

Impact of stress on the gut microbiome of free-ranging western lowland gorillas

Klára Vlčková,^{1,2,*} Kathryn Shutt-Phillips,³ Michael Heistermann,⁴ Barbora Pafčo,¹ Klára J. Petrželková,^{2,5,6} Angélique Todd,⁷ David Modrý,^{1,6,8} Karen E. Nelson,^{9,10} Brenda A. Wilson,^{11,12} Rebecca M. Stumpf,^{12,13} Bryan A. White,¹¹ Steven R. Leigh^{11,14} and Andres Gomez¹⁵

Abstract

Exposure to stressors can negatively impact the mammalian gastrointestinal microbiome (GIM). Here, we used 454 pyrosequencing of 16S rRNA bacterial gene amplicons to evaluate the impact of physiological stress, as evidenced by faecal glucocorticoid metabolites (FGCM; ng/g), on the GIM composition of free-ranging western lowland gorillas (*Gorilla gorilla gorilla*). Although we found no relationship between GIM alpha diversity (H) and FGCM levels, we observed a significant relationship between the relative abundances of particular bacterial taxa and FGCM levels. Specifically, members of the family *Anaerolineaceae* ($p=0.4$, FDR $q=0.01$), genus *Clostridium* cluster XIVb ($p=0.35$, FDR $q=0.02$) and genus *Oscillibacter* ($p=0.35$, FDR $q=0.02$) were positively correlated with FGCM levels. Thus, while exposure to stressors appears to be associated with minor changes in the gorilla GIM, the consequences of these changes are unknown. Our results may have implications for conservation biology as well as for our overall understanding of factors influencing the non-human primate GIM.

The gastrointestinal microbiome (GIM) is interconnected with overall host health [1, 2]. Disruptions in the GIM have been associated with a large array of human diseases, such as inflammatory bowel disease [3], allergies, asthma [4, 5], neurodevelopmental illnesses [6] and obesity [7]. Stress, which is defined as an acute threat to homeostasis, evokes adaptive or allostatic responses and can have both short- and long-term influences on the gastrointestinal (GI) tract. Activation of these adaptive or allostatic systems can become maladaptive because of frequent, chronic or excessive stress, leading to predisposition to disease [8]. The major effects of stress on gut physiology include: (1) alterations in GI motility; (2) increase in visceral perception; (3) changes in GI secretion; (4) increase in intestinal

permeability; (5) impaired regenerative capacity of GI mucosa and mucosal blood flow; and (6) negative effects on the GIM [9]. Chronic and acute stress models are widely employed in GI research, because stress has been identified as a risk factor for or modulator of the expression of several GI disorders [9–11].

Many factors, including exposure to stressors, can cause transient alterations in GIM composition [12–16]. Moreover, stressor-induced changes to the GIM may enhance the ability of enteric pathogens to colonize the intestine [17]. Indeed, stress can influence the outcome of bacterial infections, because enteric bacteria can respond to the release of stress-related neurochemical mediators by the host [18]. Reductions in bacterial counts have been observed in

Received 23 June 2017; Accepted 22 November 2017

Author affiliations: ¹Department of Pathology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, Brno 61242, Czech Republic; ²Institute of Vertebrate Biology, Czech Academy of Sciences, Květná 8, Brno 60365, Czech Republic; ³Fauna and Flora International, Pembroke Street, Cambridge, CB2 3QZ, UK; ⁴German Primate Centre, Endocrinology Laboratory, Kellnerweg 4, 37077 Göttingen, Germany; ⁵Liberec Zoo, Masarykova 1347/31, Liberec, 46001, Czech Republic; ⁶Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branišovská 31, České Budějovice, 37005, Czech Republic; ⁷WWF, Dzanga Sangha Protected Areas, BP 1053 Bangui, Central African Republic; ⁸CEITEC VFU, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, Brno, 61242, Czech Republic; ⁹J. Craig Venter Institute, 9714 Medical Center Drive, Rockville, MD 20850, USA; ¹⁰J. Craig Venter Institute, 4120 Capricorn Lane, La Jolla, CA 92037, USA; ¹¹Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, 1206 West Gregory Drive, Urbana, IL 61801, USA; ¹²Department of Microbiology, University of Illinois at Urbana-Champaign, 601 South Goodwin Avenue, Urbana, IL 61801, USA; ¹³Department of Anthropology, University of Illinois at Urbana-Champaign, 607 South Mathews Avenue, Urbana, IL 61801, USA; ¹⁴Department of Anthropology, University of Colorado at Boulder, 1350 Pleasant Street, Boulder, CO 80309-0233, USA; ¹⁵Department of Animal Science, University of Minnesota, 1364 Eckles Aneue, St Paul, MN 55108-6118, USA.

*Correspondence: Klára Vlčková, klari.vlckova@gmail.com

Keywords: gastrointestinal microbiome; bacteria; stress; western lowland gorilla; faecal glucocorticoid metabolites.

Abbreviations: CAR, Central African Republic; DSPA, Dzanga Sangha Protected Areas; FGCM, faecal glucocorticoid metabolites; GI, gastrointestinal; GIM, gastrointestinal microbiome; OTU, operational taxonomic unit; PCoA, principal coordinate analysis; RDP, Ribosomal Database Project; WLG, western lowland gorilla (*Gorilla gorilla gorilla*).

Five supplementary figures are available with the online version of this article.

human GIMs due to stress [19], as well as in infant rhesus monkeys, where a significant decrease in lactobacilli was recorded in response to stressors [20]. To date, the relationship between stress and the GIM has mainly been studied in humans, or in the laboratory, or in captive animals, and it has mostly been examined through qualitative evaluation of stressors [17, 21]. As part of a long-term monitoring programme of a population of western lowland gorillas [*Gorilla gorilla gorilla* (WLG)] in the Dzanga Sangha Protected Areas (DSPA), Central African Republic (CAR), we previously determined their faecal glucocorticoid metabolite (FGCM) levels, a proxy for physiological stress that shows differences related to the level of habituation to the presence of humans [22]. We also characterized the faecal bacterial communities in this DSPA gorilla population [23, 24]. Here, we evaluate the relationship between FGCM levels and GIM composition in a set of faecal samples from four WLG groups in DSPA characterized by different levels of habituation to humans and thus exposed to different levels of physiological stress. The research adhered to the legal requirements of the CAR and DSPA research protocols. Importation of the samples to the EU was approved by the State Veterinary Authority of the Czech Republic.

Faecal samples were collected from two habituated groups at two different DSPA research sites: H1 ($n=9$) and H2 ($n=12$) at Bai Hokou ($2^{\circ} 50' N$, $16^{\circ} 28' E$) and Mongambe ($2^{\circ} 55' N$, $16^{\circ} 23' E$), respectively. Faecal samples from two unhabituated groups were also collected: U1 ($n=10$) near Bai Hokou (an unhabituated group undergoing habituation) and U2 ($n=11$, an entirely wild group) at a site between two research sites (Fig. S1, available in the online version of this article; for more details about the groups see Shutt *et al.* [22]). Samples were collected from June to September 2011. Samples from habituated WLGs were obtained during gorilla follows, while unhabituated, unidentified WLGs were sampled from their nests to avoid duplication. Only fresh faeces were collected, that is, samples were taken within 3 h from the time we assumed the WLGs had left the nests, as per expert tracker assessment. Each individual was sampled noninvasively and was only sampled once. The samples were taken from within the core of the faecal bolus and stored in RNAlater (Thermo Fisher Scientific, Waltham, MA, USA) for microbiome analyses, and 90 % ethanol for FGCM analysis. Validated methods were followed to avoid variation in our FGCM measurements resulting from sampling, extraction or storage conditions [25]. The samples were shipped to the German Primate Centre Endocrinology Laboratory (Göttingen, Germany) for hormone analysis. The samples for microbiome analyses were kept at room temperature for a maximum of 1 month before being transported to the Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, where they were stored at $-20^{\circ}C$ until they were shipped to the Institute for Genomic Biology, University of Illinois at Urbana-Champaign, USA, where the genomic DNA extractions were performed. The resulting genomic DNA was shipped to the J. Craig Venter

Institute, Rockville, MD, USA, where DNA sequencing was performed.

DNA was extracted from the faecal samples using the Power Soil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's protocol. We purified obtained DNA using the QIAquick gel extraction kit (QIAGEN, Germany). The V1–V3 region of the 16S rRNA gene was amplified (35 cycles: at $95^{\circ}C$ for 30 s, at $55^{\circ}C$ for 30 s and at $72^{\circ}C$ for 30 s) using the primers 27f ($5'-AGAGTTTGTATYMTGGCTCAG-3'$, corresponding to nucleotides 27–47 of the *Escherichia coli* 16S rRNA gene) and 534r ($5'-ATTACCGCGGCTGCT GGCA-3'$, corresponding to nucleotides 534–515 of the *E. coli* 16S rRNA gene). The amplicons were multiplexed and pyrosequenced using 454 FLX-Titanium technology as described in [23]. Briefly, the sequence reads were processed using the online tool mothur and its standard 454 SOP [26]. Unique sequences were aligned against the SILVA reference alignment database and chimeras were detected using uchime [27] and removed. The sequences were then classified taxonomically using a Bayesian classifier approach implemented by mothur and reference files from the Ribosomal Database Project (RDP) [28] with a minimum cut-off of 80 %. Then sequence reads with hits corresponding to unknown, mitochondria, chloroplasts, eukaryotes and archaea were eliminated. The remaining reads were clustered *de novo* using ModalClust. Reads sharing $\geq 97\%$ 16S rRNA sequence complete-linkage similarity with the most abundant sequence were binned into an operational taxonomic unit (OTU). Taxonomic profiles were determined using the RDP classifier [28] and the phyloTYPE function within mothur. OTUs detected fewer than five times across the entire data set, and/or in fewer than three (in one and two), individuals were removed to avoid including probable sequence artifacts in the analyses.

FGCM measurements were performed using a 11β -hydroxy-etiocholanolone (3 α ,11 β -dihydroxy-CM) enzyme immunoassay as previously validated in Shutt *et al.* [25]. The inter-assay coefficients of variations for these measurements were 9.2 % (high-value quality control) and 15.1 % (low-value quality control). Any samples with known complications (e.g. seeds discovered in the faecal matrix or alcohol evaporation) were removed. We express all hormone data as hormone content per faecal wet mass (ng/g).

All statistical analyses were performed in R v3.1.2 [29]. We used the statistics package to perform linear regression models [30]; the psych package [31] for Spearman rank correlations used with false discovery rate analyses; the pfirmess package [32] to calculate Kruskal–Wallis tests adjusted for multiple comparisons; the vegan package [30] to calculate PERMANOVA, Shannon diversity indices and principal coordinate analysis (PCoA) conducted on the square root transformed relative abundance of each operational taxonomic unit (OTU), based on Bray–Curtis dissimilarity matrices; and the ggplot2 package [33] to plot the PCoA with colour key.

The median of the FGCM levels (FGCM/g of faeces) was 82.47 (min, 26.74; max, 243.61); for more details see Shutt *et al.* [22]. We did not find significant differences in FGCM levels among gorilla groups ($\chi^2=6.64$, $P=0.08$, Kruskal–Wallis rank sum test). We obtained a median value of 6495 16S rRNA pyrosequencing reads per sample (min, 2144; max, 116 361) after sequence quality control; for more details see [23, 24]. The GIM profiles of the major and minor phyla detected in each gorilla group are shown in Fig. S2. We observed significant differences in GIM profiles among the studied gorilla groups (pseudo $F=2.62$, $P<0.01$, PERMANOVA; Fig. S3). A PCoA plot showed that the GIM composition in the four WLG groups did not vary according to FGCM levels, with the exception of group U2, where individual samples clustered together (Fig. 1a). U2 was the group with the lowest FGCM levels (Fig. 1a, b). Although we observed variation in the GIM composition profiles among the groups, based on the first principal coordinate and FGCM levels, with individuals in U2 showing the lowest stress hormone metabolite values, this relationship was not significant (linear regression model: adjusted $R_2=0.01$, $t=-1.22$, $P=0.23$; Spearman rank correlation: $\rho=-0.24$, $P=0.12$; Fig. 1c). Nevertheless, we did observe significant relationships between the relative abundances of particular bacterial genera and FGCM levels across the studied gorilla groups. Namely, members of the family *Anaerolineaceae* (linear regression model: adjusted $R_2=0.12$, $t=2.55$, $P=0.02$; Spearman rank correlation: $\rho=0.4$, FDR $q=0.01$), the genus *Clostridium* XIVb (linear regression model: adjusted $R_2=0.18$, $t=3.19$, $P<0.01$; Spearman rank correlation: $\rho=0.35$, FDR $q=0.02$) and the genus *Oscillibacter* (linear regression

model: adjusted $R_2=0.19$, $t=3.21$, $P<0.01$; Spearman rank correlation: $\rho=0.35$, FDR $q=0.02$) were positively correlated with FGCM levels (Fig. 2). We did not find any differences in the relative abundances of these bacterial taxa across the entirety of the studied WLG groups, with the exception of *Oscillibacter* between U1 and U2 ($P=0.05$, Kruskal–Wallis tests adjusted for multiple comparisons, Fig. S4). Likewise, no significant relationship was detected between the relative abundance of other bacterial taxa and FGCM levels (data not shown). We also did not observe any correlations between GIM alpha diversity (Shannon diversity indices) and FGCM levels (linear regression model: adjusted $R_2<0.01$, $t=-0.03$, $P=0.98$; Spearman rank correlation: $\rho=0.03$, $P=0.86$; Fig. S5).

These results suggest that stress has minimal impact on the overall composition of the GIM in the studied WLGs, with the exception of individuals from group U2, whose GIM clustered together and showed the lowest FGCM levels. We did not observe significant differences in FGCM levels among the studied groups, which is in contrast to Shutt *et al.*'s findings [22], however, this study used a large sample set. The specific changes in the abundance of particular bacterial taxa along with FGCM levels may be noteworthy. The taxa correlated with FGCM levels have been consistently found in the GIM of great apes, and could be considered to be normal commensals in non-human primates [23, 34, 35]. Higher abundance of *Oscillibacter* has also been observed in humans on a resistant-starch and reduced-carbohydrate weight-loss diet, and is depleted in patients suffering from Crohn's disease [36, 37]. Members of the genus *Clostridium* from the cluster XIV with sub-clusters

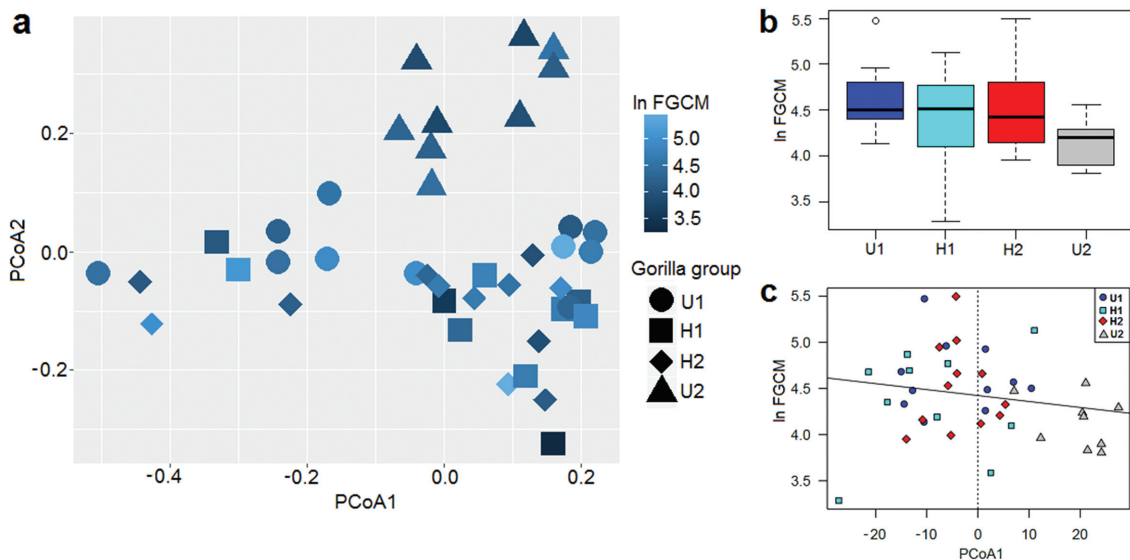


Fig. 1. Relationship of GIM composition and FGCM levels of individual WLGs from four groups. U1 and U2, unhabituated groups; H1 and H2, habituated groups. (a) PCoA plot of GIM (described with first and second principal coordinates) with colour key representing FGCM levels. (b) Box plots of natural log-transformed FGCM levels in the studied WLG groups; mean values are represented by lines in the boxes. (c) Linear regression model of GIM (first principal coordinate) and FGCM levels (natural log-transformed).

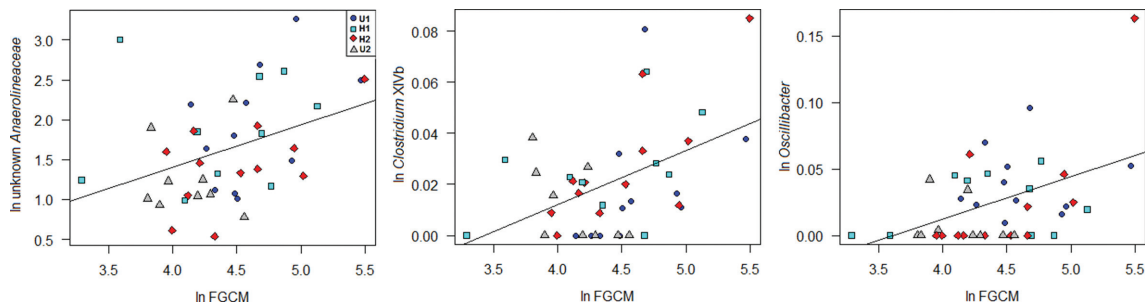


Fig. 2. Significant linear regression model of bacterial taxa at the genus level correlated with FGCM levels (natural log-transformed data). U1 and U2, unhabituated WLG groups; H1 and H2, habituated WLG groups.

XIVa and XIVb [38] include several cellulolytic *Clostridium* species that decompose and ferment various polysaccharides and disaccharides derived from plant cell walls [39]. Increases in the relative abundances of *Clostridium* spp. have also been observed in adult mice due to social stressors [21]. Unlike the clostridia detected in our study (cluster XIV), these bacteria include pathogenic *Clostridium* species known to induce inflammation [40]. Thus, it is possible that changes in the abundance of these taxa upon exposure to stressors are confounded with different dietary behaviours within these WLG groups due to small-scale geographical separation and socio-ecological reasons, such as group size difference [23, 41]. Moreover, previous studies showed that the presence of humans and the habituation process have an effect on gorilla feeding behaviour. For the group undergoing habituation, the daily path lengths were longer during the early stages of habituation, when the gorillas were avoiding observers [42], which may have an indirect impact on feeding behaviour. In habituated groups, when the number of visiting people increased, the silverbacks (at least) spent more time monitoring humans and less time feeding [41]. Future studies should thus clarify if the stress affects feeding behaviour, thereby changing the microbiome, or if there is a direct effect on the microbiome composition and function. Furthermore, as these bacterial taxa are poorly characterized in the non-human primate gut, it is hard to speculate concerning the combined effect of FGCM (stressors) and ecological factors (diet) on the abundance of these taxa in the gut of habituated and unhabituated WLGs.

Our results show that stressors, as evidenced by elevated FGCM levels, may have minor, specific effects on the overall GIM composition of free-ranging WLGs. Specifically, we observed that higher FGCM levels are associated with higher levels of members of the family *Anaerolineaceae*, genus *Clostridium* cluster XIVb and genus *Oscillibacter*. However, it is unclear whether these minor changes have a significant impact on the gut microbial communities at large (e.g. dysbiosis) or on overall host health. Although a completely unhabituated group (U2) showed the lowest incidence of physiological stress, and the GIM compositions among individuals from this group were more similar to one another than those observed in the other studied groups, it is unclear

whether the individuals with higher exposure to stressors (i.e. having the highest FGCM levels), namely those from the H1, H2 (both habituated) and U1 (undergoing habituation) groups, exhibit significant changes in their GIM composition. Our data do not support the notion that stress linked with the habituation of wild apes to humans has major effects on their GIM. However, any potential changes may be the result of several ecological and dietary factors acting together over long periods of time. It is important to control for diet-induced GIM changes within each studied gorilla group. To further assess the effect of stressors on the GIM of wild non-human primates, long-term multivariate studies that monitor fluctuation in FGCM levels together with changes in GIM profiles over the entire habituation process, in one gorilla group, are warranted.

Funding information

This work was supported by the Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic (RVO: 68081766); by the project 'CEITEC – Central European Institute of Technology' (CZ.1.05/1.100/02.0068) from the European Regional Development Fund, and co-financed from the European Social Fund and the State Budget of the Czech Republic (project OPVK CZ.1.07/2.3.00/20.0300); a NERC/ESRC interdisciplinary PhD studentship; the Primate Society of Great Britain; the International Primatological Society; the Bio-Social Society UK; Rufford Small Grants for Nature Conservation; and the US National Science Foundation (NSF BCS) 0935347.

Acknowledgements

We thank the Government of the Central African Republic and the World Wildlife Fund for granting permission to conduct our research in the Central African Republic and the Primate Habituation Programme (Dzanga-Ndoki National Park, Dzanga-Sangha Protected Areas) for logistical support in the field. We also thank all local trackers and assistants for their help with sample collection. Finally, we would like to thank Marcus Gillis for assistance in sequencing at JCVI. This publication derives from the HPI-lab, Laboratory for Infectious Diseases Common to Humans and (non-Human) Primates, Czech Republic.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

- O'Hara AM, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep* 2006;7:688–693.
- Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell* 2012; 148:1258–1270.

3. Guinane CM, Cotter PD. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. *Therap Adv Gastroenterol* 2013;6:295–308.
4. West CE. Gut microbiota and allergic disease: new findings. *Curr Opin Clin Nutr Metab Care* 2014;17:261–266.
5. Fujimura KE, Lynch SV. Microbiota in allergy and asthma and the emerging relationship with the gut microbiome. *Cell Host Microbe* 2015;17:592–602.
6. Borre YE, O’Keeffe GW, Clarke G, Stanton C, Dinan TG et al. Microbiota and neurodevelopmental windows: implications for brain disorders. *Trends Mol Med* 2014;20:509–518.
7. Ley RE. Obesity and the human microbiome. *Curr Opin Gastroenterol* 2010;26:5–11.
8. Bhatia V, Tandon RK. Stress and the gastrointestinal tract. *J Gastroenterol Hepatol* 2005;20:332–339.
9. Konturek PC, Brzozowski T, Konturek SJ. Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options. *J Physiol Pharmacol* 2011;62:591–599.
10. Collins SM. IV. Modulation of intestinal inflammation by stress: basic mechanisms and clinical relevance. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G315–G318.
11. Söderholm JD, Perdue MH. Stress and gastrointestinal tract. II. Stress and intestinal barrier function. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G7–G13.
12. Wang SX, Wu WC. Effects of psychological stress on small intestinal motility and bacteria and mucosa in mice. *World J Gastroenterol* 2005;11:2016–2021.
13. Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML et al. Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. *Infect Immun* 2009;77:2367–2375.
14. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 2008;6:e280.
15. Galley JD, Nelson MC, Yu Z, Dowd SE, Walter J et al. Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. *BMC Microbiol* 2014;14:189–13.
16. de Palma G, Blennerhassett P, Lu J, Deng Y, Park AJ et al. Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nat Commun* 2015;6:7735–13.
17. Bailey MT, Dowd SE, Parry NM, Galley JD, Schauer DB et al. Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by *Citrobacter rodentium*. *Infect Immun* 2010;78:1509–1519.
18. Lyte M, Vulchanova L, Brown DR. Stress at the intestinal surface: catecholamines and mucosa-bacteria interactions. *Cell Tissue Res* 2011;343:23–32.
19. Knowles SR, Nelson EA, Palombo EA. Investigating the role of perceived stress on bacterial flora activity and salivary cortisol secretion: a possible mechanism underlying susceptibility to illness. *Biol Psychol* 2008;77:132–137.
20. Bailey MT, Coe CL. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. *Dev Psychobiol* 1999;35:146–155.
21. Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG et al. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav Immun* 2011;25:397–407.
22. Shutt K, Heistermann M, Kasim A, Todd A, Kalousova B et al. Effects of habituation, research and ecotourism on faecal glucocorticoid metabolites in wild western lowland gorillas: Implications for conservation management. *Biol Conserv* 2014;172:72–79.
23. Gomez A, Petrzekova K, Yeoman CJ, Vlčková K, Mrázek J et al. Gut microbiome composition and metabolomic profiles of wild western lowland gorillas (*Gorilla gorilla gorilla*) reflect host ecology. *Mol Ecol* 2015;24:2551–2565.
24. Gomez A, Rothman JM, Petrzekova K, Yeoman CJ, Vlčková K et al. Temporal variation selects for diet-microbe co-metabolic traits in the gut of *Gorilla* spp. *Isme J* 2016;10:514–526.
25. Shutt K, Setchell JM, Heistermann M. Non-invasive monitoring of physiological stress in the Western lowland gorilla (*Gorilla gorilla gorilla*): validation of a fecal glucocorticoid assay and methods for practical application in the field. *Gen Comp Endocrinol* 2012;179:167–177.
26. Schloss PD, Gevers D, Westcott SL. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PLoS One* 2011;6:e27310.
27. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011;27:2194–2200.
28. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007;73:5261–5267.
29. R Development Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2008.
30. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR et al. *vegan: community ecology package: R package version 2.0-10*; 2013.
31. Revelle W. *psych: Procedures for Psychological, Psychometric, and Personality Research*. R package version 1.7.5. Northwestern University, Evanston, Illinois; 2017.
32. Giraudoux P. *pgirmess: data analysis in ecology*. R package version 1.6.7. 2017.
33. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag; 2009.
34. Lopetuso LR, Scalfaferrri F, Petito V, Gasbarrini A. Commensal Clostridia: leading players in the maintenance of gut homeostasis. *Gut Pathog* 2013;5:23.
35. Schnorr SL, Candela M, Rampelli S, Centanni M, Consolandi C et al. Gut microbiome of the Hadza hunter-gatherers. *Nat Commun* 2014;5:1–12.
36. Mondot S, Kang S, Furet JP, Aguirre de Carcer D, McSweeney C et al. Highlighting new phylogenetic specificities of Crohn’s disease microbiota. *Inflamm Bowel Dis* 2011;17:185–192.
37. Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *Isme J* 2011;5:220–230.
38. Collins MD, Lawson PA, Willems A, Cordoba JJ, Fernandez-Garayzabal J et al. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* 1994;44:812–826.
39. Düre P. Degradation of Polymers: Cellulose, Xylan, Pectin, Starch. In: Düre P (editor). *Handbook on Clostridia*. Boca Raton: CRC Press; 2005.
40. Daruwala C, Mercogliano G, Newman G, Ingerman MJ. Bacteremia due to *Clostridium difficile*: case report and review of the literature. *Clin Med Case Rep* 2009;2:5–9.
41. Klaitova M, Hodgkinson C, Lee PC. Behavioral responses of one western lowland gorilla (*Gorilla gorilla gorilla*) group at Bai Hokou, Central African Republic, to tourists, researchers and trackers. *Am J Primatol* 2010;72:897–906.
42. Cipolletta C. Ranging patterns of a western gorilla group during habituation to humans in the Dzanga-Ndoki National Park, Central African Republic. *Int J Primatol* 2003;24:1207–1226.

Edited by: K. P. Scott and D. Grainger