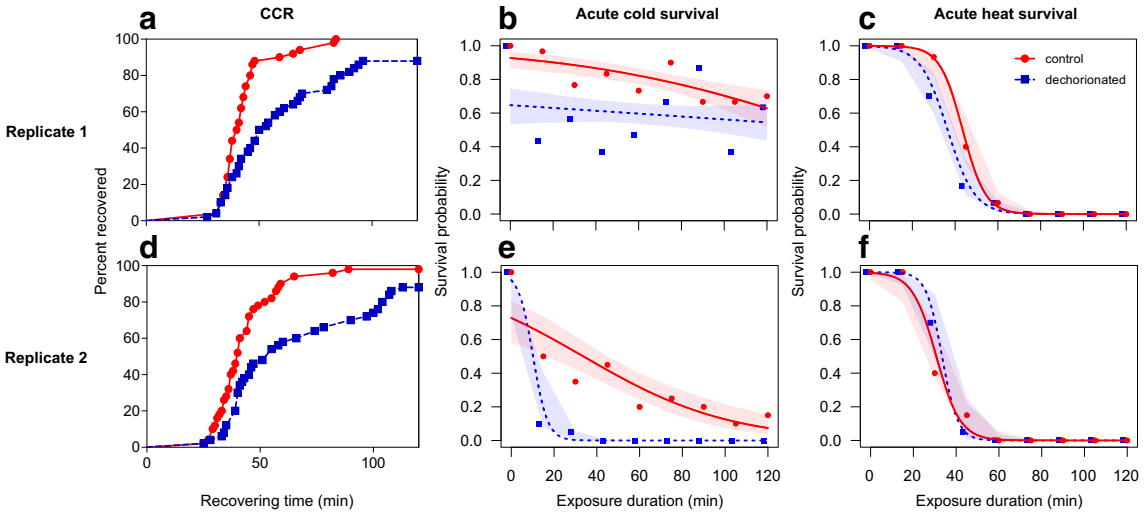

Fig. 2 Cold tolerance of *Co* vs. *De* flies. (A, D) CCR curves of female *Drosophila* flies after 12 h exposure to 0 °C ($N = 50$). (B, E) Survival curves of female *Drosophila* flies 24 h after being exposed to − 3.5 °C for increasing durations ($N = 180$–270). (C, F) Survival curves of female *Drosophila* flies 24 h after being exposed to + 38.5 °C for increasing durations ($N = 180$–270). Dots indicate raw survival proportions for 20–30 individuals at a given exposure duration. Lines indicate predicted survival probabilities based on a binomial GLM. Shaded areas indicate 95% confidence intervals around model predictions. Each row of plots represents one independent experiment

MacMillan 2017; MacMillan et al. 2017). Whether gut microbiota affects this process has not been tested, but findings suggest that complex interactions among pH, ion transporters, and bacteria do take place in the midgut (Overend et al. 2016). Nutrition is another critical determinant of cold tolerance (Colinet et al. 2013), but it also constitutes a keystone of microbiota-host relationship (Ridley et al. 2012). Elimination of microbiota was shown to induce modifications of nutrient acquisition, with sometimes pathologic consequences on triglycerides and glycogen reserves (Wong et al. 2014). With these compounds being linked to stress response, cold tolerance is likely to be affected in return. Finally, it has been proposed that yeasts, an often-neglected part of *Drosophila* microbiota, could be a major driver of microbiota-mediated thermotolerance effects (Jiménez Padilla 2016). Dechorionation is not specific to bacteria and also eliminates yeasts, which could explain part of the observed phenotypes in our experiments.

To conclude, we showed here a potential novel role of gut microbiota on thermotolerance. Whether it is under control of a specific mechanism or merely a side effect of global metabolic changes induced by quantitative reduction of commensal microorganisms still needs to be elucidated.

Acknowledgments Authors thank the GeT-PLaGe plateform for Miseq sequencing.

Compliance with ethical standards

Competing interests The authors declare that they have no conflict of interest.

References

Brummel T, Ching A, Seroude L, Simon AF, Benzer S (2004) *Drosophila* lifespan enhancement by exogenous bacteria. Proc Natl Acad Sci U S A 101:12974–12979. https://doi.org/10.1073/pnas.0405207101

Clark RI, Salazar A, Yamada R, Fitz-Gibbon S, Morselli M, Alcaraz J, Rana A, Rera M, Pellegrini M, Ja WW, Walker DW (2015) Distinct shifts in microbiota composition during *Drosophila* aging impair intestinal function and drive mortality. Cell Rep 12:1656–1667. https://doi.org/10.1016/j.celrep.2015.08.004

Colinet H, Renault D (2012) Metabolic effects of CO2 anaesthesia in *Drosophila melanogaster*. Biol Lett 8:1050–1054. https://doi.org/10.1098/rsbl.2012.0601

Colinet H, Larvor V, Bical R, Renault D (2013) Dietary sugars affect cold tolerance of *Drosophila melanogaster*. Metabolomics 9:608–622. https://doi.org/10.1007/s11306-012-0471-z

Engel P, Moran NA (2013) The gut microbiota of insects – diversity in structure and function. FEMS Microbiol Rev 37:699–735. https://doi.org/10.1111/1574-6976.12025

Jiménez Padilla Y (2016) Effects of gut-associated yeasts on *Drosophila melanogaster* performance. Electron Thesis Diss Repos

Koyle ML, Veloz M, Judd AM, Wong ACN, Newell PD, Douglas AE, Chaston JM (2016) Rearing the fruit fly *Drosophila melanogaster* under axenic and gnotobiotic conditions. J Vis Exp e54219–e54219. https://doi.org/10.3791/54219

Liu H, Dicksved J, Lundh T, Lindberg JE (2014) Heat shock proteins: intestinal gatekeepers that are influenced by dietary components and the gut microbiota. Pathogens 3:187–210. https://doi.org/10.3390/pathogens3010187

MacMillan HA, Yerushalmi GY, Jonusaite S et al (2017) Thermal acclimation mitigates cold-induced paracellular leak from the *Drosophila* gut. Sci Rep 7:8807. https://doi.org/10.1038/s41598-017-08926-7

Matos RC, Schwarzer M, Gervais H, Courtin P, Joncour P, Gillet B, Ma D, Bulteau AL, Martino ME, Hughes S, Chapot-Chartier MP, Leulier F (2017) D-Alanylation of teichoic acids contributes to *Lactobacillus* plantarum-mediated *Drosophila* growth during chronic undernutrition. Nat Microbiol 2:1635–1647. https://doi.org/10.1038/s41564-017-0038-x

McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:e61217. https://doi.org/10.1371/journal.pone.0061217

Moghadam NN, Thorshauge PM, Kristensen TN, de Jonge N, Bahrndorff S, Kjeldal H, Nielsen JL (2017) Strong responses of *Drosophila melanogaster* microbiota to developmental temperature. Fly (Austin) 12:1–12. https://doi.org/10.1080/19336934.2017.1394558

Overend G, Luo Y, Henderson L, Douglas AE, Davies SA, Dow JAT (2016) Molecular mechanism and functional significance of acid generation in the *Drosophila* midgut. Sci Rep 6. https://doi.org/10.1038/srep27242

Overgaard J, MacMillan HA (2017) The integrative physiology of insect chill tolerance. Annu Rev Physiol 79:187–208. https://doi.org/10.1146/annurev-physiol-022516-034142

R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Ridley EV, Wong AC-N, Westmiller S, Douglas AE (2012) Impact of the resident microbiota on the nutritional phenotype of *Drosophila melanogaster*. PLoS One 7:e36765. https://doi.org/10.1371/journal.pone.0036765

Ridley EV, Wong ACN, Douglas AE (2013) Microbe-dependent and nonspecific effects of procedures to eliminate the resident microbiota from *Drosophila melanogaster*. Appl Environ Microbiol 79:3209–3214. https://doi.org/10.1128/AEM.00206-13

Russell JA, Moran NA (2006) Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. Proc R Soc Lond B Biol Sci 273:603–610. https://doi.org/10.1098/rspb.2005.3348

Ryu J-H, Kim S-H, Lee H-Y, Bai JY, Nam YD, Bae JW, Lee DG, Shin SC, Ha EM, Lee WJ (2008) Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in *Drosophila*. Science 319:777–782. https://doi.org/10.1126/science.1149357

Schou MF, Mouridsen MB, Sørensen JG, Loeschcke V (2017) Linear reaction norms of thermal limits in *Drosophila*: predictable plasticity in cold but not in heat tolerance. Funct Ecol 31:934–945. https://doi.org/10.1111/1365-2435.12782

Shin SC, Kim S-H, You H, Kim B, Kim AC, Lee KA, Yoon JH, Ryu JH, Lee WJ (2011) *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin signaling. Science 334:670–674. https://doi.org/10.1126/science.1212782

Soen Y (2014) Environmental disruption of host–microbe co-adaptation as a potential driving force in evolution. Front Genet 5. https://doi.org/10.3389/fgene.2014.00168

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. https://doi.org/10.1371/journal.pone.0070749

Teets NM, Denlinger DL (2013) Physiological mechanisms of seasonal and rapid cold-hardening in insects. Physiol Entomol 38:105–116. https://doi.org/10.1111/phen.12019

Wong AC-N, Chaston JM, Douglas AE (2013) The inconstant gut microbiota of *Drosophila* species revealed by 16S rRNA gene analysis. ISME J 7:1922–1932. https://doi.org/10.1038/ismej.2013.86

Wong AC-N, Dobson AJ, Douglas AE (2014) Gut microbiota dictates the metabolic response of *Drosophila* to diet. J Exp Biol 217:1894–1901. https://doi.org/10.1242/jeb.101725

Yamada R, Deshpande SA, Bruce KD, Mak EM, Ja WW (2015) Microbes promote amino acid harvest to rescue undernutrition in *Drosophila*. Cell Rep 10:865–872. https://doi.org/10.1016/j.celrep.2015.01.018