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Gastrointestinal microbiota of wild and inbred individuals of two house mouse subspecies assessed using high-throughput parallel pyrosequencing

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Abstract

The effects of gastrointestinal tract microbiota (GTM) on host physiology and health have been the subject of considerable interest in recent years. While a variety of captive bred species have been used in experiments, the extent to which GTM of captive and/or inbred individuals resembles natural composition and variation in wild populations is poorly understood. Using 454 pyrosequencing, we performed 16S rDNA GTM barcoding for 30 wild house mice (*Mus musculus*) and wild-derived inbred strain mice belonging to two subspecies (*M. m. musculus* and *M. m. domesticus*). Sequenced individuals were selected according to a 2 × 2 experimental design: wild (14) vs. inbred origin (16) and *M. m. musculus* (15) vs. *M. m. domesticus* (15). We compared alpha diversity (i.e. number of operational taxonomic units – OTUs), beta diversity (i.e. inter-individual variability) and microbiota composition across the four groups. We found no difference between *M. m. musculus* and *M. m. domesticus* subspecies, suggesting low effect of genetic differentiation between these two subspecies on GTM structure. Both inbred and wild populations showed the same level of microbial alpha and beta diversity; however, we found strong differentiation in microbiota composition between wild and inbred populations. Relative abundance of ~ 16% of OTUs differed significantly between wild and inbred individuals. As laboratory mice represent the most abundant model for studying the effects of gut microbiota on host metabolism, immunity and neurology, we suggest that the distinctness of laboratory-kept mouse microbiota, which differs from wild mouse microbiota, needs to be considered in future biomedical research.

Keywords: domestication, hybrid zone, metagenomics, microbiome, *Mus musculus*, symbiosis

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Introduction

Vertebrates interact with bacterial communities that are both specialized and highly diversified taxonomically and spatially (Costello *et al.* 2009). Bacterial cell and active gene counts for animal-associated microbiota exceed the number of host somatic cells and genes by at least an order of magnitude (Qin *et al.* 2010). It is not surprising, therefore, that bacterial communities have a marked influence on the physiology and health of the host (Sekirov *et al.* 2010; Wallace *et al.* 2011).

Gastrointestinal tract microbiota (GTM) have been the most heavily studied vertebrate-associated bacterial community (De Filippo *et al.* 2010; Qin *et al.* 2010; Sekirov *et al.* 2010; Foster & Neufeld 2013). Functionally, GTM are often viewed as a separate metabolic organ with many causal pleiotropic effects on the host (O'Hara & Shanahan 2006). Studies using gnotobionts and germ-free animal models (i.e. animals lacking GTM) have shown

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that the presence of GTM is essential for normal development of the digestive tract (Wostmann & Bruckner-Kardoss 1959; Gordon & Bruckner-Kardoss 1961) and the immune (Macpherson & Harris 2004) and central nervous systems (Heijtz *et al.* 2011; Cryan & Dinan 2012). GTM also play an important role in host energy balance (Hooper *et al.* 2001; Bäckhed *et al.* 2005, 2007; Sonnenburg *et al.* 2005; Jumpertz *et al.* 2011). Consequently, GTM dysbiosis can lead to pathologies such as obesity (Turnbaugh *et al.* 2006), autoimmune diseases (Mathis & Benoist 2011), pathogen invasions (Koch & Schmid-Hempel 2011; Chervonsky 2012) and neurological disorders (Cryan & Dinan 2012).

At higher taxonomic levels, GTM are typically dominated by two bacterial phyla, the Bacteroidetes and Firmicutes (Ley *et al.* 2008; Ochman *et al.* 2010; Muegge *et al.* 2011). At the phylotype (i.e. operational taxonomic unit; OTU) level, interindividual differences are much more pronounced, with the majority of taxa being unique to a given individual (Turnbaugh *et al.* 2009; Qin *et al.* 2010). It is possible, however, to identify a group of bacterial taxa that are shared between individuals and that enable the key functions of the GTM (Turnbaugh & Gordon 2009; Jalanka-Tuovinen *et al.* 2011).

Factors contributing to high levels of interindividual difference in GTM composition are still the subject of intensive research, as well as severe controversy. Maternal transfer during the early stages of ontogeny has a significant long-lasting effect on GTM composition (Lucas & Heeb 2005; Turnbaugh *et al.* 2009; Friswell *et al.* 2010). In addition, GTM composition is influenced by environmental conditions during early life (Sanchez *et al.* 2011) and diet composition (Faith *et al.* 2011; Wang *et al.* 2014). Finally, it has also been suggested that genetic background has an effect on GTM structure (Rawls *et al.* 2006; Benson *et al.* 2010).

At the population level, GTM profiles tend to cluster together based on the geographic origin of the host (De Filippo *et al.* 2010; Nam *et al.* 2011; Banks *et al.* 2009; see also Wang *et al.* 2014). Geographic distance has also been found to be the most significant factor explaining patterns of diversity in the GTM of house mice, with host population structure and genetic distance having a comparatively weaker influence (Linnenbrink *et al.* 2013).

The effect of host phylogeny on GTM composition is not yet completely resolved. During mammalian evolution, GTM composition appears to have been unstable and shaped through independent acquisitions of bacterial taxa in animals with convergent dietary strategies (Ley *et al.* 2008; Muegge *et al.* 2011).

The wild house mouse (*Mus musculus*) is a polytypic species often used as a model species in population and evolutionary biology (Macholán *et al.* 2012). In particular, the European hybrid zone between the *M. m. musculus* (hereafter Mmm) and *M. m. domesticus* (Mmd) subspecies is an important model for studying the evolution of speciation (Baird & Macholán 2012; Dureje *et al.* 2012). Limited hybridization between the two subspecies has been put down to various reproductive barriers (Dufková *et al.* 2011; Duvaux *et al.* 2011; Macholán *et al.* 2011; Vošlajerová-Bímová *et al.* 2011; Albrechtova *et al.* 2012; Janousek *et al.* 2012; Turner *et al.* 2012). While the genetic component of decreased hybrid fitness has frequently been studied (reviewed in Forejt *et al.* 2012; Dzur-Gejdosova *et al.* 2012; Flachs *et al.* 2012), studies of the epigenetic component of reproductive isolation are rather scarce (see for example Schütt *et al.* 2003), and possible differences in gut microbiota composition between mouse subspecies have not been considered at all. Nevertheless, previous studies on nonmammalian species suggest that differences in GTM composition may contribute to assortative mating and promote reproductive barriers (Sharon *et al.* 2010).

Laboratory strains of the house mouse are the most widely used animal model for biomedicine and human genetics (Fox *et al.* 2007), including research focused on metabolic and physiological effects of GTM on the host (reviewed in Sekirov *et al.* 2010; Staubach *et al.* 2012). However, the diversity and genetic composition of laboratory strains are not truly representative of wild house mice (Yang *et al.* 2011). This is mainly due to a founder effect (i.e. most laboratory strains are of Mmd origin), inbreeding and, sometimes, the hybrid origin of strains. It is probable that similar processes may also have influenced GTM composition in laboratory mice, and hence 'laboratory GTM' will not represent 'wild GTM'. Differences between laboratory and wild GTM may become further pronounced through the exposure of these two groups to completely different environmental conditions (e.g. temporal and spatial energy intake or presence of pathogens and stress; Sudakaran *et al.* 2012; Nelson *et al.* 2013). Interindividual variability in GTM composition in wild populations vs. inbred strains is also of interest as this can strongly influence effect size estimates and the significance of experimental results. While knowledge of GTM composition in classical laboratory mouse strains is growing rapidly (Benson *et al.* 2010; Friswell *et al.* 2010; Krych *et al.* 2013), it is still lacking for wild-derived mouse strains with well-defined breeding histories and genetic origin. There has been just one study of GTM composition in wild house mice, undertaken by Linnenbrink *et al.* (2013). A direct comparison of GTM between wild populations and captive bred strains, therefore, has yet to be performed.

In this study, we perform 16S rRNA barcoding, to assess gut microbiota composition in wild and wild-derived inbred individuals of Mmm and Mmd origin.

We evaluate interindividual variation and differences in GTM diversity and composition between wild populations and inbred mouse strains, as well as between the two subspecies. Our results allow us to address questions regarding the role of genetic background vs. environmental conditions on diversity and interindividual variation in GTM. We also used 16S rRNA sequences to predict GTM functional potential (i.e. genes encoded by GTM) based on the recently developed algorithm of Langille *et al.* (2013). This enabled us to gain preliminary insights into metagenomic variation between Mmm and Mmd and between wild vs. wild-derived inbred individuals.

Materials and methods

Mouse samples

Each of the 14 wild mice used in this study was captured at a different locality along the Czech–Bavarian border region (length of east–west transection = 142 km; Fig. 1). Subadult mice and mice in bad condition were not included in the study. In addition, a male and female from each of eight wild-derived inbred strains kept at the conventional breeding facility of the Studenec Research Facility (Institute of Vertebrate Biology, Academy of Sciences, Czech Republic) were divided into pairs based on their source localities. Details of Mmm strains BUSNA, BULS (source locality Buskovice) and STUF, STUS (Studenec) and Mmd strains STRA, STRB (Straas) can be found in Piálek *et al.* (2008); the two remaining Mmd strains, SCHEFE and SCHUNT, originated from Schweben in Central Germany [50°26′N, 9°35′E]. Thus, the 16 inbred mice had their origin in eight inbred strains from no more than 4 wild localities, giving a high probability of decreased genetic diversity due to a founder effect. See Table S1 (Supporting information) for more details on individual samples.

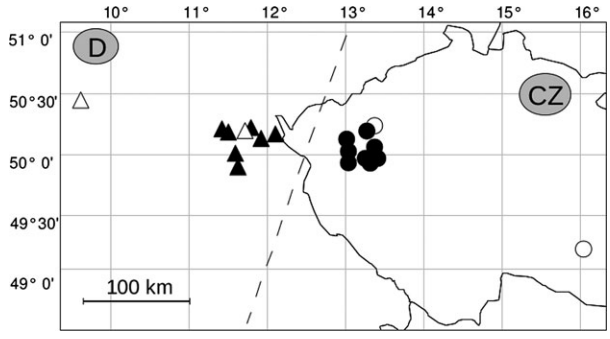

Fig. 1 Sampling localities for wild (full symbols) and inbred (open symbols) populations of *Mus musculus musculus* (circles) and *Mus musculus domesticus* (triangles). The dashed line represents the house mouse hybrid zone.

The wild-derived strains were kept in 16 cm×28 cm×15 cm Perspex cages with sawdust bedding under the following constant conditions: a 14:10 light:dark cycle, 22 °C temperature, 40% humidity. Standard mouse pellets (ST1, VELAZ) and tap water were available *ad libitum*. The breeding facility is licensed for keeping small mammals according to Czech law (3OZ31162/2011-17214).

Laboratory procedures

Wild mice were live-trapped and caged singly until dissection in a field laboratory. Sterile dissection was performed no more than 24 h after the mouse was trapped. Colons, including faeces, were stored in 70% EtOH at room temperature. DNA was isolated from the faeces at the Institute of Parasitology, Academy of Sciences, Czech Republic, as described in Sak *et al.* (2011). Inbred individuals were euthanized and dissected at the breeding facility in Studenec. Faeces were placed into extraction buffer and DNA extracted using the same commercial kit as for wild mice (i.e. QIAamp® DNA Stool Mini Kit, QIAGEN). All DNA samples were diluted to a concentration of 5 ng/μL (Qubit fluorometer, Invitrogen).

To perform bacterial barcoding, we used MPRK341F (CCTAYGGGRBGCASCAG) and MPRK806R (GGACT ACNNGGGTATCTAAT) primers to amplify the 466 bp fragment consisting of the V3 and V4 regions of *Escherichia coli* 16S rDNA (Yu *et al.* 2005). Both the fusion primers needed for 454 sequencing and the amplification protocol were kindly provided by Lars Hansen (University of Copenhagen). The reverse fusion primer consisted of the Titanium adaptor A sequence (Lib A) and the MPRK806R primer sequence. Forward fusion primers differed between the individuals sequenced within a single sequencing run and consisted of adaptor B sequence (Lib A), a unique tag sequence from the Roche MID library and the MPRK341F primer sequence. 16S rDNA was amplified in two independent PCRs. A first PCR was performed with the MPRK341F and MPRK806R primers twice for each sample and the two PCRs pooled. The PCR mix (20 μL) consisted of 0.02 U/μL Phusion Hot Start DNA Polymerase II (Finnzymes), 1× Phusion HF buffer with MgCl2, 0.2 mM dNTP, 0.5 μM of each primer and 0.5 μL of DNA. The PCR conditions consisted of an initial denaturation at 98 °C for 30 s, followed by 32 cycles of 98 °C (5 s), 56 °C (20 s) and 72 °C (20 s), and a final extension at 72 °C (5 min). PCR products were incubated at 70 °C for three minutes and moved to ice. The samples were run on 1% agarose gel, PCR bands were excised from the gel, purified using the QIAquick gel extraction kit (QIAGEN) and eluted in 30 μL of elution buffer. A

second PCR was performed to add sequencing adaptors and sample specific tags to the amplicons. This PCR was performed in 40 μL and was the same as the previous one, except that fusion PCR primers were used, 1 μL of the first PCR was used as a template and the number of PCR cycles was cut down to 15. PCR products were warmed (70 °C/3 min) and incubated on ice, separated onto agarose gel, excised from the gel and purified as described above, and their concentration was measured using PicoGreen (Invitrogen) and normalized.

Amplicons were then pooled (separate pools for wild and for inbred samples) and pyrosequenced using GS Junior System (Roche) in two independent sequencing runs according to the manufacturer's instructions. To achieve unidirectional sequencing starting from the tagged forward primers, only B beads were used in the emulsion PCR.

Data analysis

Ampliconnoise pipeline (Quince *et al.* 2011) was used for denoising and quality and chimera filtering of the original sff files. The resulting high-quality sequences were further clustered into OTUs based on 97% sequence similarity, using the CLUSTOM algorithm (Hwang *et al.* 2013).

CLUSTOM outputs were converted to QIIME input format using in-house R scripts and further processed using QIIME pipeline (Caporaso *et al.* 2010a), whereby OTU taxonomy was assigned using the RDP classifier (Wang *et al.* 2007; confidence to record assignment >0.80). Representative sequences were aligned using PyNast and Greengenes Core Set Alignment (Caporaso *et al.* 2010b). A minimum evolution phylogenetic tree was constructed using FASTTREE (Price *et al.* 2009).

To assess variation in alpha diversity between individual subspecies (Mmm vs. Mmd) and between individuals from wild populations vs. inbred strains, we used rarefaction analysis (10 rarefied subsamples of sequences for each individual and sequence counts per individual sample ranging between 100 and 3500). Three diversity measures were computed on the subsampled data: (i) the number of OTUs in a given rarefied subsample, (ii) the Chao1 index (Chao & Shen 2003), and (iii) a phylogenetic diversity index computed as total branch length.

We further computed weighted and unweighted UNIFRAC distances (Lozupone & Knight 2005) between individuals and visualized the overall pattern of interindividual variation using Nonmetric Multidimensional Scaling (NMDS). To avoid potential bias associated with unequal sequencing depth in the samples, UNIFRAC analysis is based on a random subsample corresponding to 1200 reads (~minimum achieved sequencing depth) per individual. Next, we assessed to what extent (i) subspecies identity (Mmm vs. Mmd), (ii) mouse origin (wild vs. inbred), and (iii) the interaction between these variables contribute to variation in microbiome composition. To do this, we used multivariate ANOVA based on dissimilarities (ADONIS) implemented in the R (v. 2.14.1, R Development Core Team 2011) VEGAN package (Oksanen *et al.* 2013). The BETADISPER function (analogous to Levene's test) was used to test whether the interindividual variation in UNIFRAC distances was the same for samples originating from different subspecies and for inbred vs. wild individuals. ADONIS was further used to test for the effect of source population on GTM composition in inbred strains. Using the Mantel test, we assessed the correlation between UNIFRAC distances and geographic distance in wild individuals.

Differences in taxonomic composition between subspecies and inbred vs. wild individuals at the phylum level were further evaluated using the Mann–Whitney *U*-test and the Šidak multiple test correction method. The same comparison based on Welsh's t test provided identical results (not shown).

Further, we used permutation ANOVAs to analyse variation in taxonomic composition at the level of individual OTUs. We chose an arbitrary OTU threshold of those with >50 high-quality sequences (n = 81) as these OTUs represented the vast majority (95.7%) of all high-quality reads. First, we converted read counts for individual OTUs within sampled individuals into proportions. We further generated 1000 randomized community matrices by resampling (without replacement) OTU proportions across samples. Using custom R scripts, separate factorial ANOVA models were fitted for individual OTUs and for both original and resampled community matrixes, including sample origin (wild vs. inbred), species identity (Mmm vs. Mmd) and their interactions as explanatory variables. Significance of these variables for a given OTU was assessed based on a comparison of t values computed for original data with the distribution of simulated t values (Lepš & Šmilauer 2003). The Q value method for multiple tests correction (QVALUE software; Storey & Tibshirani 2003) was used to minimize the risk of false discovery rates.

PICRUST pipeline (Langille *et al.* 2013) was used to predict gene content of individual GTM samples. In short, this software uses 16S RNA data on taxonomic composition of a microbiome, together with information on bacterial gene content that have been whole-genome-sequenced and an ancestral-state reconstruction algorithm to predict functional content (in terms of encoded genes) of given community. First, we mapped our high-quality sequences against reference OTUs from the Green Gene reference database (version:

gg_13_5_otus; DeSantis *et al.* 2006; 97% similarity threshold) using the closed reference algorithm implemented in QIIME. The resulting OTU table was used as the PICRUST input. PICRUST was run using the default setup recommended by the authors. The resulting database included information on predicted abundance of individual Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Kanehisa & Goto 2000) in individual samples. This data set was further analysed using permutation-based ANOVAs, as described above.

Results

General description of house mouse GTM

We identified 107999 sequences that passed quality filtering and did not correspond to chimeric OTUs, with the number of high-quality sequences per individual ranging between 1230 and 6811 (mean = 3599). These sequences were clustered in 1374 OTUs based on 97% sequence similarity. The RDP classifier successfully classified 865 OTUs (which included 10 336/>95%/ high-quality sequences) to family level. RDP failed to classify just 157 OTUs to phylum level. These taxonomically unclassified OTUs, however, represented just a small minority (~0.5%) of the high-quality sequences.

The house mouse microbiome was dominated by bacteria of the phyla *Firmicutes* and *Bacteroidetes*. Other bacterial phyla identified included *Proteobacteria*, *Fusobacteria*, *TM7*, *Tenericuides*, *Deferribacteres* and 10 others. Compared to *Firmicutes* and *Bacteroidetes*, however, their relative abundances were low (see Fig. 2; details on taxonomic composition is available in Appendix S1 and Table S2, Supporting information).

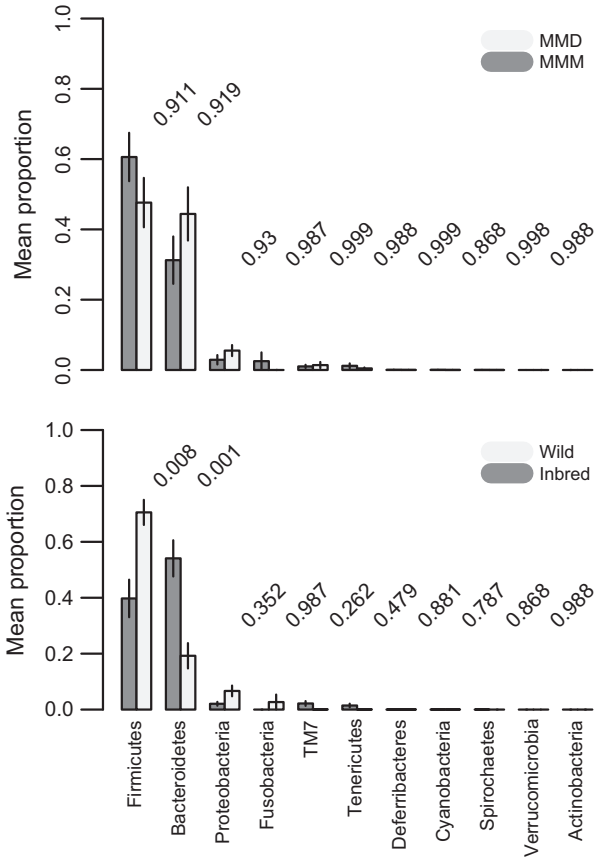

Fig. 2 Differences in taxonomic composition at the phylum level, expressed as mean proportion of sequences in *Mus musculus musculus* (MMM) vs. *Mus musculus domesticus* (MMD) individuals (upper graph) and wild vs. inbred individuals (lower graph). Error lines indicate standard errors for individual groups. *P* values above bars correspond to Welsh's *t*-tests and the Šidak multiple test correction method.

GTM diversity

The number of OTUs per individual, as predicted by the Chao1 index, ranged between 650 and 354, with the exception of one inbred outlier with a considerably lower alpha diversity level (Table S1, Supporting information). Rarefaction analysis suggested that the sequencing depth was insufficient to capture all GTM diversity (Fig. 3); nevertheless, rarefaction curves indicated no pronounced difference in alpha diversity between Mmm and Mmd (mean Chao1 ± SE = 390 ± 40 and 320 ± 27, respectively) or between samples from inbred strains and wild population (mean Chao1 ± SE = 359 ± 32 and 350 ± 38, respectively; Fig. 3; Table S1, Supporting information). We tested the effect of these variables, and their interaction, on observed alpha diversity using linear mixed effect models (fitted using R package lme4; Bates *et al.* 2012). Alpha diversity indices computed for rarefied GTM subsamples ($n = 10$ for each individual and each sequence count category), including 1000 (~minimum sequencing depth) and 3000 (~median sequencing depth) randomly selected sequences, were used as response variables. Identity of individuals, corresponding to the replicated rarefied subsamples, was included as a random intercept. None of these explanatory variables had a significant effect on observed alpha diversity (Table 1), although rarefaction plots for number of observed OTUs and phylogenetic diversity suggest slightly decreased alpha diversity for inbred individuals (see Fig. 3 and Appendix S2, Supporting information).

Variation in community structure

The NMDS plot based on both weighted and unweighted UNIFRAC distances revealed differences in faecal microbiome composition between individuals originating from inbred individuals and wild populations

Table 1 Effect of subspecies identity (Mmm vs. Mmd), origin (inbred vs. wild) and interaction between these variables on three alpha diversity measures (i.e. number of OTUs, Chao1 index and Phylogenetic diversity index) for rarefied subsamples of the original data set. n = 1000 and 3000 randomly selected sequences per individual. Data were analysed using linear mixed effect models. Significance of explanatory variables was assessed using the likelihood ratio test (e.g. Crawley 2007)

			n = 1000		n = 3000	
Diversity index	Explanatory variable	d.f.	χ^2	P	χ^2	P
Number of OTUs	Subspecies	1	1.131	0.288	0.015	0.903
	Origin	1	1.116	0.291	0.027	0.869
	Subspecies × Origin	1	0.028	0.868	0.010	0.993
Chao1	Subspecies	1	0.066	0.797	3.114	0.078
	Origin	1	0.219	0.640	2.803	0.094
	Subspecies × Origin	1	0.112	0.738	2.945	0.086
Phylogenetic diversity	Subspecies	1	1.540	0.215	0.184	0.668
	Origin	1	0.195	0.195	0.682	0.409
	Subspecies × Origin	1	0.010	0.922	0.136	0.712

(Fig. 4). We detected one outlying point in the NMDS plot that exhibited decreased alpha diversity (corresponding to sample 'I' in Table S1, Supporting information). Subsequently, ADONIS and BETADISPER models were evaluated for distance matrices that included (presented in Appendix S2, Supporting information) and excluded (presented in this section) this sample. If not explicitly stated, both options provided consistent results.

In accordance with the NMDS plots, ADONIS confirmed significant differentiation between inbred and wild individuals. Conversely, ADONIS failed to detect significant differences between Mmm and Mmd. The effect of interaction between species identity and origin was significant in the case of weighted UNIFRAC, but not in the case of unweighted UNIFRAC (Table 2).

Separate ADONIS analyses for wild and inbred samples do not suggest GTM differentiation between Mmm and Mmd (Wild samples: $F_{(1,12)} = 0.903$, $R^2 = 0.070$, $P = 0.424$ and $F_{(1,13)} = 0.768$, $R^2 = 0.060$, $P = 0.654$, Inbred samples: $F_{(1,13)} = 1.042$, $R^2 = 0.070$, $P = 0.366$ and $F_{(1,13)} = 1.186$, $R^2 = 0.080$, $P = 0.194$, for weighted and unweighted UNIFRAC, respectively). On the other hand, we observed significant differentiation between wild and inbred Mmm individuals using weighted UNIFRAC ($F_{(1,12)} = 12.868$, $R^2 = 0.520$, $P = 0.003$), while the results were nonsignificant in the case of Mmd ($F_{(1,14)} = 1.448$, $R^2 = 0.100$, $P = 0.223$). Unweighted UNIFRAC revealed significant differentiation between inbred and wild samples for both Mmd and Mmm ($F_{(1,13)} = 4.259$, $R^2 = 0.250$, $P = 0.002$ and $F_{(1,13)} = 2.650$, $R^2 = 0.180$, $P = 0.004$, respectively).

Using ADONIS, we found no significant effect on GTM composition of source population used for establishment of individual strains (weighted UNIFRAC: $F_{(3,12)} = 1.259$, $R^2 = 0.230$, $P = 0.144$ and unweighted UNIFRAC: $F_{(3,12)} = 1.402$, $R^2 = 0.250$, $P = 0.234$) or sex (weighted UNIFRAC: $F_{(1,14)} = 0.692$, $R^2 = 0.050$, $P = 0.882$ and unweighted UNIFRAC: $F_{(1,14)} = 0.842$, $R^2 = 0.060$, $P = 0.472$).

BETADISPER revealed no difference in interindividual variation in GTM composition between inbred and wild individuals (weighted UNIFRAC: $F_{(1,27)} = 0.300$, $P = 0.598$, unweighted UNIFRAC: $F_{(1,27),}$ $R^2 = 0.568$, $P = 0.457$) or between Mmm and Mmd (weighted UNIFRAC: $F_{(1,27)}$, $R^2 = 0.128$, $P = 0.719$, unweighted UNIFRAC: $F_{(1,27),}$ $R^2 = 0.335$, $P = 0.568$).

The correlation between geographic distance between wild specimens and dissimilarity indices for their GTM was not significant (Mantel test; weighted UNIFRAC: $r = -0.0134$, $P = 0.506$ and unweighted UNIFRAC: $r = -0.0684$, $P = 0.759$).

Variation in the abundance of taxonomic signatures

Significant differences were detected in the proportion of reads assigned to the *Bacteroidetes* and *Firmicutes* phyla between wild and inbred individuals using the multiple Mann–Whitney *U*-test. The proportions of other phyla did not differ significantly between wild and inbred individuals. In addition, we found no significant variation in taxonomic composition between Mmm and Mmd at the phylum level (Fig. 2).

Permutation-based ANOVAs did not support variation in the relative abundance of individual OTUs between Mmm and Mmd, with OTUs represented differently in the two subspecies. Conversely, we observed pronounced differences between wild and inbred individuals, with 16% of OTUs varying significantly. Specifically, OTUs corresponding to the *Lachnospiraceae, Lactobaciallaceae, Ruminococaceae* and *Porphyromonadaceae* families were more abundant in wild individuals and

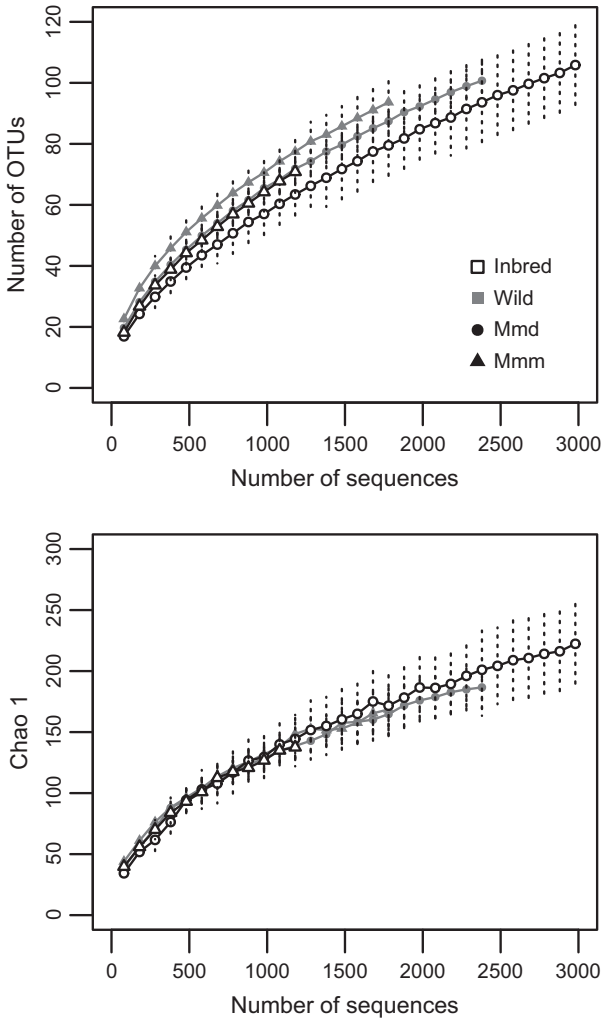

Fig. 3 Mean number of OTUs and Chao1 index (± SE) per number of reads detected in *Mus musculus musculus* (circles) and *Mus musculus domesticus* (triangles), and wild (full symbols) and inbred (open symbols) individuals. Rarefaction curves presented only for the range that does not exceed minimum number of high-quality sequences per individual detected within a given category.

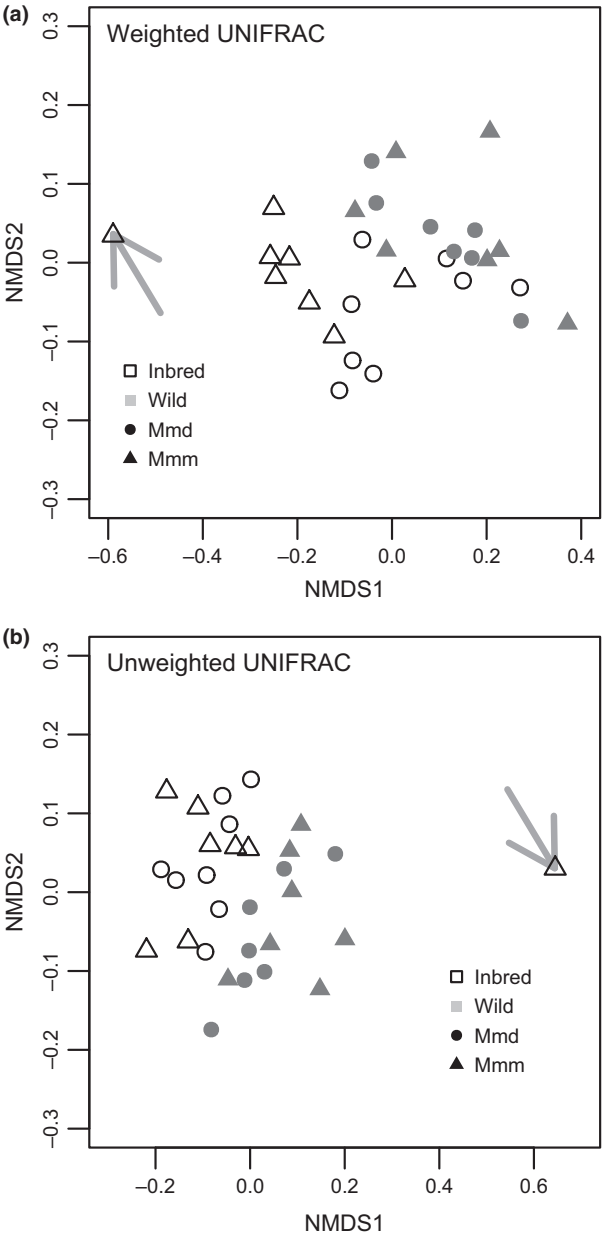

Fig. 4 Nonmetric multidimensional scaling plots for (a) weighted and (b) unweighted UNIFRAC distances for wild (full symbols) and inbred (open symbols), and *Mus musculus musculus* (circles) and *Mus musculus domesticus* (triangles) individuals. Outlying sample is indicated by arrow.

Rikenellaceae and *Bacteroidaceae* in inbred individuals. Finally, there was low support for an interactive effect between sample origin and subspecies identity, with a significant interactive effect being detected in a single OTU (Table S2, Supporting information).

Permutation ANOVAS were performed separately for individual subspecies. Following q-value adjustment, we detected four and one OTUs differing significantly between wild vs. inbred individuals in Mmm and Mmd samples, respectively. The standardized effect sizes for individual OTUs were positively correlated between Mmd and Mmm ($F_{(2,77)} = 27.180$, $R^2 = 0.250$, $P < 0.001$).

Metagenomic predictions

We were unable to map 9104 sequences (8.5% of high-quality reads) to GreenGenes reference database, when constructing input OTU table for PICRUST. The proportion of unmappable sequences did not differ dramatically between wild ($n = 3499$; 7.4%) and inbred samples ($n = 5605$; 9.3%). ADONIS analysis based on Bray–Curtis distances for individual metagenomes revealed significant

Table 2 Results for multivariate ANOVA based on dissimilarities (ADONIS). Weighted and unweighted UNIFRAC distance matrices were used as independent variables. Variables with a significant effect are in italics. The outlier from Fig. 2 was not considered in this analysis; its exclusion, however, did not change the results (for more details, see Appendix S2, Supporting information)

Distance measure	Explanatory variable	d.f.	MSS	F	R^2	P
Weighted UNIFRAC	*Origin*	*1*	*0.162*	*7.923*	*0.211*	*0.003*
	Subspecies	1	0.032	1.573	0.042	0.180
	Subspecies × Origin	*1*	*0.063*	*3.090*	*0.082*	*0.043*
	Residuals	25	0.020		0.665	
Unweighted UNIFRAC	*Origin*	*1*	*0.017*	*6.781*	*0.199*	*<0.001*
	Subspecies	1	0.003	1.162	0.034	0.277
	Subspecies × Origin	1	0.003	1.074	0.032	0.334
	Residuals	25	0.002		0.735	

differences in the functional potential of the wild vs. inbred group ($F_{(1,26)} = 7.349$, $R^2 = 0.200$, $P < 0.005$). Differences between Mmd and Mmm, as well as the interactive effect of subspecies identity vs. sample origin, were not significant ($F_{(1,26)} = 1.842$, $R^2 = 0.050$, $P = 0.153$ and $F_{(1,26)} = 2.061$, $R^2 = 0.060$, $P = 0.130$, respectively). We repeated this analysis using z-score transformed relative abundances of individual KEGG categories to compute a Euclidean distance matrix as the ADONIS input. This normalization caused all KEGG categories to have the same weight during analysis, irrespective of their relative abundances in predicted metagenomes. Despite this, the ADONIS results remained unchanged, revealing highly significant differences between wild and inbred samples ($F_{(1,26)} = 6.116$, $R^2 = 0.170$, $P < 0.001$) and no effect of subspecies identity ($F_{(1,26)} = 1.946$, $R^2 = 0.050$, $P = 0.077$) or interaction of these two variables ($F_{(1,26)} = 1.620$, $R^2 = 0.040$, $P = 0.130$). Subsequently, PICRUST analysis, followed by permutation-based tests, predicted >35% KEGG categories to be significantly over- or under-represented in wild compared to inbred individuals, whereas the abundances of only ~2% KEGG categories varied between Mmm and Mmd and ~6.5% KEGG categories changed interactively according to subspecies vs. origin identity (Table S3, Supporting information).

Discussion

Taxonomic assignment of 16S rRNA sequences indicated that the house mouse GTM consists of six bacterial phyla and is dominated by *Bacteroidetes* and *Firmicutes*. This is in agreement with previous studies on GTM composition in wild mice of Mmd origin (Linnenbrink *et al.* 2013), laboratory mouse strains (Staubach *et al.* 2012; Ubeda *et al.* 2012) and most mammalian taxa studied so far (Ley *et al.* 2008; Muegge *et al.* 2011). GTM diversity ranged between 131 and 633 OTUs per individual, as predicted using the Chao1 index.

Our GTM data set benefits from the fact that samples from wild Mmm and Mmd populations were obtained at a small spatial scale, resulting in a lack of correlation between geographical distance and GTM similarity among individuals (as assessed using Mantel's test). Hence, the comparison of GTM structure between the two subspecies is unlikely to be affected by confounding factors, such as clinal variation in environmental conditions (e.g. Banks *et al.* 2009; Linnenbrink *et al.* 2013). Consequently, our data allow direct testing of the hypothesis that genetic differences alone underline potential GTM diversification between Mmm and Mmd.

We found that both Mmm and Mmd subspecies harbour the same level of GTM alpha diversity. In addition, interindividual variation (i.e. beta diversity) in GTM composition between both subspecies was comparable. This implies a low effect of host genetic diversity on GTM structure, given the higher genetic diversity and higher expected interindividual variation in Mmm genetic background compared to Mmd (Geraldes *et al.* 2008; Phifer-Rixey *et al.* 2012).

There is growing evidence that the host genome interacts with the GTM community and shapes its taxonomic composition (Rawls *et al.* 2006). Nevertheless, we found no pronounced difference in GTM taxonomic composition between Mmd and Mmm mice. This indicates a limited effect of both separate evolutionary history and differences in life history traits and ecology between Mmm and Mmd (Macholán *et al.* 2012) on the functional divergence of genes underlining GTM content. It is worth noting that weighted UNIFRAC analysis indicated a significant interactive effect of subspecies identity and captive vs. wild origin on GTM composition. Separate analyses indicated a higher difference in wild vs. inbred microbiota for Mmm than for Mmd suggesting a pronounced response to captive breeding in Mmm. Nevertheless, the relative abundance of individual OTUs varied between wild and captive individuals in a similar way in both house mouse subspecies,

suggesting that the GTM of Mmm and Mmd tend to exhibit a congruent response to captive conditions.

Previous experimental studies have shown that differences in bacteria inhabiting *Drosophila* result in a strong assortative mate choice, which may contribute to the establishment of precopulatory reproductive barriers (Sharon *et al.* 2010). Our data, however, suggest that assortative mating based on GTM composition is unlikely to contribute to the reproductive isolation of Mmm and Mmd as GTM composition is almost identical in both Mmm and Mmd mice, at least in wild populations.

It is to be expected that both alpha (*sensu* OTU richness) and beta (*sensu* interindividual variation) GTM diversity will be higher in wild populations compared to wild-derived inbred strains. First, inbred mice in a breeding facility are kept solely on a standardized diet, while the diet of wild animals is likely to exhibit much higher temporal and spatial variation. According to niche differentiation and resource partitioning theory (Gause 1932; MacArthur 1958), which assumes a link between environmental heterogeneity (variability of diet in this particular context) and community richness (GTM composition in our case), diet uniformity should lead to low GTM diversity in inbred strains. Secondly, a decrease in genetic diversity in host populations due to a founder effect and inbreeding may be manifested in decreased GTM beta diversity in inbred strains if the host genotype has a direct effect on GTM composition. Thirdly, decreased GTM beta diversity in inbred strains is expected due to the sampling of GTM from fewer source localities for inbred strains compared to wild populations. Finally, limited social contact between captive individuals compared to free interaction in wild individuals is likely to result in decreased GTM beta diversity in inbred strains due to limited horizontal transfer of bacteria (Koch & Schmid-Hempel 2011). Our results, however, oppose these expectations as alpha and beta GTM diversities in both inbred strains and wild population were the same. Interestingly, Nelson *et al.* (2013) recently found GTM alpha diversity of southern elephant seals (*Mirounga leonina*) to be even higher in captive breeds compared to wild populations. What is more, the number of sequences that were successfully mapped against the database of annotated reference 16S rRNA sequences was high and comparable for both wild and inbred individuals (92.6% and 90.7% in the case of wild and inbred samples), suggesting that the proportion of unknown or poorly described microbial diversity is unexpectedly low in wild house mouse populations.

Despite comparable levels of GTM diversity between wild and wild-derived inbred populations, GTM taxonomic composition varied markedly between these two categories. To our knowledge, there have been only few studies focusing on differences in GTM composition in wild populations vs. captive individuals (Sudakaran *et al.* 2012; Nelson *et al.* 2013; Kohl & Dearing 2014). Most of them observed pronounced differentiation between the two categories. Similarly, a previous study on laboratory mouse GTM (Friswell *et al.* 2010) showed that individuals tended to be clustered together based on origin (breeding facility) rather than on genetics (mouse strain).

We found that, at higher taxonomic levels, the GTM of inbred individuals was dominated by members of the *Bacteroidetes* phylum, whereas *Firmicutes* dominated in the GTM of wild populations. It has been documented that both obesity and efficiency of nutrient harvest are associated with an increasing ratio of *Firmicutes* vs. *Bacteroidetes* in both mice and humans (Ley *et al.* 2005, 2006; Turnbaugh *et al.* 2006, 2009; Jumpertz *et al.* 2011).

Marked difference in the metabolism of wild and inbred individuals is suggested by the fact that KEGG pathways corresponding to lipid and protein metabolism were predicted to be overrepresented in wild vs. inbred individuals. Note, however, that interpretation of differences in metabolic potential between wild and inbred house mice cannot rely solely on the association between obesity and GTM in the laboratory mouse model. For example, we have shown, consistently with Turnbaugh *et al.* 2006, that several KEGG pathways associated with the obese genotype, such as 'glycerolipid metabolism' or 'ABC transporters', are more abundant in wild populations. On the other hand, other obesity associated pathways, such as 'the Cytrat cycle', 'glycosphingolipid' and 'glycosaminoglycan metabolism' are more abundant in inbred individuals. A number of other pathways known to vary between obese and lean mice, such as 'galactose metabolism', 'pyruvate metabolism', 'starch' and 'sucrose metabolism', did not exhibit significant levels of variation between wild and inbred populations in our study.

A more detailed insight into GTM composition at lower taxonomic scales suggests that relative abundance of ~16% of OTUs differs between wild and inbred mice. These differences are likely to mirror different environmental conditions, such as diet, species pool of environmental bacteria and/or housing. Several *Ruminococcus* OTUs, for example, enable cellulose digestion in some mammalian species (Michalet-Doreau *et al.* 2002), and these were more abundant in wild populations, suggesting differences in diet composition. Similarly, three *Lactobacilaceae* OTUs were more abundant in wild population. Many members of this taxon are important for normal functioning of the digestive tract and immune system as they exhibit anti-inflammatory properties (Mohamadzadeh *et al.* 2011) and may reduce the severity of bacterial infection in the digestive tract (Naaber

et al. 2004). Depletion of these bacteria in the GTM is associated with 'western lifestyle type diseases' such chronic inflammatory disorders (Michail 2009; Harb *et al.* 2013) and diabetes (Honda *et al.* 2012). Interestingly, in line with these findings, diabetes-associated KEGG pathways were predicted to be overrepresented in inbred samples within our study.

Currently, laboratory mouse strains of different origin (both with respect to genetic background and source breeding facility) are used in experimentally focused biomedical research. Both Ivanov *et al.* (2009) and Friswell *et al.* (2010) have previously reported that, despite being isogenic, mouse strains of different provenance possess different GTM (see also Rogers *et al.* 2014), which could contribute to conflicting results in some manipulative experiments (reviewed in Hooper *et al.* 2012). It follows, therefore, that there is a need for the establishment of isogenic mouse strains with standardized GTM (Orcutt *et al.* 1987; Hooper *et al.* 2012).

Our results indicate a pronounced difference in GTM composition and its metabolic potential between wild-derived inbred strains and wild house mouse populations. Given the profound effect of GTM composition on host phenotype (Bäckhed *et al.* 2005; Gaskins *et al.* 2008; Sekirov *et al.* 2010; Jumpertz *et al.* 2011; Koch & Schmid-Hempel 2011; Cryan & Dinan 2012; Hooper *et al.* 2012), it is probable that at least some variation between wild and inbred individuals is influenced by this factor. We suggest, therefore, that differences in wild and inbred GTM should be taken into account when undertaking biomedical research studies that use house mice as an animal model for studying the association between GTM and various metabolic, neural and behavioural disorders. In particular, further research should be undertaken to validate the usage of individuals with captive bred GTM as appropriate control groups for this type of experiment. Alternatively, based on our results, experimental groups that mirror the composition and interindividual variability found in wild GTM may further improve the robustness of such studies.

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References

Albrechtova J, Albrecht T, Baird SJE *et al.* (2012) Sperm-related phenotypes implicated in both maintenance and breakdown of a natural species barrier in the house mouse. *Proceedings of the Royal Society B-Biological Sciences*, **279**, 4803–4810.

Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI (2005) Host-bacterial mutualism in the human intestine. *Science*, **307**, 1915–1920.

Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 979–984.

Baird SJE, Macholán M (2012) What can the *musculus/domesticus* hybrid zone tell us about speciation? In: *Evolution of the House Mouse* (eds Macholán M, Baird SJE, Munclinger P, Piálek J), pp. 334–372. Cambridge University Press, Cambridge.

Banks JC, Cary SC, Hogg ID (2009) The phylogeography of *Adelie penguin* faecal flora. *Environmental Microbiology*, **11**, 577–588.

Bates D, Maechler M, Bolker B (2012) lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-0. http://CRAN.R-project.org/package=lme4 .

Benson AK, Kelly SA, Legge R *et al.* (2010) Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 18933–18938.

Caporaso JG, Kuczynski J, Stombaugh J *et al.* (2010a) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, **7**, 335–336.

Caporaso JG, Bittinger K, Bushman FD *et al.* (2010b) PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics*, **26**, 266–267.

Chao A, Shen TJ (2003) Nonparametric estimation of Shannon's index of diversity when there are unseen species in sample. *Environmental and Ecological Statistics*, **10**, 429–443.

Chervonsky AV (2012) Intestinal commensals: influence on immune system and tolerance to pathogens. *Current Opinion in Immunology*, **24**, 255–260.

Costello EK, Lauber CL, Hamady M *et al.* (2009) Bacterial community variation in human body habitats across space and time. *Science*, **326**, 1694–1697.

Crawley MJ (2007) *The R Book*. John Wiley & Sons, Chichester.

Cryan JF, Dinan TG (2012) Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, **13**, 701–712.

De Filippo C, Cavalieri D, Di Paola M *et al.* (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 14691–14696.

DeSantis TZ, Hugenholtz P, Larsen N *et al.* (2006) Greengenes, a Chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, **72**, 5069–5072.

Dufková P, Macholán M, Piálek J (2011) Inference of selection and stochastic effects in the house mouse hybrid zone. *Evolution*, **65**, 993–1010.

Dureje L, Macholán M, Baird SJE, Piálek J (2012) The mouse hybrid zone in Central Europe: from morphology to molecules. *Folia Zoologica*, **61**, 308–318.

Duvaux L, Belkhir K, Boulesteix M, Boursot P (2011) Isolation and gene flow: inferring the speciation history of European house mice. *Molecular Ecology*, **20**, 5248–5264.

Dzur-Gejdosova M, Simecek P, Gregorova S, Bhattacharyya T, Forejt J (2012) Dissecting the genetic architecture of F1 hybrid sterility in house mice. *Evolution*, **66**, 3321–3335.

Faith JJ, McNulty NP, Rey FE, Gordon JI (2011) Predicting a human gut microbiota's response to diet in gnotobiotic mice. *Science*, **333**, 101–104.

Flachs P, Mihola O, Simecek P *et al.* (2012) Interallelic and intergenic incompatibilities of the *Prdm9* (*Hst1*) gene in mouse hybrid sterility. *PloS Genetics*, **8**, e1003044.

Forejt J, Pialek J, Trachtulec Z (2012) Hybrid male sterility genes in the mouse subspecific crosses. In: *Evolution of the House Mouse* (eds Macholan M, Baird SJE, Muclinger P, Pialek J), pp. 482–503. Cambridge University Press, Cambridge.

Foster JA, Neufeld KA (2013) Gut–brain axis: how the microbiome influences anxiety and depression. *Trends in Neurosciences*, **36**, 305–312.

Fox JG, Barthold SW, Davisson MT, Newcomber CE, Quimby FW, Smith AL (2007) *The Mouse in Biomedical Research*, Vol. **I–IV**. Academic Press, New York.

Friswell MK, Gika H, Stratford IJ *et al.* (2010) Site and strain-specific variation in gut microbiota profiles and metabolism in experimental mice. *PLoS ONE*, **5**, e8584.

Gaskins HR, Croix JA, Nakamura N, Nava GM (2008) Impact of the intestinal microbiota on the development of mucosal defense. *Clinical Infectious Diseases*, **46**, S80–S86.

Gause GF (1932) Experimental studies on the struggle for existence I. Mixed population of two species of yeast. *Journal of Experimental Biology*, **9**, 389–402.

Geraldes A, Basset P, Gibson B *et al.* (2008) Inferring the history of speciation in house mice from autosomal, X-linked, Y-linked and mitochondrial genes. *Molecular Ecology*, **17**, 5349–5363.

Gordon H, Bruckner-Kardoss E (1961) Effect of normal microbial flora on intestinal surface area. *American Journal of Physiology*, **201**, 175–178.

Harb H, van Tol EAF, Heine H *et al.* (2013) Neonatal supplementation of processed supernatant from *Lactobacillus rhamnosus* GG improves allergic airway inflammation in mice later in life. *Clinical and Experimental Allergy*, **43**, 353–364.

Heijtz DR, Wang S, Anuar F *et al.* (2011) Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 3047–3052.

Honda K, Moto M, Uchida N, He F, Hashizume N (2012) Anti-diabetic effects of lactic acid bacteria in normal and type 2 diabetic mice. *Journal of Clinical Biochemistry and Nutrition*, **51**, 96–101.

Hooper LV, Wong MH, Thelin A *et al.* (2001) Molecular analysis of commensal host-microbial relations hips in the intestine. *Science*, **291**, 881–884.

Hooper LV, Littman DR, Macpherson AJ (2012) Interactions between the microbiota and the immune system. *Science*, **336**, 1268–1273.

Hwang K, Oh J, Kim T-K *et al.* (2013) CLUSTOM: a novel method for clustering 16S rRNA next generation sequences by overlap minimization. *PLoS ONE*, **8**, e62623.

Ivanov II, Atarashi K, Manel N *et al.* (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*, **139**, 485–498.

Jalanka-Tuovinen J, Salonen A, Nikkila J *et al.* (2011) Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. *PLoS ONE*, **6**, e23035.

Janousek V, Wang L, Luzynski K *et al.* (2012) Genome-wide architecture of reproductive isolation in a naturally occurring hybrid zone between *Mus musculus musculus* and *M. m. domesticus*. *Molecular Ecology*, **21**, 3032–3047.

Jumpertz R, Le DS, Turnbaugh PJ *et al.* (2011) Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *American Journal of Clinical Nutrition*, **94**, 58–65.

Kanehisa M, Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, **28**, 27–30.

Koch H, Schmid-Hempel P (2011) Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 19288–19292.

Kohl KD, Dearing MD (2014) Wild-caught rodents retain a majority of their natural gut microbiota upon entrance into captivity. *Environmental Microbiology Reports*, **6**, 191–195.

Krych L, Hansen CHF, Hansen AK, van den Berg FWJ, Nielsen DS (2013) Quantitatively different, yet qualitatively alike: a meta-analysis of the mouse core gut microbiome with a view towards the human gut microbiome. *PLoS ONE*, **8**, e62578.

Langille MGI, Zaneveld J, Caporaso JG *et al.* (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, **31**, 814–821.

Lepš J, Šmilauer P (2003) *Multivariate Analysis of Ecological Data Using CANOCO*. Cambridge University Press, Cambridge.

Ley RE, Bäckhed F, Turnbaugh P *et al.* (2005) Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 11070–11075.

Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology - Human gut microbes associated with obesity. *Nature*, **444**, 1022–1023.

Ley RE, Hamady M, Lozupone C *et al.* (2008) Evolution of mammals and their gut microbes. *Science*, **320**, 1647–1651.

Linnenbrink M, Wang J, Hardouin EA *et al.* (2013) The role of biogeography in shaping diversity of the intestinal microbiota in house mice. *Molecular Ecology*, **22**, 1904–1916.

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, **71**, 8228–8235.

Lucas FS, Heeb P (2005) Environmental factors shape cloacal bacterial assemblages in great tit *Parus major* and blue tit *P. caeruleus* nestlings. *Journal of Avian Biology*, **36**, 510–516.

MacArthur RH (1958) Population ecology of some warblers of Northeastern coniferous forests. *Ecology*, **39**, 599–619.

Macholán M, Baird SJE, Dufková P *et al.* (2011) Assessing multilocus introgression patterns: a case study on the mouse X chromosome in Central Europe. *Evolution*, **65**, 1428–1446.

Macholán M, Baird SJE, Munclinger P, Piálek J (eds.) (2012) *Evolution of the House Mouse*. Cambridge University Press, Cambridge.

Macpherson AJ, Harris NL (2004) Interactions between commensal intestinal bacteria and the immune system. *Nature Reviews Immunology*, **4**, 478–485.

Mathis D, Benoist C (2011) Microbiota and autoimmune disease: the hosted self. *Cell Host & Microbe*, **10**, 297–301.

Michail S (2009) The role of probiotics in allergic diseases. *Allergy, Asthma, and Clinical Immunology*, **5**, 5.

Michalet-Doreau B, Fernandez I, Fonty G (2002) A comparison of enzymatic and molecular approaches to characterize the cellulolytic microbial ecosystems of the rumen and the cecum. *Journal of Animal Science*, **80**, 790–796.

Mohamadzadeh M, Pfeiler EA, Brown JB *et al.* (2011) Regulation of induced colonic inflammation by *Lactobacillus acidophilus* deficient in lipoteichoic acid. *Proceedings of the National Academy of Sciences of the United States of America*, **108**(Suppl 1), 4623–4630.

Muegge BD, Kuczynski J, Knights D *et al.* (2011) Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science*, **332**, 970–974.

Naaber P, Smidt I, Štšepetova J *et al.* (2004) Inhibition of *Clostridium difficile* strains by intestinal *Lactobacillus* species. *Journal of Medical Microbiology*, **53**, 551–554.

Nam Y-D, Jung M-J, Roh SW, Kim M-S, Bae J-W (2011) Comparative analysis of Korean human gut microbiota by barcoded pyrosequencing. *PLoS ONE*, **6**, e22109.

Nelson TM, Rogers TL, Carlini AR, Brown MV (2013) Diet and phylogeny shape the gut microbiota of Antarctic seals: a comparison of wild and captive animals. *Environmental Microbiology*, **15**, 1132–1145.

Ochman H, Worobey M, Kuo C-H *et al.* (2010) Evolutionary relationships of wild hominids recapitulated by gut microbial communities. *PLoS Biology*, **8**, e1000546.

O'Hara AM, Shanahan F (2006) The gut flora as a forgotten organ. *Embo Reports*, **7**, 688–693.

Oksanen J, Blanchet FG, Kindt R *et al.* (2013) vegan: Community Ecology Package. R package version 2.0-6. http://CRAN.R-project.org/package=vegan

Orcutt RP, Gianni FJ, Judge RJ (1987) Development of an "Altered Schaedler Flora" for NCI gnotobiotic rodents. *Microecology Therapy*, **17**, 59.

Phifer-Rixey M, Bonhomme F, Boursot P *et al.* (2012) Adaptive evolution and effective population size in wild house mice. *Molecular Biology and Evolution*, **29**, 2949–2955.

Piálek J, Vyskočilová M, Bímové B *et al.* (2008) Development of unique house mouse resources suitable for evolutionary studies of speciation. *Journal of Heredity*, **99**, 34–44.

Price MN, Dehal PS, Arkin AP (2009) FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution*, **26**, 1641–1650.

Qin J, Li R, Raes J *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, **464**, 59–65.

Quince C, Lanzen A, Davenport RJ, Turnbaugh PJ (2011) Removing noise from pyrosequenced amplicons. *BMC Bioinformatics*, **12**, 38.

R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rawls JF, Mahowald MA, Ley RE, Gordon JI (2006) Reciprocal gut microbiota transplants from zebrafish and mice to germfree recipients reveal host habitat selection. *Cell*, **127**, 423–433.

Rogers GB, Kozlowska J, Keeble J *et al.* (2014) Functional divergence in gastrointestinal microbiota in physically-separated genetically identical mice. *Scientific Reports*, **4**, 5437.

Sak B, Kváč M, Květonová D, Albrecht T, Piálek J (2011) The first report on natural *Enterocytozoon bieneusi* and *Encephalitozoon* spp. infections in wild East-European House Mice (*Mus musculus musculus*) and West-European House Mice (*M. m. domesticus*) in a hybrid zone across the Czech Republic-Germany border. *Veterinary Parasitology*, **178**, 246–250.

Sanchez E, De Palma G, Capilla A *et al.* (2011) Influence of environmental and genetic factors linked to Celiac disease risk on infant gut colonization by *Bacteroides* species. *Applied and Environmental Microbiology*, **77**, 5316–5323.

Schütt S, Florl AR, Shi W *et al.* (2003) DNA methylation in placentas of interspecies mouse hybrids. *Genetics*, **165**, 223–228.

Sekirov I, Russell SL, Antunes LCM, Finlay BB (2010) Gut microbiota in health and disease. *Physiological Reviews*, **90**, 859–904.

Sharon G, Segal D, Ringo JM *et al.* (2010) Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 20051–20056.

Sonnenburg JL, Xu J, Leip DD *et al.* (2005) Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science*, **307**, 1955–1959.

Staubach F, Kuenzel S, Baines AC *et al.* (2012) Expression of the blood-group-related glycosyltransferase B4galnt2 influences the intestinal microbiota in mice. *Isme Journal*, **6**, 1345–1355.

Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 9440–9445.

Sudakaran S, Salem H, Kost C, Kaltenpoth M (2012) Geographical and ecological stability of the symbiotic mid-gut microbiota in European firebugs, *Pyrrhocoris apterus* (*Hemiptera, Pyrrhocoridae*). *Molecular Ecology*, **21**, 6134–6151.

Turnbaugh PJ, Gordon JI (2009) The core gut microbiome, energy balance and obesity. *Journal of Physiology-London*, **587**, 4153–4158.

Turnbaugh PJ, Ley RE, Mahowald MA *et al.* (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, **444**, 1027–1031.

Turnbaugh PJ, Hamady M, Yatsunenko T *et al.* (2009) A core gut microbiome in obese and lean twins. *Nature*, **457**, 480–484.

Turner LM, Schwahn DJ, Harr B (2012) Reduced male fertility is common but highly variable in form and severity in a natural house mouse hybrid zone. *Evolution*, **66**, 443–458.

Ubeda C, Lipuma L, Gobourne A *et al.* (2012) Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. *Journal of Experimental Medicine*, **209**, 1445–1456.

Vošlajerová-Bímová B, Macholán M, Baird SJE *et al.* (2011) Reinforcement selection acting on the European house mouse hybrid zone. *Molecular Ecology*, **20**, 2403–2424.

Wallace TC, Guarner F, Madsen K *et al.* (2011) Human gut microbiota and its relationship to health and disease. *Nutrition Reviews*, **69**, 392–403.

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, **73**, 5261–5267.

Wang J, Linnenbrink M, Künzel S *et al.* (2014) Dietary history contributes to enterotype-like clustering and functional metagenomic content in the intestinal microbiome of wild mice. *Proceedings of the National Academy of Sciences*, **111**, E2703–E2710.

Wostmann B, Bruckner-Kardoss E (1959) Development of cecal distention in germ-free baby rats. *American Journal of Physiology*, **197**, 1345–1346.

Yang H, Wang JR, Didion JP *et al.* (2011) Subspecific origin and haplotype diversity in the laboratory mouse. *Nature Genetics*, **43**, 648–655.

Yu Y, Lee C, Kim J, Hwang S (2005) Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnology and Bioengineering*, **89**, 670–679.

J.P. provided samples; J.P. and J.V. contributed reagents; D.C. and J.K. conceived and designed the experiments; D.C., J.V. and J.K. performed the experiments; J.K. analysed the data; and J.K., D.C. and J.P. wrote the manuscript.

Data accessibility

Demultiplexed unfiltered ssf files: http://www.ebi.ac.uk/ena/data/view/PRJEB6634 Clusters of quality filtered sequences and OTU table: Dryad doi:10.5061/dryad.018 g4.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Detailed listing of house mouse samples used

Table S2 Taxonomic classification of dominant OTUs and their variation between sample groups

Table S3 Variation of predicted KEGG pathways between sample groups

Appendix S1 Taxonomic composition of house mouse samples

Appendix S2 Extended statistical analysis