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Disentangling the effect of host genetics and gut microbiota on resistance to an intestinal parasite

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ABSTRACT

Resistance to infection is a multifactorial trait, and recent work has suggested that the gut microbiota can also contribute to resistance. Here, we performed a fecal microbiota transplant to disentangle the contribution of the gut microbiota and host genetics as drivers of resistance to the intestinal nematode *Heligmosomoides polygyrus*. We transplanted the microbiota of a strain of mice (SJL), resistant to *H. polygyrus*, into a susceptible strain (CBA) and vice-versa. We predicted that if the microbiota shapes resistance to *H. polygyrus*, the FMT should reverse the pattern of resistance between the two host strains. The two host strains had different microbiota diversities and compositions before the start of the experiment, and the FMT altered the microbiota of recipient mice. One mouse strain (SJL) was more resistant to colonization by the heterologous microbiota, and it maintained its resistance profile to *H. polygyrus* (lower parasite burden) independently of the FMT. On the contrary, CBA mice harbored parasites with lower fecundity during the early stage of the infection, and had an up-regulated expression of the cytokine IL-4 (a marker of *H. polygyrus* resistance) after receiving the heterologous microbiota. Therefore, while host genetics remains the main factor shaping the pattern of resistance to *H. polygyrus*, the composition of the gut microbiota also seems to play a strain-specific role.

1. Introduction

The outcome of a host-parasite interaction, whether the host clears the infection, succumbs to it, or the pathogen persists in a chronic stage, depends on host genetics, parasite genetics and the environment where the interaction takes place. During recent years, the gut microbiota has emerged as another factor likely to determine the outcome of host-parasite interactions (Abt and Pamer, 2014). In mammals, the gut microbiota is environmentally acquired but it is also vertically transmitted from mother to offspring (Funkhouser and Bordenstein, 2013), and the host genetic background (including polymorphic immune genes) has been shown to shape its composition (Bolnick et al., 2014; Davenport, 2016). The gut microbiota, therefore, lies between being an intrinsic host characteristic and an environmental trait.

A healthy gut microbiota can confer protection against infection at different scales, through local and systemic effects. Within the gut, the microbiota plays a role in pathogen resistance through a phenomenon called colonization resistance, which involves different mechanisms of competitive exclusion between commensal and pathogenic microorganisms (Lawley and Walker, 2013). Outside the intestine, the gut microbiota can shape the dynamics of infections occurring at distant organs, through pervasive effects on the immune response (Schroeder and Backhed, 2016).

Gastrointestinal helminths are involved in particularly tight interactions with the gut microbiota (Glendinning et al., 2014; Reynolds et al., 2015; Gause and Maizels, 2016; Zaiss and Harris, 2016; Midha et al., 2017). Early work already stressed the importance of the gut microbiota for the outcome of infection with gastrointestinal helminths. In germfree, outbred mice, the intestinal nematode *Heligomosmoides polygyrus* produces infections that are cleared faster compared with conventional hosts, and have smaller adult population sizes (Wescott and Todd, 1964). More recently, it has been shown that infection with the nematode *Trichuris muris*

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requires a microbiota because, in the absence of gut bacteria, parasite eggs cannot hatch (Hayes et al., 2010). Accumulating evidence shows that while the composition and diversity of the microbiota affect helminth life history traits (Reynolds et al., 2014), helminths also alter the composition of the microbiota (Walk et al., 2010; Lee et al., 2014; Holm et al. 2015,; Houlden et al., 2015; Cattadori et al., 2016). However, the composition and the diversity of the microbiota also depend on several host and environmental factors, and disentangling the relative contribution of host factors and the microbiota in helminth life history traits requires an experimental approach. The gut microbiota is an amenable model system for such an approach because it can be successfully transplanted from one individual to another (Borody and Khoruts, 2012). Several fecal microbiota transplant (FMT) experiments have been conducted during recent years (Willing et al., 2011) and FMT is nowadays one of the most effective treatments against recurrent *Clostridium difficile* infection in humans (Gough et al., 2011).

The aim of the present study was to disentangle the relative contribution of host genetics and microbiota composition in resistance to infection with the nematode *Heligmosomoides polygyrus*, a natural parasite of house mice (*Mus musculus*). For this purpose, we used two inbred mouse strains (CBA and SJL) previously known to differ in their resistance to *H. polygyrus*, mediated by the Th2 immune response (Filbey et al., 2014; Lippens et al., 2016), and we reciprocally transplanted the gut microbiota between the two strains. In addition to heterologous (between strains), we also performed homologous (within strain) transplants, as controls. We predicted that if host genetics is the main determinant of resistance to *H. polygyrus*, the expression of parasite life history traits (survival and fecundity) should only depend on host strain (hosts harboring parasites with low survival and fecundity being considered as resistant). On the contrary, if the gut microbiota plays a role in resistance to *H. polygyrus*, we predicted that mice in the heterologous transplant groups should acquire the resistance profile of the donor strain.

2. Materials and methods

2.1. Experimental procedure

Eight-week old CBA ($n = 36$) and SJL ($n = 36$) female mice were purchased from Janvier Labs, (Laval, France), and housed in the mouse house of the Université de Bourgogne, France, under a standardized temperature (21 °C) and light:dark cycle (12:12 h). They were kept in plastic cages with shelters (18.5 × 38 × 22.5 cm; six individuals per cage) and given ad libitum commercial mouse pellets and filtered tap water. The study was approved by the Comité d'Ethique de l'Expérimentation Animale Grand Campus Dijon, France (CNREEA n° C2EA – 105; project N° 13731) and by the "Ministère de la Recherche et de l'Enseignement Supérieur", France, in compliance with the national guidelines ("Charte nationale portant sur l'éthique de l'expérimentation animale") on the use of animals for research purposes. The experiment started when mice were 11 week old. Within each strain, mice were randomly assigned to the experimental groups: donors ($n = 12$), homologous recipients ($n = 12$), or heterologous recipients ($n = 12$). Homologous and heterologous recipients refer to individuals that received the FMT from donors belonging to the same or the other strain, respectively. Prior to the start of the fecal transplant (day 0), recipient mice received an antibiotic treatment (100 µl per os, 250 mg of streptomycin/ml of drinking water). Three individuals out of 72 died during the experimental procedure, reducing the sample size to 69 mice.

2.2. FMT

Recipient mice received the FMT six times during the 8 days that followed the antibiotic treatment (days 1, 2, 3, 4, 7, 8 post-antibiotic treatment). On each of these days, donor mice were placed in clean (autoclaved) cages in the morning (09:00 h). Feces were immediately collected, placed in sterile tubes, homogenized in sterile PBS (1 g of feces/5 ml of PBS) and immediately centrifuged (3 min, 900*g*, 4 °C). The supernatant was collected and given to recipient mice per os (200 µl) according to the specific treatment (homologous or heterologous transplant).

2.3. Infection with Heligmosomoides polygyrus and infection dynamics

At day 8 (the last day of FMT), all mice were infected per os with 150 L3s of the nematode *H. polygyrus*, suspended in 200 µl of drinking water. At day 10 p.i., we counted the number of parasite eggs shed in the faeces in half of the mice ($n = 12$ donors, 11 homologous recipients, 10 heterologous recipients). Mice were transferred at 08:00 h into clean (autoclaved) individual cages with a humidified paper towel at the bottom to prevent desiccation of faeces. Mice were left for approximately for 3 h, faeces were collected and 300 mg of those were smashed and suspended in 2.5 ml of water. Thereafter, 5 ml of salted water (75% saturation, 0.27 g of NaCl per ml of water) were added to allow eggs to float. After agitation, a fraction of this suspension was transferred into a McMaster chamber for egg counting. Each faecal egg count was expressed as the number of eggs per mg of faeces.

After having collected their faeces, these mice were euthanized by cervical dislocation under isoflurane anaesthesia. The intestine was removed and placed in a tube with a formaldehyde solution (4%); the spleen was frozen in liquid nitrogen for further assessment of cytokine gene expression (see Section 2.6). Intestines were washed within 3 days post-collection and stored in ethanol (70%) at room temperature.

We used dry biomass to assess the burden of adult *H. polygyrus*. Intestines were longitudinally opened under a stereo microscope (×10) and all worms were removed and placed in Eppendorf tubes that had been previously weighed (±0.01 mg) using a precision balance (Precisa 262SMA-FR) and subsequently filled with absolute ethanol. The tubes were placed at 40 °C for 48 h and then weighed again using the same precision balance. The difference between the weight of empty tubes and tubes containing dehydrated worms was used as a measure of parasite dry biomass. To make sure that dry biomass reliably correlates with the number of worms, we also counted them in a subsample of hosts ($n = 27$).

The other half of the mice was monitored up to day 29 p.i. for SJL mice and up to day 73 p.i. for CBA. The monitoring of infection dynamics was stopped earlier for SJL mice because at day 29, almost half of the mice (seven out of 18) shed no parasite eggs in their faeces. For SJL mice, we counted parasite eggs at days 13, 16, 21, 24 and 29 p.i. using the same procedure described above. At day 29 p.i., all SJL mice were euthanized, the intestines collected and the dry biomasses of adult worms measured as described above. For CBA mice, we counted parasite eggs at days 13, 16, 21, 24, 29, 36, 52 and 73 p.i. using the same procedure described above. At day 73 p.i., all CBA mice were euthanized, the intestines collected and the dry biomasses of adult worms measured as described above.

2.4. Quantification of fecal bacteria abundance

To assess the effect of the antibiotic treatment on the abundance of gut bacteria, feces were collected before the antibiotic treatment and up to 4 days post-treatment, as described in Section 2.2. Two feces per mouse were placed in a microcentrifuge

tube containing 700 µl of sterile water and three glass beads (1 mm), vortexed at maximum speed for 2 min and centrifuged at 800 *g* for 3 min. The total number of aerobic colony forming units (CFUs) was determined by plating serial dilutions of the supernatant on Brain Heart Infusion (BHI) agar (Biokar Diagnostics) and incubating at 37 °C overnight.

2.5. Microbiota analysis

To assess the diversity and composition of the gut microbiota, faeces were collected at day 0 (prior to the antibiotic treatment) and at days 8, 18 and 32. Fresh faeces were collected as previously described and immediately stored at −80 °C. The composition of the gut microbiota was assessed by high-throughput sequencing of a part of the 16S rRNA bacterial gene as a molecular marker.

We extracted bacterial DNA from fecal samples using a Qiamp stool kit (Qiagen) following the protocol of the manufacturer. We amplified a 251 bp length fragment of the V4 region of the 16S rRNA bacterial gene using a slightly modified version of the dual-index method (Kozich et al., 2013), detailed in Galan et al. (2016). Briefly, the forward and reverse V4 primers included 8 bp index and Illumina adapters. The combinations of forward and reverse indexed primers allow identification of each PCR product that has been pooled and loaded onto the same MiSeq flow cell. Using different tagged primers, DNA amplification was replicated for four positive controls (*Bartonella, Borrelia*, *Mycoplasma mycoides* and *Mycoplasma putrefaciens*), for three ZymoBIOMICS Microbial community standard (Zymo Research, Irvine, CA, USA) diluted by 1, 1/10 and 1/100 and composed of mixed known quantities of DNA from eight bacteria species, for eight negative controls from the DNA extraction step and for 247 DNA fecal samples, except five whose amplifications were done in triplicate. We also performed amplification of seven PCR negative controls (i.e. DNA-free water instead of DNA). The conditions of DNA amplification, purification and pooling of PCR products are detailed in Galan et al. (2016). The final library (i.e. the pool of all PCR products) was loaded on a MiSeq (Illumina) flow cell (expected cluster density, 700,000–800,000/mm^2) and performed with a reagent kit 500v2 (Illumina) (500 cycles). The high throughput sequencing comprised a total of 536 PCR products corresponding to 247 samples (242 replicated twice and five replicated thrice), seven replicated positive controls, eight replicated extraction negative controls and seven PCR negative controls. Finally, six unused tagged primer combinations allowed estimation of the mistagging rate in the entire procedure. The assembling, denoising, demultiplexing and clustering of the MiSeq dataset obtained have been processed through Mothur v.1.34 (Schloss et al., 2009) following the standard Mothur MiSeq system operating procedure (https://www.mothur.org/wiki/MiSeq_SOP). Briefly, we assembled R1 and R2 contigs considering a phred score quality >25 and we reattributed each sequence to the corresponding sample based on the exact specific index combination. Only sequences with no ambiguous nucleotide and a maximal sequence length of 275 bp were considered. Then, we processed to sequence demultiplexing and we aligned the sequence variants to Silva short subunit Reference alignment v119 (Schloss et al., 2009). The sequences misaligned to the Silva alignment (and the sequences matching in this database as Chloroplast, Mitochondria, Archaea and unclassified) were removed. We merged sequences with two nucleotides differences, considered as artefactual errors, and we removed the remaining singletons (unique sequences at the sequencing run level that likely correspond to sequencing errors). The chimera sequences were detected and eliminated using the Uchime algorithm (Edgar et al., 2011) implemented in Mothur. A total of 6,141,537 denoised sequences was obtained. We clustered variants into 8 740 operating taxonomic units (OTUs) using the average hierarchical method with 97% similarity. For further analyses, using the phylogenetic diversity index, we generated a phylogenetic tree of the representative sequence of each OTU (i.e. the most abundant sequence) using FastTree implemented in QIIME v. 1.9.1 (Caporaso et al., 2010).

2.5.1. Validation of molecular and bioinformatics procedure

We obtained between 15,875 and 28,733 denoised sequences for each replicate of the mock community and we estimated an averaged 0.03% global discrepancy resulting from both PCR and Illumina errors. We observed that 97%–99% of the sequences of each mock community matched the eight expected OTUs.

We obtained between 165 and 10,017 denoised sequences for the eight duplicated negative extraction controls; and between 97 and 14,093 denoised sequences for the seven negative amplification controls. However, OTUs detected in the negative controls did not correspond to any systematic contamination. Finally, the detection of four to 37 sequences assigned to unused tagged primers combinations indicated a negligible mistagging rate.

To normalize data samples for sequencing depth, we performed a rarefying procedure that consists of randomly re-sampling, without replacement, sequences for each sample to 5000 sequences. The procedure was repeated 1000 times to avoid potential biases due to the randomization process. For these 1000 OTU tables produced, we estimated the Good's coverage index and the alpha diversity indices: Shannon (binary logarithm) and Phylogenetic diversity (Lozupone and Knight, 2008). Then, for each index, we considered the mean value estimated for the 1000 OTU tables. The mean Good's coverage was estimated at 0.992, indicating that our sequencing depth allowed coverage of the high diversity of the microbiota studied. We found strong correlations between technical replicates for Shannon (Pearson $r = 0.98$) and Phylogenetic diversity indices (Pearson $r = 0.92$), validating the repeatability of the PCR amplification and bioinformatics pipeline. Therefore, we merged the abundance of sequences obtained for each OTU between technical replicates for further analyses.

2.6. Cytokine expression

Spleen tissue was transferred to a 2 ml fast-prep tube containing 1.4 mm ceramic spheres (lysing matrix D, MP Biomedicals, Illkirch-Graffenstaden, France) and 1 ml of TRIzol Reagent (Invitrogen, Saint Herblain, France). Tissue was disrupted in a Precellys homogenizer (Bertin instruments, Montigny-le-Bretonneux, France) for 2 × 30 s at 6500 rpm. Nucleic acids were extracted in a 0.2 volume of chloroform (Sigma, Saint-Quentin Fallavier, France) and purified by precipitation in a 0.5 volume of isopropanol (Sigma). RNA pellets were dried and resuspended in 40 µl of RNAse-free water. Nucleic acid concentrations were determined by measuring absorbance at 260 nm using a NanoDrop spectrophotometer (ThermoScientific, Saint Herblain, France). One microgram of total RNA was treated for reverse transcription with the iScript cDNA synthesis kit (Bio-Rad, Marnes-la-Coquette, France) according to the manufacturer's instructions. Quantitative PCR was performed in 12.5 µl containing 1X iTaq Universal SYBER Green Supermix (Bio-Rad), 0.6 µM of each primer (Supplementary Table S1) and 2.5 µl of cDNA diluted five times. The thermal cycling conditions comprised 30 s at 95 °C and then 40 cycles of 95 °C for 10 s and 60 °C for 30 s, followed by a standard melting curve analysis. Gene expression was calculated based on the ΔΔCt method (Pfaffl, 2001), ΔCt values were calculated using the Ct values from the amplification of a reference gene (β-actin, housekeeping gene). Quantitative real-time PCR was performed on the CFX96 PCR system (Bio-Rad).

2.7. Statistical analyses

We estimated the microbiota diversity within mice (α diversity) using the Shannon index, and the dissimilarity of the composition of microbiota between mice (β diversity) using the following metrics: Bray–Curtis, Jaccard, unweighted and weighted Unifrac. We used OTUs as the taxonomic level to estimate both α and β diversity.

Differences in α diversity between strains prior to the start of the experiment were analyzed using a one-way ANOVA. Changes in α diversity over time for the different experimental groups were analyzed using a linear mixed effects model. Strain, time post-FMT, treatment (donor, homologous recipient, heterologous recipient) and the two- and three-way interactions were included as fixed effects and mouse identity as a random effect. Degrees of freedom were estimated using the Satterthwaite approximation.

We characterized the variation of β diversity using a Principal Coordinate Analysis for each dissimilarity index. We used PERMANOVA (function Adonis through Vegan package, 10,000 permutations) to test the effect of host strain, FMT and their interaction on the dissimilarity of the microbiota composition.

We explored whether the abundance of bacteria families differed between strains, prior to FMT. This was done using non-parametric *t* tests (10,000 permutations) implemented in QIIME v. 1.9.1, based on averaged abundances of 1000 rarefied tables. We then focused on bacteria families whose relative abundance significantly differed between host strains prior to FMT and investigated how FMT altered the relative abundance of these families in heterologous transplanted mice. To do this, we computed the changes in relative abundance with respect to the same-strain donors, and analyzed these changes over time using a linear mixed effects model. This model included mouse strain, time post-FMT, and the interaction between the two as fixed effects, and mouse identity as a random effect. Degrees of freedom were estimated using the Satterthwaite approximation.

The effect of the strain and FMT on parasite life history traits and host cytokine expression was analyzed using two-way ANOVAs. Fecal egg counts and cytokine expression values were log-transformed to normalize the distribution of model errors.

P values below 0.05 were considered significant. All statistical analyses were performed with SAS (9.4).

3. Results

3.1. Strain-specific microbiota

Prior to the start of the experiment, the α diversity of the microbiota was slightly higher in SJL than in CBA mice (Shannon index, mean ± S.E., CBA: 5.29 ± 0.37; SJL: 5.46 ± 0.30; one-way ANOVA: $F_{1,69} = 4.60$, $P = 0.0354$).

Before the FMT, CBA and SJL mice had very different compositions of their microbiota, as shown by the highly significant strain effect in the PERMANOVA model (Table 1; Fig. 1). The relative abundance of key bacteria families also differed between strains, prior to the start of the experiment. CBA mice had higher relative abundance of Lactobacillaceae ($t = 3.64$, False Discovery Rate (FDR) $P < 0.001$), Bacteroidaceae ($t = 7.63$, FDR $P < 0.001$), and Lachnospiraceae ($t = 6.51$, FDR $P < 0.001$); while SJL mice had higher relative abundance of Bacteroidales S24-7 ($t = -9.01$, FDR $P < 0.001$), Prevotellaceae ($t = -7.22$, FDR $P < 0.001$), and Clostridiales vadinBB60 ($t = -7.21$, FDR $P < 0.001$) (Supplementary Fig. S1).

The antibiotic treatment was effective in reducing bacterial abundance, which recovered following the FMT (Supplementary Fig. S2).

Therefore, the two mouse strains initially had different diversities and compositions of their gut microbiota and the antibiotic treatment successfully reduced bacteria abundance.

3.2. FMT altered strain-specific microbiota

Changes in the α diversity of the microbiota over the course of the experiment were affected by the three-way interaction between mouse strain, FMT and time post-FMT ($P < 0.0001$; Supplementary Table S2). In SJL mice, there was a strong difference in α diversity between donors and recipients at day 8 post-FMT, suggesting that recipient mice had not fully recovered from the antibiotic treatment by that time (Fig. 2A). The reduction in α diversity of CBA mice at day 8 post-FMT was not as pronounced as in the SJL strain, suggesting a faster recolonization after the antibiotic treatment (Fig. 2B).

We analyzed β diversity at days 8, 18 and 32 post-FMT. On each of these sampling dates, PERMANOVA showed significant interactions between mouse strain and FMT (Table 1). This indicates that

Table 1
PERMANOVA exploring the effect of mouse strain and fecal microbiota transplant on dissimilarity of the gut microbiota. The model included mouse strain (CBA, SJL) at day 0 (prior to the FMT); mouse strain, FMT (donors, homologous recipients, heterologous recipients), and the interaction between the two at day 8, 18 and 32 after the start of the FMT. The terms were added sequentially and the estimation of pseudo F values was based on 10,000 permutations.

		Dissimilarity metrics											
		Bray–Curtis			Jaccard			Unweighted unifrac			Weighted unifrac		
	df	*Pseudo F*	R^2	*P*	*Pseudo F*	R^2	*P*	*Pseudo F*	R^2	*P*	*Pseudo F*	R^2	*P*
Day 0													
Mouse strain	1	28.78	0.29	<0.001	13.97	0.17	<0.001	20.73	0.23	<0.001	41.8	0.38	<0.001
Day 8													
Mouse strain	1	9.46	0.086	<0.001	4.14	0.049	<0.001	5.84	0.06	<0.001	10.03	0.09	<0.001
FMT	2	8.53	0.155	<0.001	4.17	0.099	<0.001	6.15	0.13	<0.001	8.04	0.15	<0.001
Mouse strain × FMT	2	9.81	0.178	<0.001	3.75	0.089	<0.001	5.14	0.11	<0.001	8.95	0.17	<0.001
Day 18													
Mouse strain	1	21.15	0.22	<0.001	5.64	0.07	<0.001	7.75	0.10	<0.001	19.12	0.20	<0.001
FMT	2	2.81	0.06	0.0012	1.80	0.05	<0.001	2.22	0.05	<0.001	2.83	0.06	0.0022
Mouse strain × FMT	2	4.22	0.09	<0.001	2.61	0.07	<0.001	3.25	0.08	<0.001	3.50	0.07	<0.001
Day 32													
Mouse strain	1	5.37	0.10	<0.001	2.64	0.07	<0.001	3.66	0.09	<0.001	4.22	0.08	0.007
FMT	2	1.46	0.06	0.0946	1.36	0.07	0.0030	1.73	0.08	<0.001	1.29	0.05	0.2359
Mouse strain × FMT	2	6.80	0.26	<0.001	2.06	0.10	<0.001	2.67	0.13	<0.001	8.72	0.32	<0.001

df, degrees of freedom.

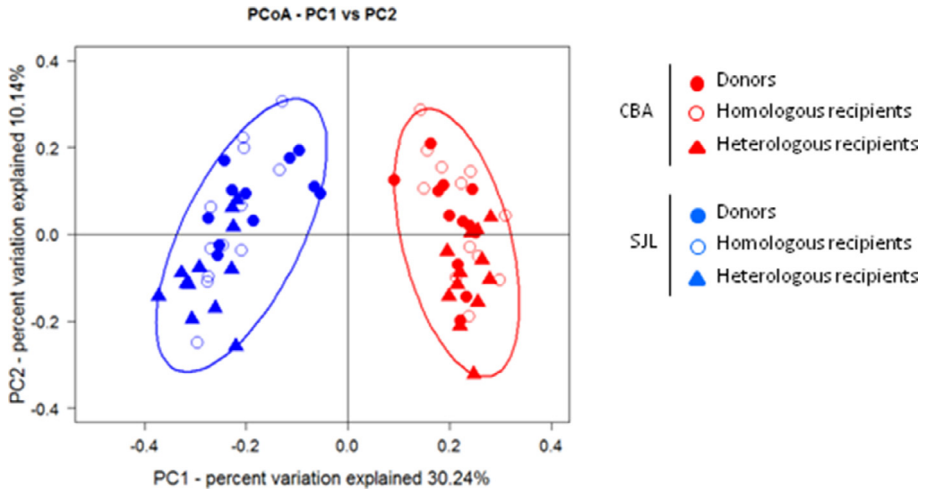

Fig. 1. Principal Coordinate Analysis of β diversity (Bray–Curtis distances) of fecal microbiota of SJL (blue symbols) and CBA (red symbols) mice prior to the fecal microbiota transplant. Filled dots, empty dots and triangles refer to mice that have been subsequently allocated to the three FMT groups: donors, homologous and heterologous recipients.

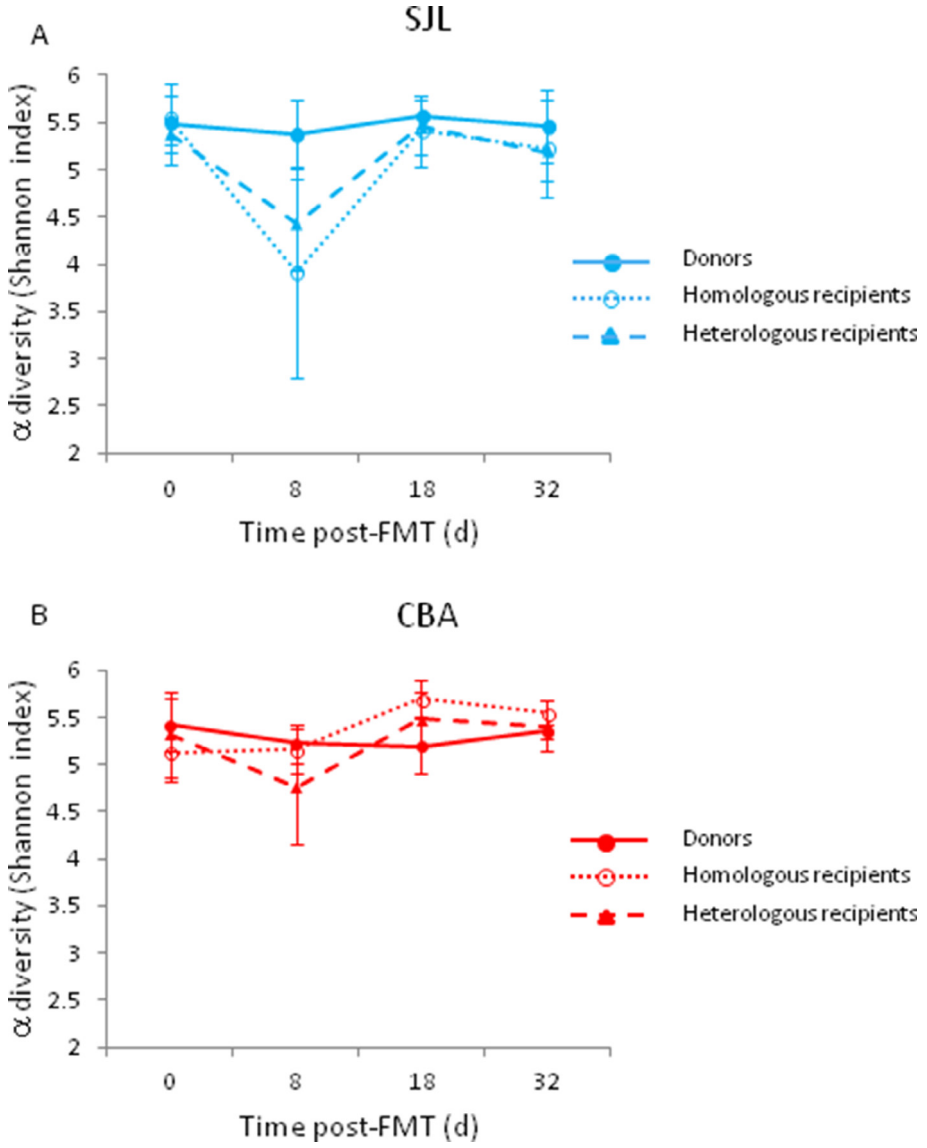

Fig. 2. Changes in α diversity (Shannon index) of the fecal microbiota over time for the three experimental groups (donors, homologous recipients, heterologous recipients) for two mouse strains. (A) SJL; (B) CBA. Means ± S.E. Time post-fecal microbiota transplant refers to the time elapsed since the start of the FMT. d, days.

the FMT altered the composition of the microbiota in a strain-specific manner, with CBA heterologous recipients having more similar microbiota composition to SJL donors than SJL heterologous recipients had to CBA donors (Fig. 3).

In addition to β diversity, we analyzed the changes in the relative abundance of bacteria families that were found to differ between mouse strains prior to the FMT. For bacteria families that were initially more abundant in the SJL strain (Bacteroidales *S24-7*, Prevotellaceae, Clostridiales vadin*BB60*), we expected that their abundance should increase in CBA heterologous recipients and decrease in SJL heterologous recipients. Similarly, for bacteria families that were initially more abundant in the CBA strain (Lactobacillaceae, Bacteroidaceae, Lachnospiraceae), we expected that their abundance should increase in SJL heterologous recipients and decrease in CBA heterologous recipients. In agreement with the predictions, we found statistically significant time × strain interactions for five out of six bacteria families (Table 2), the only exception being the Lactobacillaceae. Changes in relative abundance were stronger when CBA mice received the SJL microbiota than when SJL mice received the CBA microbiota (Fig. 4).

Overall, these findings suggest that while the FMT successfully altered the strain-specific composition of the microbiota, the CBA strain was more susceptible to invasion by the SJL microbiota, and the SJL was more resistant to colonization by the CBA microbiota.

3.3. Heligmosomoides polygyrus life history traits

We measured four *H. polygyrus* life history traits during the early phase of the infection (up to day 29 p.i.): the dry biomass of adult worms at day 10 p.i., the number of eggs shed in the feces at day 10 p.i., the per capita fecundity at day 10 p.i., and the cumulative egg output from day 13 to day 29 p.i. Given that at day 29 p.i., the parasite burden was already very low in SJL mice, we stopped the monitoring of fecal egg counts at this time point in this mouse strain. For CBA mice, monitoring of the infection dynamics was kept up to day 73 p.i., and therefore for this strain we also had values of dry biomass of adult worms, number of eggs shed in the feces, and per capita fecundity at day 73 p.i.

The dry biomass of adult worms was strongly correlated with the number of individual nematodes counted in a subsample of hosts (Pearson $r = 0.97$, $P < 0.0001$, $n = 27$), showing that biomass is a reliable indicator of parasite burden. Worm dry biomass at day 10 p.i. varied as a function of the interaction between mouse strain and FMT (two-way ANOVA: strain, $F_{1,27} = 8.67$, $P = 0.0066$; FMT, $F_{2,27} = 1.82$, $P = 0.1808$; strain × FMT, $F_{2,27} = 4.01$, $P = 0.0298$; Fig. 5A). At day 29 p.i., dry biomass of worms recovered in the intestine of SJL mice did not differ between FMT groups ($F_{2,15} = 1.58$, $P = 0.238$). Similalry, the dry biomass of worms recovered at day 73 p.i. in CBA mice did not differ between FMT groups ($F_{2,15} = 0.26$, $P = 0.7715$).

Fecal egg counts at day 10 p.i. were consistently higher in the CBA strain and were not affected by the FMT (two-way ANOVA: strain, $F_{1,27} = 12.69$, $P = 0.0014$; FMT, $F_{2,27} = 1.86$, $P = 0.1754$, strain × FMT, $F_{2,27} = 0.93$, $P = 0.4086$; Fig. 5B). At day 29 p.i., numbers of eggs shed in the feces of SJL mice did not differ between FMT groups ($F_{2,15} = 1.50$, $P = 0.2539$). Similarly, at day 73 p.i., numbers of eggs shed in the feces of CBA mice did not differ between FMT groups ($F_{2,15} = 0.53$, $P = 0.5992$).

We computed per capita fecundity at day 10 p.i. as the strain-specific residuals of the regression of fecal egg count on worm dry biomass (CBA: slope ± S.E. = 32.69 ± 5.11, $P < 0.0001$, $n = 18$; SJL: slope ± S.E. = 22.74 ± 4.95, $P = 0.0005$, $n = 15$). Per capita fecundity varied as a function of the interaction between mouse strain and the FMT (two-way ANOVA: strain, $F_{1,27} = 0.00$, $P = 0.9514$; FMT, $F_{2,27} = 2.59$, $P = 0.0936$; strain × FMT, $F_{2,27} = 3.65$, $P = 0.0395$; Fig. 5C). At day 29 p.i., per capita fecundity of worms in SJL mice did not differ between FMT groups ($F_{2,15} = 0.22$, $P = 0.8033$). Similarly, at day 73 p.i., per capita fecundity of parasites infecting CBA mice did not differ between FMT groups ($F_{2,15} = 0.70$, $P = 0.5101$).

Cumulative parasite egg output was computed as the sum of fecal egg counts between day 13 and day 29 p.i. Cumulative egg outputs varied as a function of the interaction between mouse strain and the FMT (two-way ANOVA: strain, $F_{1,29} = 62.22$, $P < 0.0001$; FMT, $F_{2,29} = 3.54$, $P = 0.0423$; strain × FMT, $F_{2,29} = 4.18$, $P = 0.0253$; Fig. 5D). For CBA mice we also investigated whether cumulative parasite egg outputs up to day 73 p.i. were affected by the FMT, and found no differences between groups ($F_{2,15} = 0.11$, $P = 0.8991$).

To summarise, while SJL mice maintained their resistance profile against *H. polygyrus* independently of the FMT, CBA mice receiving the SJL microbiota harbored worms with reduced per capita fecundity during the early phase of the infection, suggesting a possible microbiota-dependent acquired resistance.

3.4. Cytokine expression in response to H. polygyrus infection

Cytokine expression in the spleen at day 10 p.i. was strongly strain-dependent. SJL mice had higher IFN-γ, IL-6 and IL-10 expression in response to *H. polygyrus* infection, and this strain-specific expression was not altered by the FMT (Table 3;

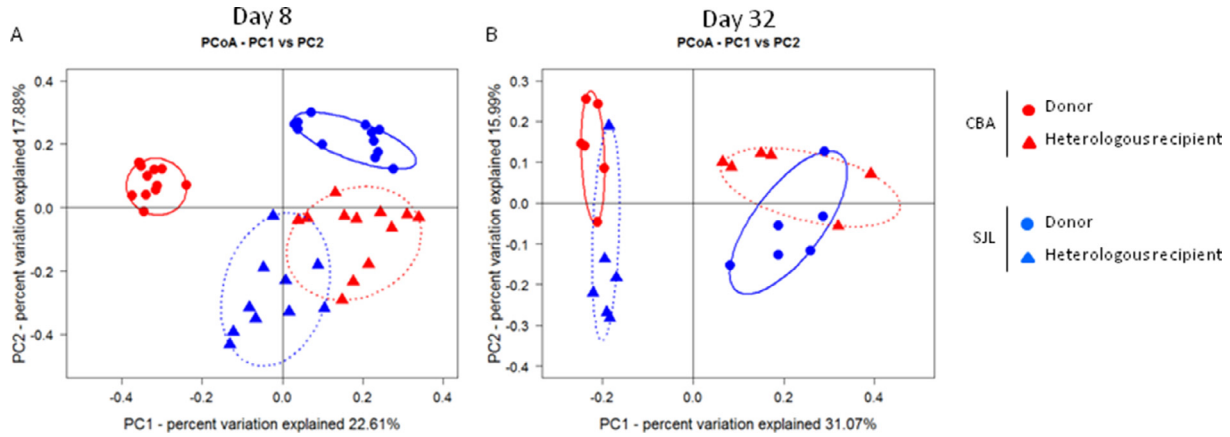

Fig. 3. Principal Coordinate Analysis of β diversity (Bray–Curtis distances) of fecal microbiota of SJL (blue symbols) and CBA (red symbols) mice at days 8 (A) and 32 (B) post-fecal microbiota transplant. Dots refer to donors and triangles to heterologous recipients.

Table 2
Linear mixed effects models exploring the effect of mouse strain (CBA, SJL), time since the start of the fecal microbiota transplant and their interaction on changes in relative abundance of bacterial families in heterologous recipients. The six bacterial families correspond to those with different relative abundance between strains prior to the FMT. Families on the left side of the table had higher relative abundance in SJL mice prior to FMT; families on the right side of the table had higher relative abundance in CBA mice prior to FMT. In each model, mouse ID was also included as a random factor to take into account the repeated nature of the data.

Bacterial family							
Bacteroidales S24-7				Lachnospiraceae			
	df	*F*	*P*		*df*	*F*	*P*
Mouse strain	1,72	58.67	<0.001	Mouse strain	1,20.2	6.7	0.018
Time post-FMT	3,72	0.76	0.518	Time post-FMT	3,52.7	4.18	0.001
Time post-FMT × mouse strain	3,72	8.97	<0.001	Time post-FMT × mouse strain	3,52.7	11.3	<0.001
Prevotellaceae				Bacteroidaceae			
Mouse strain	1,22.4	29.57	<0.001	Mouse strain	1,72	35.53	<0.001
Time post-FMT	3,54.7	5.16	0.003	Time post-FMT	3,72	5.19	0.003
Time post-FMT × mouse strain	3,54.7	5.28	0.003	Time post-FMT × mouse strain	3,72	4.7	0.005
Clostridiales vadinBB60				Lactobacillaceae			
Mouse strain	1,23.2	11.22	0.003	Mouse strain	1,25.5	7.04	0.014
Time post-FMT	3,55.4	16.79	<0.001	Time post-FMT	3,57	0.19	0.905
Time post-FMT × mouse strain	3,55.4	9.65	<0.001	Time post-FMT × mouse strain	3,57	1.94	0.134

df, degrees of freedom.

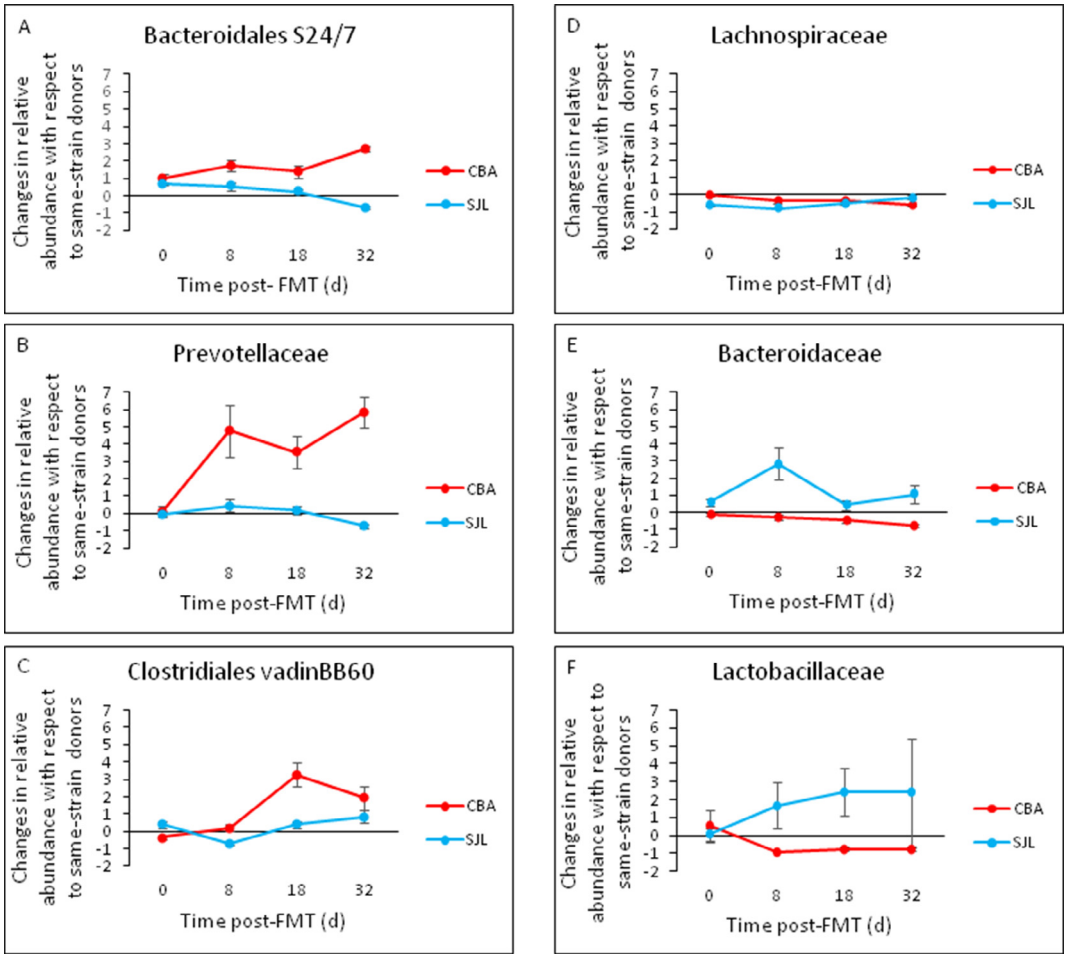

Fig. 4. Changes over time in relative abundance of six bacterial families in microbiotas of heterologous recipient mice with respect to same-strain donor mice. The six bacterial families correspond to those that had significant differences in abundance between mouse strains prior to the fecal microbiota transplant. (A–C) Bacterial families that had higher initial abundance in SJL mice; (D–F) Bacterial families that had higher initial abundance in CBA mice. Each panel reports means ± S.E. for the two strains.

Fig. 6A–C). A different result was obtained for IL-4 expression which was affected by the interaction between mouse strain and the FMT (Table 3; Fig. 6D).

Therefore, while FMT did not alter IFN- γ, IL-6 and IL-10 expression, CBA heterologous recipients tended to acquire the profile of IL-4 expression of SJL donors.

4. Discussion

The aim of this study was to investigate the role played by the gut microbiota and the host genetic background in resistance to the gastrointestinal nematode *H. polygyrus*. For this purpose, we performed a reciprocal FMT experiment between two mouse

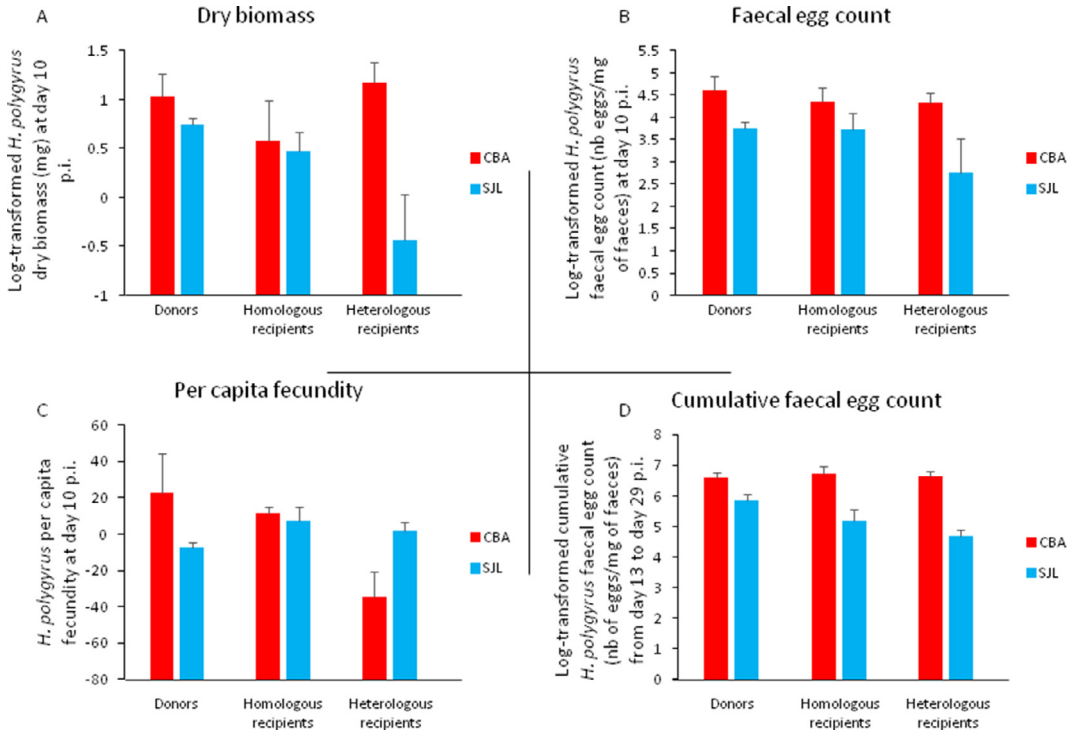

Fig. 5. *Heligmosomoides polygyrus* life history traits (mean ± S.E.) in CBA and SJL mice in the three experimental groups (donors, homologous and heterologous recipients. (A) log-transformed dry biomass at day 10 p.i., (B) log-transformed fecal egg count (number of eggs/mg of feces) at day 10 p.i., (C) per capita fecundity at day 10 p.i., (D) log-transformed cumulative fecal egg count (number of eggs/mg of feces) from day 13 to day 29 p.i.

Table 3
ANOVAs exploring the effect of mouse strain (SJL, CBA), fecal microbiota transplant (donors, homologous recipients, heterologous recipients) and their interaction on gene expression of IL-6, IFN-γ, IL-10 and IL-4 (relative to β-actin) in the spleen of *Heligmosomoides polygyrus*-infected individuals. For each model, we also report the overall proportion of variance explained (R^2).

	IL-6		
	R^2 = 0.71		
Sources of variation	*Df*	*F*	*p*
Mouse strain	1,26	57.05	<0.001
FMT	2,26	1.21	0.313
Mouse strain × FMT	2,26	0.49	0.615
	IFN-γ		
	R^2 = 0.69		
Sources of variation	*df*	*F*	*p*
Mouse strain	1,26	54.87	<0.001
FMT	2,26	0.74	0.485
Mouse strain × FMT	2,26	1.13	0.257
	IL-10		
	R^2 = 0.88		
Sources of variation	*df*	*F*	*p*
Mouse strain	1,26	198.20	<0.001
FMT	2,26	2.34	0.116
Mouse strain × FMT	2,26	0.03	0.975
	IL-4		
	R^2 = 0.70		
Sources of variation	*df*	*F*	*p*
Mouse strain	1,26	47.35	<0.001
FMT	2,26	0.78	0.469
Mouse strain × FMT	2,26	4.28	0.025

df, degrees of freedom.

strains with different patterns of resistance to *H. polygyrus*. We found that (i) the composition of the microbiota differed between the two strains prior to the start of the experiment; (ii) the strain (CBA) that was susceptible to *H. polygyrus* was also more susceptible to colonization by the heterologous microbiota; (iii) the SJL strain maintained its resistance profile against *H. polygyrus* independently of the FMT; (iv) the CBA strain receiving the microbiota from the resistant SJL strain harbored parasites with lower per capita fecundity during the early phase of the infection, and had higher expression of the Th2 cytokine IL-4, suggesting a microbiota-dependent acquired resistance to *H. polygyrus* in this mouse strain.

The idea that the microbiota can confer protection against infectious diseases has been substantiated by several studies and the mechanisms underlying this protective effect have been identified in some cases (Stecher and Hardt, 2008; Baumler and Sperandio, 2016; Brown and Clarke, 2017). Gastrointestinal helminths are certainly involved in very tight interactions with the gut microbiota because they essentially share the same ecological niche within the intestine (Glendinning et al., 2014; Gause and Maizels, 2016). Actually, it has long been known that the host's microbiota is an essential component of a helminth's environment, and alteration of this component can result in compromised (Wescott and Todd, 1964; Wescott, 1968) or improved helminth fitness (Reynolds et al., 2014). Therefore, it is possible to envisage that inter-individual variation in the host's capacity to deal with a helminth infection is partly due to a difference in the diversity and composition of the microbiota. Using a FMT approach, we provide support for this hypothesis; however, the results also showed that the efficacy of the transplant and the associated effects on the nematode life history traits were host strain-dependent, and that host strain per se remained the main factor that shaped the infection profile.

FMT has been used to investigate the involvement of the gut microbiota in several diseases (Kuethe et al., 2016; Turnbaugh et al., 2006). In animal models, a microbial transplant is usually done either in germ-free individuals or in conventional mice that have been previously treated with antibiotics, with bowel cleans-

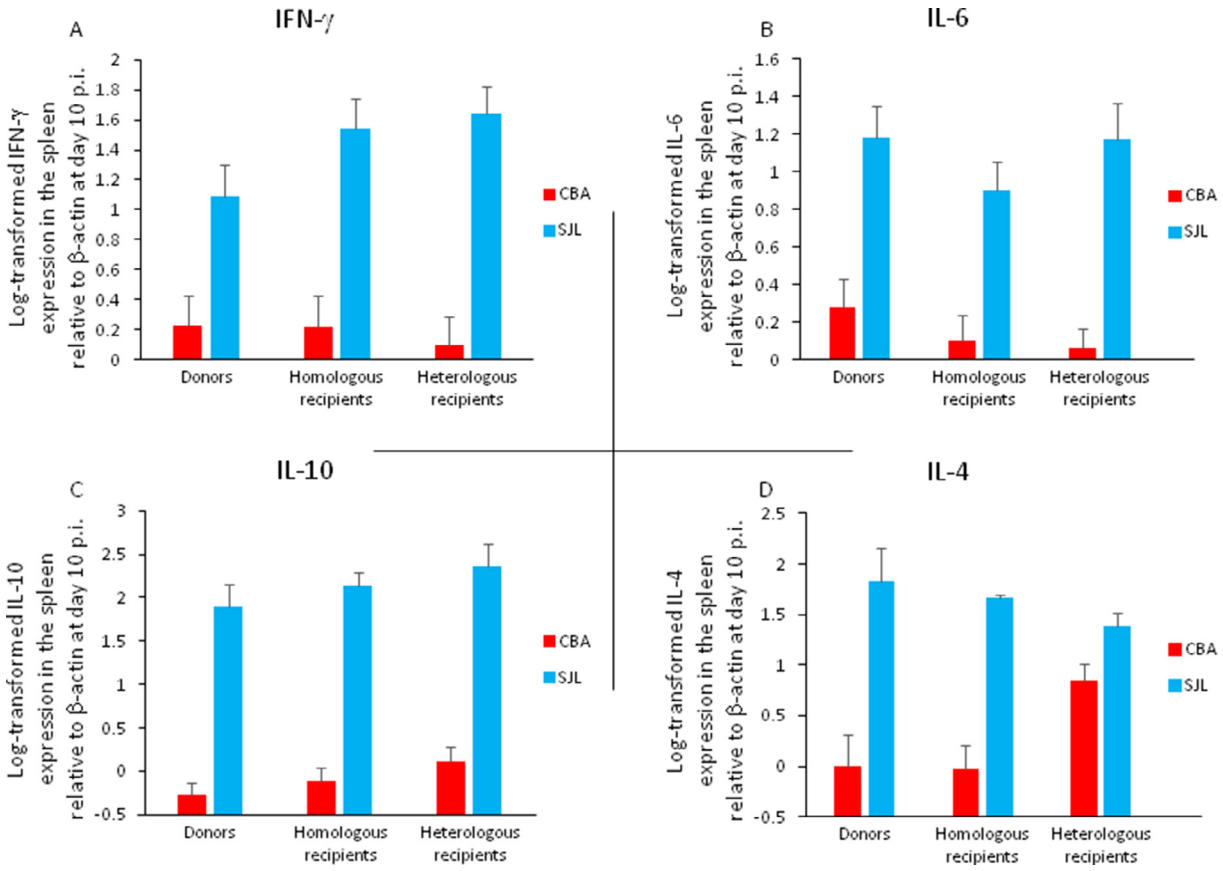

Fig. 6. Log-transformed gene expression (mean ± S.E.) of IFN-γ (A), IL-6 (B), IL-10 (C) and IL-4 (D) relative to β-actin in the spleen, at day 10 p.i., of *H. polygyrus*-infected CBA and SJL mice.

ing solutions or left untreated (Ishikawa et al., 2017). Antibiotic treatment is supposed to disrupt the resident microbiota and facilitate the transfer of the new microbiota, and it usually provides the best results in terms of colonization efficiency post-FMT (Ji et al., 2017). It is, however, important to acknowledge that single or repeated antibiotic administrations never succeed in eradicating the resident microbiota. Antibiotic treatment should then be seen more as an environmental perturbation of the resident microbiota that should facilitate colonization by the new one (Ji et al., 2017). Another potential caveat with the common procedure used to transplant the gut microbiota, which also applies to our study, is that some bacterial taxa, especially anaerobic ones, might be lost during the transfer.

In agreement with the asymmetrical effect of the FMT, we found that SJL mice maintained their resistance profile against *H. polygyrus*, whichever microbiota they received. Similarly, the expression of several cytokines was consistently higher in SJL mice independent of the FMT. On the contrary, while the more receptive CBA mice still harbored more parasites than the resistant SJL. *Heligmosomoides polygyrus* infecting heterologous transplanted CBA mice had lower per capita fecundity compared with worms infecting CBA donors; however, this effect was restricted to the early stage of the infection and the FMT did not modify per capita fecundity 2 months p.i. In addition, heterologous CBA recipients had higher expression of the Th2 cytokine IL-4, known to be a marker of resistance towards *H. polygyrus* (Pelly et al., 2016). Host strain-dependent effects of the microbiota on resistance to *H. polygyrus* have been suggested in previous work. Using outbred ICR mice, Wescott (1968) found reduced parasite burdens at day 30 p.i. in germ-free compared with conventional individuals; whereas in BALB/c mice, treatment with vancomycin increased the number of parasites recovered in the intestine at day 28 p.i. (Reynolds et al., 2014). Strain-dependent interactions between the microbiota and the infection with *H. polygyrus* are further corroborated by the finding that while infection increases the relative abundance of *Lactobacillus* in C57BL/6 hosts, it reduces *Lactobacillus* abundance in BALB/c mice (Reynolds et al., 2014). More generally, variable donor- or recipient-specific success of a fecal transplant has been reported in studies involving both mice and humans (Li et al., 2016; Vermeire et al., 2016; Ericsson et al., 2017). The asymmetrical effect of FMT in the two host mouse strains also questions the effectiveness of teasing apart the host genetic background from the microbiota effect, at least in SJL mice. It is indeed possible that, in this strain, the resistance profile is due to a direct effect of host genes (immunity) or to an indirect effect of host genes on the microbiota diversity and composition. Further studies using axenic SJL mice infected with *H. polygyrus* might help in elucidating this point.

The mechanisms underlying the asymmetrical effects between the two host mouse strains are unknown, although one might speculate about the differential permeability of specific bacterial taxa to colonize the different strains, possibly linked to strain-specific immune responses or genes (Leung and Loke, 2013; Pham et al., 2014; Turpin et al., 2016). In agreement with this view, we found that the two host strains had very different cytokine profiles (a finding that has already been reported for SJL and CBA mice; Filbey et al., 2014), with SJL having a higher propensity to produce pro-inflammatory cytokines such as IFN- γ and IL-6. This might make SJL mice prone to resist colonization by any microorganism, whatever the potential effect of the microbe (pathogenic or com-

mensal). This also would imply that using another parasite/-pathogen (instead of *H. polygyrus*) might provide a different picture of the strain-dependent effect of FMT. Actually, it seems plausible that the effectiveness of FMT as a way to promote resistance might depend on several, independent or synergistic, factors (not only host genetic background but also pathogen species, the initial diversity and composition of the transferred microbiota). This last point raises the question of the reproducibility and generality of the results reported in this study. In this respect, certainly no general conclusion can be drawn based on a single experiment and we advocate that more studies using the same, as well as other, parasites and host strains are needed to have a better understanding of the involvement of the microbiota in the process of resistance to infection. As other studies are made available in the literature, robust and quantitative approaches (meta-analyses) will allow conclusions on whether a consistent pattern in the relationship between host-microbiota-infection emerges or not. In the meantime, we believe that it is important to highlight the point that using FMT as a therapeutic tool to cure infection should not be considered without a better understanding of the intrinsic (genetic) and extrinsic (ecological) factors potentially shaping the effectiveness of the treatment. This might contribute to elucidating why FMT does not always result in symptom remission in certain pathologies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2019.06.001.

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